

Biochemical and Molecular Characterization of Indigenous Leafy Vegetables of Jorhat district of Assam

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By

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This is to certify that the thesis entitled “**Biochemical and Molecular Characterization of Indigenous Leafy Vegetables of Jorhat district of Assam**” submitted to the Faculty of Agriculture, Assam Agricultural University in partial fulfillment for the degree of **Doctor of Philosophy (Agriculture) in Agricultural Biochemistry** is a record of research work carried out by **Billal Hoque Choudhury** under my personal supervision and guidance.

All helps received by him have been duly acknowledged.

No part of this thesis has been reproduced elsewhere for any degree.

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ABSTRACT

Twenty five indigenous leafy vegetables were selected on the basis of their popularity of uses and collected from various places of Jorhat district. These plants were used for biochemical and molecular investigation. These plant species showed significant variations in various biochemical constituents such as moisture (76.40- 92.77%), ash (7.80-17.70%), K (0.64-9.58%), Na (52.0-233.00 mg/100 g), Ca (200.7-2104.96 mg/100 g), P (38.00-603.00 mg/100 g), Fe (31.00-401.00 mg/100 g, Cu (0.003 -0.020%), Zn (0.013-7.55 mg/100 g), Mn (12.00-132.00 mg/100 g), As (0.00-1.70 mg/100 g), crude fat (1.13-7.10%), crude fibre (2.50-26.10%), crude protein (15.77-39.38%), total free amino acid (129.27-992.00 mg/100 g), starch (18.78-56.42%), TSS (7.37-18.37%), reducing sugar (4.19-18.58%), non-reducing sugar (0.72-7.36%), soluble protein (1.10-4.13%), vitamin C (39.00-119.67 mg/100 g), riboflavin (0.17-8.80 mg/100 g), thiamine (0.00-0.08 mg/100 g), niacin (0.15-2.63 mg/100 g), total alkaloid (0.13-3.23 mg/100 g), saponin (0.23-0.61%), tannin (0.37-2.82%), phytic acid (0.03-0.32%), oxalate (0.02-0.13%), total phenol (0.25-2.2%), free radical scavenging activity (74.81-91.72%), total flavanoid (0.86-3.40%), total flavanol (0.33-1.63%), super oxide dismutase specific activity (0.11-0.24 unit min⁻¹ mg⁻¹protein), catalase specific activity (0.053-0.13 unit min⁻¹ mg⁻¹protein), peroxidase specific activity (0.01-0.08 unit min⁻¹ mg⁻¹protein). The RAPD analysis of *Alternanthera* spp. land races revealed the genetic diversity among the land races. The maximum level of polymorphism was generated by OPA-04 primer (100%) followed by OPB-05 primer (87.50%). The RAPD analysis of *Amaranthus* spp. land races revealed the genetic diversity among the land races. The maximum level of polymorphism was generated by OPB-01 primer (71.42%) followed by OPB-07 primer (66.67%). The RAPD analysis of *Chenopodium* spp. land races revealed the genetic diversity among the land races. The maximum level of polymorphism was generated by OPA-04 primer (100%) followed by OPB-02 primer (77.21%). The RAPD analysis of *Enhydra* spp. land races revealed the genetic diversity among the land races. The maximum level of polymorphism was generated by OPC-01 primer (100%) followed by OPB-01 primer (80.00%).

From the present study it was observed that all the indigenous leafy vegetables are rich in various nutritionally significant biochemical constituents and genetic diversity exists

among the land races of studied plant species though no distinct morphological variation was seen.

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ABBREVIATIONS USED

%	: Per cent
μ	: Micron(s)
°C	: Degree celcius
g	: Gram(s)
cm	: Centimetre(s)
mm	: Millimetre(s)
mg	: Milligram(s)
ml	: Millilitre(s)
sec	: Second(s)
min	: Minute(s)
h	: Hour(s)
mM	: Milli molar
M	: Molar
N	: Normal
BHA	: <u>Butylated hydroxyanisole</u>
BHT	: Butylated hydroxytoluene
CTAB	: N-Cetyl –N,N,N-Trimethyl Ammonium Bromide
EDTA	: Ethylene diamine tetra acetic acid
DPPH	: 2,2 diphenyl-1-picryl-hydrazyl
DNS	: 3, 5-dinitrosalicylic acid
NBT	: Nitroblue tetrazolium
OD	: Optical density
kcal	: Kilo calorie
v/v	: Volume/volume
g/l	: Gram/litre
e.g	: Exempli gratia(for example)
Fig.	: Figure
viz.	: Videlicet(namely)
LDL	: Low-density lipoprotein
NTSYS	: Numerical Taxonomy System
PCR	: Polymerase Chain Reaction

CHAPTER I

INTRODUCTION

Food plants are crops that grow wild or are cultivated and which are gathered or harvested for food within a particular ecosystem. Most of the traditional leafy vegetables are abundant in marginal lands that commonly increase in areas with disturbed soils or agricultural activity (Cunningham, 1988). Wild foods grow naturally in the bush and do not have to be tended for them to produce edible parts. Semi-wild or semi-cultivated foods are protected when they grow close to the home. Plants have been essential for human existence for millennia. They are sources of food in the form of leaves, seed, berries, fruit, roots, tubers, stems, rhizomes and to a lesser degree gums (Chweya and Eyzaguirre, 1999; Maundu, 1997). According to the FAO (1988), traditional vegetables are all plants whose leaves, roots or fruits are acceptable and used as routine vegetables by rural and urban communities through tradition, custom and habit. They were widely consumed; especially play crucial role during famines or natural disasters. Traditional vegetables might not be indigenous to a country, but they can be associated with traditional production systems, local knowledge and usually have a long history of local selection and usage (Keller *et al.*, 2004) and are described as indigenized foods (Phillips - Howard, 1999). Wild plants have been formed an integral part of the regular diet, culture and tradition of many indigenous communities of the world. In many developing countries most of the rural communities still depend on wild plants to meet-up their dietary requirements, especially during scarcity and that plays an important role in their food security (FAO, 2004; Balemie and Kebebew, 2006). Inclusion of wild edible plants as regular dietary supplement by the ethnic groups is mainly for sustenance in their dwelling environment and also intrinsically linked to their traditional and cultural life. They also sell some wild edible plants in the local markets for additional income (Angami *et al.*, 2006).

Traditional leafy vegetables have several advantages over the exotic crops that are promoted extensively by research and extension. Traditional leafy vegetables have a short growing period as they can be harvested within 3-4 weeks, they can tolerate abiotic and biotic

stress and they respond well to organic fertilizers (Maundu, 1997) and marginal land conditions. Many traditional crops grow in marginal areas, where exotic crops struggle to survive (Abukutsa - Onyango, 2007). The bulk of this production never reaches the market as they are mainly used for household consumption, leading to an underestimation of the importance of these crops to household food security (Hart and Vorster, 2006; Shackleton *et al.*, 1998). These crops are growing under rainfed conditions as intercrops or very often as weeds with local staples in home gardens or fields, and management thereof is relatively low (Hart and Vorster, 2006; Mnzava, 1997). With amaranth reported as a host for stem borers, which is a major pest in maize (Vorster *et al.*, 2007), it could have a negative impact. There is an indication that amaranth might suppress nematodes in soils but researchers also report that *Amaranthus cruentus* is susceptible to root knot nematodes and could have a negative allelopathic effect on tomatoes. Kordylas (1990) reported that traditional leafy vegetables are mainly consumed when fresh, but are preserved by using their traditional drying methods or using a solar drying method. Traditional leafy vegetables are often used and make up a large percentage of the food intake, even if substitute products are for sale. This leads to an increase of diversity of the diet (Hart and Vorster, 2006; Shackleton *et al.*, 1998), with those used as supplements adding flavour to dishes (Maundu, 1997). Constant downgrading of this vast edible plant resource and the associated knowledge by research and extension in India, new generation particularly the youth fails to appreciate their taste and quality preferring 'imbalanced modern' food or 'fast food' in the face of income induced diet diversification and diet globalization. Thus these vegetables are not underutilized but undervalued and lack of nutritional, anti-nutritional and nutraceutical data for providing scientific support for their large scale preservation, collection, popularization and careful and sustainable exploitation of these vast resources for human subsistence in this stage of rapid environmental, economic and demographic transition.

Man is using plants since the dawn of civilization to treat various diseases, disorders and as food. This interaction of man and plants leads to the establishment of the traditional knowledge of plants. A plant in one place may be useful as food, feed, fiber and medicine, while at others it may be a weed. Thus, plant species cannot be considered as weed under all circumstances (Ullah *et al.*, 2006). Assam state of India is one of the few places on earth with such a unique biodiversity, comprising different climatic zones with a wide range of plant species. This traditional knowledge of ethnic groups around the globe forms the basis of

modern plant based manufacturing especially allopathic medicine as well as food processing industry. Certain practices are unique to a given culture of a society and vary between countries, regions, villages and even communities, and this knowledge of the people has been termed as indigenous knowledge (Nagnur *et al.*, 2006). India is a country of national food self-sufficiency and able to export food. However, hunger and malnutrition are still found in many rural and urban areas. Leafy vegetables (greens) play a major role in the Assamese diet, probably due to the influence of traditional herbal medicine, easy accessibility and low cost.

Further, green leaves are considered as a main source of vitamins, minerals and fiber for local consumers. Due to their dietary importance, many scientific studies have been carried out on the nutritive values of green leaves. Information as well as misinformation regarding the true nutritional and/or undesirable effects plays a major role in this process of selection. There are plenty of reports published worldwide about the risk of misidentification of culinary herbs as well as the consumption of toxic herbs, which can cause irreparable damage to vital organs and body functions including death. Exotic vegetable species cannot be grown under the harsh climatic and resource- poor conditions encountered in many of rural areas with this problem. However, there are a number of indigenous and traditionally grown plant species which could help to alleviate this situation. The use of wild plant foods in the region during flood periods contributes to food security and provides dietary supplements to the staple diet. Vegetables are of great importance in helping to alleviate this problem as they significantly contribute the amount of calories and other nutrients in the diet. The scarcity of vegetables, or their scarcity in the diet, is a major cause of vitamin A deficiency, which causes blindness and even death of small children throughout the developing countries. The use of wild plants as leafy vegetables is very common in Assam. Some of these species are also very popular but some species are sought-after more than others.

The north eastern region of India belongs to the Indian Eastern Himalayas – a global biodiversity hot spot which endowed with a great wealth of plant genetic resources. There is an estimated 3895 species of flowering plants found in Assam alone (Gopichand, 2008). Out of the vast floral diversity of the region, a large number of indigenous plant species and their wild forms constitute the routine vegetables of the inhabitants which provide sufficient nutrients, novel nutraceuticals and medicinal value for the people. Wild vegetables have formed a year round food security strategies for generations, particularly women, children, poor household and

tribal communities who rely on consumption of wild traditional vegetables to fulfill their daily requirement of vitamins and minerals especially vitamin A and iron.

The knowledge, values and beliefs of the caregivers, most importantly women, will determine the nutritional contribution of these wild vegetable plants through their multiple uses as foods, feeds or medicines. Distribution, seasonality and availability of these vegetables are governed by the agro-ecological factors while, identification, collection and production, parts used, preference, method of processing and preservation, culinary practices, extent of use, perception about the content of anti-nutritional and medicinal values of a given plant species etc. are associated with the traditional knowledge and cultural beliefs.

Even though the current exploitation of many plants by rural and tribal population is restricted in the areas of their origin, some of them have gained significant place in urban market for their equal acceptability among the urban population. The valorization of local products is an opportunity to safeguard marginal land for biodiversity preservation, to improve economy of the local populations and to enlarge the food basket to obtain a more diversified diet. Perhaps, due to the long association of the local people of this region with the vast natural flora and fauna, the occurrence and prevalence of malnutrition diseases associated with vitamins and micronutrients such as anemia, night-blindness and goiter are comparatively less in this region compared with the national average.

Over the ages, the local community have discovered and developed indigenous use of many wild plants/plant parts within their environment as food sources (Ramkrishnan, 1992; Das, 1998). However, many of these plant species of this region virtually unknown outside the land (Ramkrishnan, 1998). Nutritional studies have indicated that many of these vegetables are rich in carbohydrates, proteins, minerals and vitamins (Ismail, 2000). Some of the vegetables are also be claimed to have medicinal properties such as blood cleansing, induction of uterine contractions and preventing or care of ailments such as diabetes, high blood pressure, cardiovascular diseases, arthritis, fever and cough. In addition, it is also believed that vegetables play a vital role in lowering the incidence of cancer as well as the control of ageing and age related diseases (Abas *et al.*, 2005).

The search of lesser known crops, many of which are potentially valuable as human and animal food has been identified to maintain a balance between population growth and

agricultural productivity, particularly in tropical and sub tropical areas of the world. In those regions indigenous vegetables are abundant immediately after the rainy season and very scarce during the rainy season. India being blessed with a variety of natural surroundings and varying climates and seasons, has a number of edible green leafy vegetables, some of which are locally grown and utilized.

Green leafy vegetables are rich sources of vitamins such as β -carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as Fe, Ca, and P. Green leafy vegetables are also recognized for their characteristics colour, flavour and therapeutic value. Apart from these, there are various types of underutilized leafy vegetables which are available seasonally and practically no information is available on the nutrient content and anti-nutritional factor of such vegetables consumption. Consumption of such food material is confined to the people living in the areas where they grow. In general they contained less oxalates compounds to cultivated species (Shingade *et al.*, 1995).

However, antinutritional factors *viz.*, oxalates, tannin and saponins were found in underutilized green leafy vegetables (Gupta and Wagle, 1978). Dietary antioxidant intake has been associated with reduced risk of cardiovascular disease (Hertog *et al.*, 1997), diabetes (Montonen *et al.*, 2004), cancer (Ekstrom *et al.*, 2000), neurodegenerative diseases (Gilgunsherki *et al.*, 2004). In traditional societies, nutrition and health care are interconnected and many plants are consumed as food in order to benefit health (Etkin, 1996; Pieroni, 2000; Pieroni *et al.*, 2002a, 2002b; Pieroni and Price, 2005). The nutraceutical value and the antioxidant activity of wild, semi-cultivated or neglected vegetables is regarded worldwide as an important area of the nutritional and phyto-therapeutic research (Lionis *et al.*, 1998; Grivetti and Ogle, 2000; Karakaya *et al.*, 2001; Ogle *et al.*, 2001a, 2001b, 2001c; Johnson and Grivetti, 2002; Owen and Johns, 2002; Coulaidis *et al.*, 2003).

On the other hand, oxidative stress is intricately linked with ageing related diseases (ARDs) and longevity: antioxidant activity was recently studied in relation to CNS disorders. The metabolism of phospholipids is in fact associated with neural death in Alzheimer's diseases (AD) and antioxidant such as vitamin E play an important role in β -amyloid ($A\beta$) aggregation, while very recently the Cache County study demonstrated that the use of vitamin and vitamin C

supplements in combination is associated with a reduced prevalence and incidence of AD (Zandi *et al.*, 2004).

Looking into the prevalence of higher level of micronutrient malnutrition among the valuable sections in developing countries and increasing prevalence of chronic degenerative diseases globally, the need for exploration of underutilized foods is significant to overcome the nutritional disorders. The diet and food based approach in combating micronutrient malnutrition is essential for its role in increasing the availability and consumption of micronutrient rich foods (FAO, 1997). Increasing the utilization of green leafy vegetables in our diet which are known to be rich sources of micronutrients as well as dietary fibre can be a food based approach for ensuring the intake of these nutrients. It is essential that the locally available green leafy vegetables, which are in expensive and easy to cook be used in the diets to eradicate micronutrient malnutrition and also to prevent degenerative diseases.

Food security is a condition related to the ongoing availability of food. Vegetables are ‘protective foods’ and contribute in essential micronutrients and vitamins of daily diet. They are rich in phyto-chemicals which are associated with lowering the risk of cardiovascular, digestive, colon cancer, anemia, fatigue, blindness and other immunity associated diseases. Indigenous vegetables are region specific, limited acceptance, part of subsistence production system, no established market and never undergone for any breeding program (Singh and Singh, 2012). Further, they are native to or originating from a particular region or and naturalized or evolved from introduced materials over a period of time. They are abundant, easily accessible, locally adaptable, cost effective and acceptable in custom and traditions of tribal and settler communities. Many of them are rich in ‘protective elements’ than exotic counterparts and also have antibacterial, antiviral, antiinflammatory, antithrombotic, vasodilatory actions and antioxidant activities (Olembo *et al.*, 1995; WHO, 2003). India is very diverse in climate, agro-ecologies, tribes, topography, altitudes and agri-biodiversity where promotion of local indigenous vegetables fit well in the concepts of ‘fresh is better’ and also ‘variety is important’ and this can reduce the burden on government account for targeting the malnutrition. In this line, the indigenous vegetables have significant role in food and nutritional needs of the tribes and rural communities (Singh *et al.*, 2012). However, systematic information was required for promotion of indigenous vegetables for ensuring sustainable food and nutritional security to marginal communities.

Thus, traditional leafy vegetables are plants whose leaves are socially accepted, used and consumed for various purposes as food, nutritional and medicinal values and additives. In Assam they appear as uncultivated and semi cultivated crops or weedy and wild plants, with ecological, social and cultural values, playing a significant role in the daily food and nutritional requirement of local people mainly in the rural and it is also getting importance in urban zone. Thus, it is of paramount importance that the agro-biodiversity of these important unexplored indigenous leafy vegetables be precisely characterized with the use of modern molecular tools. Genetic resource must be preserved based on this accurate characterization for the benefit of local people. The assessment of intra-specific genetic diversity is prerequisite for further action.

Systematic evaluation of this vast resource of wild edible plants of the state including their nutritional, antinutritional and potential benefit of phyto-chemicals, formal intervention as well as awareness creation can contribute to change in perception among the youth and adult. This is not only to promote the conservation, and ensuing of sustainable use of these diversified plant resources through enhanced utilization but also can play great role in improvement of rural health sector, tackling malnutrition and in food security in the face of environmental and societal changes in rural domains. Moreover, DNA finger printing of the wild vegetables of the region will certainly help in proper identification, authentication and documentation and in overcoming the ambiguity that arises out of different names of the same species by different tribes/ communities or localities. Further, it will also help in studying the molecular diversity as well as in addressing the related Intellectual Property Right and bio-piracy issues and Geographical Indication, if any. Keeping in view of these considerations, the present investigation is designed with the following fundamental objectives:

1. Survey, collection and preparation of database of traditional leafy vegetables of Assam.
2. Biochemical characterization of traditional leafy vegetables.
3. RAPD analysis of few important traditional leafy vegetables.

CHAPTER II

REVIEW OF LITERATURE

2.1 Floral biodiversity of North Eastern Region

The North Eastern region of India comprising the states of Arunachal Pradesh, Assam, Meghalaya, Manipur, Mizoram, Nagaland, Tripura and Sikkim (21.5° N - 29.5° N latitudes and 85.5° E - 97.3° E longitudes) represents a distinct agro-climatic zone of the country. The region is bounded by China in the north of Sikkim and Arunachal Pradesh, Bhutan in the east of Sikkim, China and Myanmar to the east and north of Arunachal Pradesh, Nagaland, Manipur and Mizoram and Myanmar and Bangladesh in the south of Manipur, Mizoram, Tripura and Meghalaya. It has a total geographical area of 262179 km², which is nearly 8% of the total geographical area of the country (Census, 2001). The NE region possesses the characteristics of inaccessibility (a product of altitude and terrain), fragility (a product of steep slopes and other biophysical conditions), marginality (a product of natural and manmade factors having both physical and socio-economic dimension) and rich in biodiversity and ethnicity and social set up.

Morphologically, NE region of the country is marked by the development of a series of ridges and valleys, terraces, scraps, several geomorphologic surfaces at different elevations up to 1,500 m and above. The mean annual rainfall exceeds 2000 mm. Out of 21 agro-ecological zones of the country; four zones are covered exclusively by NE region. The zones represent wide variation of climate ranging from cold to warm per-humid. In the whole of NE region, about 35% area is plain and the remaining 65% area is under hills. In Assam, plains account for 84.44% of its total geographical area and the remaining 15.56% area is under hills. Net sown area is highest in Assam (34.12%) followed by Tripura (23.48%). Arunachal Pradesh has lowest net sown area in the region. Cropping intensity is highest in Tripura (156.5%) followed by Manipur (152.1%), Mizoram (136.36%) and Assam (123.59%). About 0.5 million hectare area is under shifting cultivation in whole NE region. Out of 4.4 million hectare net sown area of the region, roughly 1.4 million hectare lies in hilly sub region and at least 1.3 million

hectare suffer from serious soil erosion problem due to its geographical situations, climatic variations and differences in altitudes.

No systematic and accurate estimate of area and production of different vegetable crops in the North Eastern Region are available and the estimates made by various sources vary considerably. North Eastern Council compiled the data available from different sources. According to Agricultural Research Data Book ICAR (2002) the total area under horticultural crops is around 822.5 thousand hectare, which is around 3.14% of the total geographical area of the region and it gives a total production of 6818.4 thousand tones (Yadav *et al.*, 2009).

2.2 Bio-diversity of vegetable crops in North Eastern region

The North Eastern region is considered to be the richest reservoir of genetic variability of large number of horticultural crops. The enormous diversity makes the region a gene pool for the varietal improvement but in spite of this potential much remains to be done for development in the field of horticulture in the region. It may be mentioned that in hill area particularly horticultural crop cultivation as an alternative to *jhuming* (shifting cultivation) may prove to be a boon in the regional economy. In NEH region, farming being the main stay of the people and development of horticulture will markedly improve the economy of the people. Establishment of orchards and planting of vegetable crops on hill slopes will prevent soil erosion of shifting cultivation areas and migration of people to towns. The diversity present among different vegetable groups is as follows:

Solanum group: There is wide range of *Solanum* spp. found in various parts of the region. The tribals grow a vegetable having red tomato like fruits slightly bitter in taste but related to brinjal which belongs to the *Solanum gilo* group. In Manipur another kind of brinjal having round fruit and intermediate in appearance between tomato and brinjal is grown which is botanically known as *Solanum macrocarpon*. Apart from these, *Solanum khasianum*, *Solanum torvum*, *Solanum*

mimosum, *Solanum insanum*, *Solanum kurzii* etc. are also found abundantly in the region. *Solanum khasianum*, which contains highest amount of solasodine, is found to be grown wild (Yadav *et al.*, 2009).

Tomato and Chilli: Tomato was introduced roughly in 18th century and most of the introductions are bred varieties, which have adapted to this region. Germplasm of wild species of tomato *Lycopersicon esculentum pimpinellifolium*, has been found in NE region. Chillies are usually grown in warm to hot and humid climate in Manipur, Mizoram, Meghalaya, Nagaland, Tripura and Arunachal Pradesh. Due to the long history of cultivation, out crossing nature and popularity of the crop; large genetic diversity including local landraces have evolved. In hot chilli great range of variability for several attributes (fruit shape, size, colour, and bearing habit and semi-perennial, perennial and pungency) occurs throughout the North Eastern Region. Bird Eye Chilli is native to Central and South America. Fruits are very small, but highly pungent and easily separated from the calyx and are dispersed by birds. *Dulle Khurshani* is common in Sikkim. Fruits are small round, highly pungent. It is liked by local people due to its peculiar aroma. Raja Mircha, which is also called as King chilli in Nagaland and Manipur and Bhut (Ghost) Jholakia in Assam. It is known as hottest chilli of the world. Tejpur Chilli is also a type of Raja Mircha (Yadav *et al.*, 2009).

Cucurbitaceous Vegetables: This is a large group of vegetable crops, consisting of more than 15 types, grown and consumed within the region. In North East, many species of cucurbits are found as vegetables and fruits. These include: *Cucurbita*, *Momordica*, *Luffa* and several lesser known cucurbitaceous crops. Pumpkin varieties abound in number with variation in fruit size, fruit skin, flesh colour, thickness, sweetness etc. The wild species, *Cucumis hardwickii*, the likely progenitor of cultivated cucumber, is found growing in natural habitats in the foothills of NE region particularly Meghalaya. *C. sativus* var. *sativus* is cultivated throughout North Eastern region in tropical and sub tropical condition. However, *C. sativus* var. *sikkimensis* has adapted in temperate and humid climate. In Nagaland one type of cucumber (*C. sativus*) is grown which is very soft and sweet in taste and has got tremendous potential for commercialization. Apart from

these, *C. hystrix*, which is round in shape and resistant to Downey mildew and *C. trigonus*, which is triangular in shape, are also found in the region. Among gourds, in North East India maximum variability has been recorded for bottle gourd in fruit shape and size. The Indian gene center has rich diversity in genetic resources of ridge gourd (*L. acutangula*) and sponge gourd (*L. cylindrica*) especially in North Eastern Region. In bitter gourd small as well large sized forms are available (Yadav *et al.*, 2009).

Crucifers: There are several cruciferous vegetables namely Cauliflower, Cabbage, Knolkhol, etc. which have been introduced from the days of East India Company in 14th-15th century when European traders visited this region. The genetic resources in these crops are essentially bred varieties introduced during the last 3-4 centuries and now acclimatization and adaptation has taken place. In North East, variability in Indian cauliflower widely exists in Assam and Meghalaya (Yadav *et al.*, 2009).

Leguminous vegetables: Wide variability of French bean, cowpea, Indian bean and broad bean are found in the various parts of the region. In French bean, climbing or pole type is popular among the tribal since it is used for mix cropping with maize, the stem of which act as the support for the bean. One of the interesting species of *Vigna*, namely *V. vexillata* is grown by the tribals of Tripura, which forms edible tuber. Sword bean (*Canavalia gladiata*), a bushy plant of papilionaceae family is also cultivated on limited scale in the North Eastern region of the country. Winged bean (*Psophocarpus tetraonolobus*), jack bean (*Canavalia ensiformis*), Lima bean (*Phaseolus lunatus*), mucuna bean or velvet bean (*Mucuna pruriens*), Rice bean (*Vigna umbellata*) are confined to humid sub tropical parts of NE region (Yadav *et al.*, 2009).

Leafy vegetables: The important leafy vegetables include lai (*Brassica juncea* var. *rugosa*), lafa (*Malva verticillata*) and palak (*Spinacea oleracea*). In addition to these a wide variety of indigenous leafy vegetables are also available. These are amaranth (*Amaranthus* spp.) poi sag (*Basell rubra* and *B. alba*), sorrel (*Rumex rasicarius*) etc. Other indigenous leafy vegetables used occasionally are Jilmil sag (*Chenopodium album*) and Kolmou sag (*Ipomea reptans*).

Amaranthus viridis, *A. lividus*, *A. retroflexus* and *A. spinosus* are important leafy vegetable types grown in North East India including Assam (Yadav *et al.*, 2009).

Tubers and Rhizomatous Crops: A number of dioscorea species i.e. *Dioscorea alata*, *D. bulbifera*, *D. brevipetiolata*, *D. esculenta*, *D. hamiltonii*, *D. hispida*, *D. kamaonensis*, *D. nummularia*, *D. pentaphylla*, *D. puber* and *D. quinata* were recorded in the NE Region. *D. hamiltonii* occurs in humid forests of NE hills. *D. floribunda* and *D. deltoidea* are certain medicinal types, which are used for steroid. Tapioca, or cassava has also been cultivated to some extent. In Colocasia also there is a wide variability even in one species such as *Colocasia*, *Allocasia* and *Xanthosoma*. Sweet potato white and red skinned are cultivated and around 50% is consumed as animal feed (Yadav *et al.*, 2009).

Lesser-known vegetables: There are a large number of indigenous vegetables crops that are used particularly by the tribal population. Maximum such varieties are available in the Arunachal Pradesh. Tree bean (*Parkia roxburghii*) is one of the most common of multipurpose tree species in the Manipur and Mizoram. In the hilly areas, tree tomato (*Cyphomandra betacea*), a perennial shrub producing egg shaped, reddish yellow in various shades, smooth skinned and weighing 30-50 g is also grown and used as such in chutney. It is grown as backyard venture crop in Meghalaya. Another vegetable tree growing in the lower altitude zones and popular among the people is drumstick or horse radish locally called Sajina (*Moringa oleifera*). Chow-chow or Chayote (*Sechium edule*) a native of tropical America is a very popular vegetable in the region commonly called Squash and grown abundantly without much care and attention. Sweet gourd or Kheksa (*Momordica cochinchinesis*) and spine gourd or Kakrol or Kartoli (*M. dioica*) are widely spread in Assam, the Garo hills of Meghalaya. *Flemingia vestita* known as Sohphlong (in Khasi), which belongs to family leguminosae, is consumed raw after peeling. It is a weak climbing/trailing type and produces underground tubers, distributed the humid to sub tropical regions of NE India up to 1500 m (Yadav *et al.*, 2009).

Assam is rich in flora and diverse in its vegetation types. This coincides with the variability of physical features, climate, soil, etc. There are tropical moist- evergreen, tropical semi-evergreen, moist deciduous, dry deciduous and sub-tropical moist evergreen forests. In addition to these, degraded forests, scrub jungles, grassland and savannahs, bamboo thickets, hydrophytes in wetlands and wastelands also occur. There is an estimated 3895 species of flowering plants found in Assam (Baruwa, 2006). The main inhabitants of the hilly area of the state are tribal people belonging to six major tribes, viz. *Karbi*, *Bodo*, *Mishing*, *Hmar*, *Kuki* and *Garo* (Borooah, 1985). Among them, the *Karbi* (*Mikir*) is a prominent tribe inhabiting mainly in Karbi-Anglong district of Assam. Karbi-Anglong lies between 92° 50' to 94° 25' East longitude and 25°05' and 26°15' North latitudes (Guha, 2002). *Karbi* people prefer to live on the hill slopes and are pile dwellers (Pegu, 1981). They belong to the Mongoloid racial stock (Bey, 2004). The tribes living in this district have been leading an intricate life that depended on wild plants. Their knowledge about the plants and their properties are immense. These plants are used as food plants, medicine, fodder, fuel, dye, and for festivals, rituals and other functions. Many have a high moisture and low dry matter content. Most of them also have high medicinal value. Major contributions of vegetables to human health are the large quantity of vitamin C, vitamin A and folic acid as well as good amount of dietary fibres and minerals. Folic acid helps in multiplication and maturation of cells, and its deficiency results in certain types of anemia, especially of infants and pregnant women (Sanwal *et al.*, 2007). The phyto-chemicals in vegetables protect the human body from a large number of ailments. That is why vegetables are often known as protective foods (Rai *et al.*, 2004).

2.3 Traditional leafy vegetables of Assam

Food plants are crops that grow wild or are cultivated and which are gathered or harvested for food within a particular ecosystem. Most of the traditional leafy vegetables are ruderals that commonly increase in areas with disturbed soils or agricultural activity (Cunningham, 1988). Wild foods grow naturally in the bush and do not have to be tended for them to produce edible parts. Semi-wild or semi-cultivated foods are protected when they grow

close to the home. Plants have been essential for human existence for millennia. They are sources of food in the form of leaves, seed, berries, fruit, roots, tubers, stems, rhizomes and to a lesser degree gums. According to the FAO (1988), traditional vegetables are all plants whose leaves, roots or fruits are acceptable and used as vegetables by rural and urban communities through tradition, custom and habit. They were widely consumed as routine vegetables, especially during famines or natural disasters. Traditional vegetables might not be indigenous to a country, but they can be associated with traditional production systems, local knowledge and usually have a long history of local selection and usage (Keller *et al.*, 2004) and are described as indigenized foods (Phillips-Howard, 1999). Traditional leafy vegetables are indigenous or traditional vegetables whose leaves, young shoots and flowers are consumed (Chweya and Eyzaguirre, 1999; Maundu, 1997).

Traditional leafy vegetables have several advantages over the exotic crops that are promoted extensively by research and extension. Traditional leafy vegetables have a short growing period as they can be harvested within 3-4 weeks, they can tolerate abiotic and biotic stress and they respond well to organic fertilizers (Maundu, 1997). Many traditional crops grow in marginal areas, where exotic crops struggle to survive (Abukutsa-Onyango, 2007b). The bulk of this production never reaches the market as they are mainly used for household consumption, leading to an underestimation of the importance of these crops to household food security (Hart and Vorster, 2007; Shackleton *et al.*, 1998). These crops are growing under rainfed conditions as intercrops with local staples in home gardens or fields, and management thereof is relatively low (Hart and Vorster, 2006; Mnzava, 1997). With amaranth reported as a host for stem borers, which is a major pest in maize (Vorster *et al.*, 2007a), it could have a negative impact. There is an indication that amaranth might suppress nematodes in soils but researchers also report that *Amaranthus cruentus* is susceptible to root knot nematodes and could have a negative allelopathic effect on tomatoes. Kordylas (1990) reported that traditional leafy vegetables are mainly consumed when fresh, but are preserved by using their traditional drying methods or using a solar method. Traditional leafy vegetables are often used and make up a large percentage

of the food intake, even if substitute products are for sale. This leads to an increase of diversity of the diet (Hart and Vorster, 2006; Shackleton *et al.*, 1998), with those used as supplements adding flavour to dishes (Maundu *et al.*, 1997).

Traditional foods contribute to household food supplies on a seasonal, emergency and supplemental basis (Rubaihayo, 1997; Shackleton *et al.*, 2000). Use of wild food during drought or in marginal areas increases and represents a part of the rural safety net against poverty and disaster (Shackleton, 2003). Communities often face a shortage of vegetables during the dry season. Preservation of edible leaves is one of the strategies developed to help face these times of shortage (Mnzava, 2005). During periods of unemployment, these plants become very important for the affected families (Dovie *et al.*, 2002). Leafy vegetables are very important to women and children in poor rural areas, as they are more dependent on the natural resource base. According to Mnzava (1997) improving the cultural practices, processing and status of the plants can improve the cash income and status of the plants. Lev (1981) stated that food sometimes competes for resources and that the distinction between food and cash crops is sometimes very indistinct. Lev (1981) also stated that cash income is sometimes converted to food and that these cases need to be studied on an individual basis as there is so much variety. Harnmeijer and Waters-Bayer (1993) found that the increase in cash crop and modern foods production lowers the diversity on the plate. Pagezy (1985) found that small scale cash cropping helped to overcome the seasonal fluctuation of food supply by providing the cash to buy food during this time, thus overcoming the nutritional imbalance in the lean periods. Some crops (amaranth, nightshade) that were traditionally women's crops and have become commercially viable, have been taken over by men (Abukutsa-Onyango, 2007b; Moore and Raymond, 2006). In an effort to develop a holistic perspective on food the nutritional culture of a society, it is necessary to connect it to a scientific study of nutrients of the same society (Khare, 1980). Several studies report on the vulnerability of women and young children (especially female children) due to the marginalization of their nutritional needs through indigenous food distribution practices (Fleuret and Fleuret, 1980). Poverty and hunger are closely related. Insufficient income leads to the

inability to buy food while hunger contributes to poverty by lowering the effectiveness of the immune system (less resistant to disease), lowering labour productivity and having a negative effect on educational achievements (Dixon *et al.*, 2001).

Green Leafy Vegetables are rich sources of vitamins such as B-carotene, ascorbic acid, riboflavin and folic acid as well as minerals such as Fe, Ca, and P. Green leafy vegetables are also recognized for their characteristics color, flavor and therapeutic value (Gupta *et al.*, 2005). Apart from these there are various types of underutilized leafy vegetables which are available seasonally and practically no information is available on the nutrient content and anti-nutritional factor of such vegetables consumption. Consumption of such food material is confined to the people living in the areas where they grow.

However, antinutritional factors *viz.* oxalates, tannin dietary fibre and saponins were found in underutilized green leafy vegetables (Gupta and Wagle, 1978). Dietary antioxidant intake has been associated with reduced risk of cardiovascular disease (Hertog *et al.*, 1997), diabetes (Montonen *et al.*, 2004), cancer (Ekstrom *et al.*, 2000), neurodegenerative diseases (Gilgunsherki *et al.*, 2004). In traditional societies, nutrition and health care are interconnected and many plants are consumed as food in order to benefit health (Etkin, 1996; Pieroni, 2000; Pieroni *et al.*, 2002a, 2002b; Pieroni and Price, 2005). The nutraceutical value and the anti oxidant activity of wild, semi-cultivated or neglected vegetables is regarded worldwide as an important area of the nutritional and phyto-therapeutic research (Lionis *et al.*, 1998; Grivetti and Ogle, 2000; Karakaya *et al.*, 2001; Ogle *et al.*, 2001a, 2001b, 2001c; Johnson and Grivetti, 2002; Owen and Johns, 2002; Coulaidis *et al.*, 2003; Zeghichi *et al.*, 2003).

The different wild vegetables are distributed throughout the state and are become available at various season and used by Assamese peoples. Some major wild vegetables extensively used by the local population are *Alternanthera sessilis* (Amaranthaceae), *Amaranthus spinosus* (Amaranthaceae), *Basella alba* var. *rubra* (Basellaceae), *Boerhavia diffusa* (Nyctaginaceae), *Centella asiatica* (Apiaceae), *Chenopodium album* (Chenopodiaceae), *Celosia*

argentea (Amaranthaceae), *Colocasia esculenta* var. *black* (Araceae), *Eichhornia crassipes* (Pontederiaceae), *Enhydra fluctuans* (Asteraceae), *Oxalis corniculata* (Oxalidaceae), *Solanum indicum* (Solanaceae), *Hydrocotyle javanica* (Apiaceae), *Nasturtium officinale* (Brassicaceae), *Nelumbo nucifera* (Nymphaeaceae), *Nymphaea stellata* (Nymphaeaceae), *Paederia foetida* (Rubiaceae), *Sagittaria sagittifolia* (Alismataceae), *Ipomoea aquatica* (Convolvulaceae), *Calamus erectus* (Arecaceae), *Portulaca oleracea* (Portulacaceae), *Diplazium esculentum* (Athyriaceae), *Hedyotis corymbosa* (Rubiaceae), *Drymaria cordata* (Caryophyllaceae), *Commelina benghalensis* (Commelinaceae), *Solanum nigrum* (Solanaceae), *Sida rhombifolia* (Malvaceae), *Eclipta alba* (Asteraceae), *Albizia lucida* (Mimosaceae), *Alpinia allughas* (Zingiberaceae), *Anthocephalus chinensis* (Rubiaceae), *Ntidesma acidum* (Euphorbiaceae), *Ntidesma bunius* (Euphorbiaceae), *Bischofia javanica* (Euphorbiaceae), *Calamus rotang* (Arecaceae), *Clerodendrum cloebrookianum* (Verbenaceae), *Commelina benghalensis* (Commelinaceae), *Cyclosorus extensa* (Thelypteridaceae), *Diplazium esculentum* (Athyriaceae), *Embelia subcoriacea* (Myrsinaceae), *Ficus auriculata* (Moraceae), *Garcinia lancifolia* (Clusiaceae), *Hedyotis diffusa* (Rubiaceae), *Hedyotis scandens* (Rubiaceae), *Hydrocotyl sibithorpioides* (Apiaceae), *Kalanchoe pinnatum* (Crassulaceae), *Lasia spinosa* (Araceae), *Leucas plukenetii* (Lamiaceae), *Mackaya neesiana* (Acanthaceae) (Pegu *et al.*, 2013; Kar and Borthakur, 2008).

Food security is a condition related to the ongoing availability of food. Vegetables are ‘protective foods’ and contribute in essential micronutrients and vitamins of daily diet. They are rich in phytochemicals which are associated with lowering the risk of cardiovascular, digestive, colon cancer, anaemia, fatigue, blindness and other immunity associated diseases. Indigenous vegetables are region specific, limited acceptance, part of subsistence production system, no established market and never undergone for any breeding program (Singh and Singh, 2012). Further, they are native to or originating from a particular region or and naturalized or evolved from introduced materials over a period of time. They are abundant, easily accessible, locally adaptable, cost effective and acceptable in custom and

traditions of tribal and settler communities. Many of them are rich in 'protective elements' than exotic counterparts and also have anti-bacterial, anti-viral, antiinflammatory, antithrombotic, vasodilatory actions and antioxidant activities (Olembo *et al.*, 1995; WHO, 2003). The India is very diverse in climate, agro-ecologies, tribes, topography, altitudes and agri-biodiversity where promotion of local indigenous vegetables fit well in the concepts of 'fresh is better' and also 'variety is important' and this can reduce the burden on government account for targeting the malnutrition. In this line, the indigenous vegetables have significant role in food and nutritional needs of the tribes and rural communities (Singh *et al.*, 2012). However, systematic information was required for promotion of indigenous vegetables for ensuring sustainable food and nutritional security to marginal communities.

Wild plants have been formed an integral part of the regular diet, culture and tradition of many indigenous communities of the world. In many developing countries most of the rural communities still depend on wild plants to meet-up their dietary requirements, especially during scarcity and that plays an important role in their food security (FAO 2004; Balemie and Kebebew, 2006). Inclusion of wild edible plants as regular dietary supplement by the ethnic groups is mainly for sustenance in their dwelling environment and also intrinsically linked to their traditional and cultural life. They also sell some wild edible plants in the local markets for additional income (Angami *et al.*, 2006).

The indigenous leafy vegetables are distributed throughout the state during various seasons. These different wild vegetables become available during various seasons throughout the state.

Potentials of wild foods of Assam need conservation. Many wild plant foods reported from various regions of Assam have multiple functions like medicine, animal feed, building materials, religious and material needs and source of livelihoods. Many plants double as food and medicine while some food plants treble as food, medicine and feed. This invaluable dynamic link illustrates the indirect benefits of wild edible plants to mankind through

consumption of livestock or its products. Many households collect food plants and other minor products and sell them in local markets to earn cash income. Wild vegetables are indispensable in terms of food security, livelihoods and ecological stability various region of Assam. The wild foods like leafy vegetables, therefore, need urgent prioritization for conservation for maintaining goods and services, and ecological stability.

These wild plants are identified for effective conservation and utilization of plant genetic resources for improvement, It is also important that these wild plant genetic resources are evaluated for productivity and its components, crop duration, resistance to biotic and abiotic stress and quality of produce. The wild plant genetic resources available at different parts of Assam has been evaluated and utilized for crop improvement by employing traditional breeding programme and molecular breeding technique.

Systematic evaluation of this vast resource of wild edible plants of the state including their nutritional, anti-nutritional and potential benefit of phyto-chemicals, formal intervention as well as awareness creation can contribute to change in perception among the youth and adult leading to not only the promotion of conservation, and ensuing of sustainable use of these diversified plant resources through enhanced utilization but also can play great role in improvement of rural health sector, tackling malnutrition and in food security in the face of environmental and societal changes in rural domains. Moreover, DNA finger printing of the wild vegetables of the region will certainly help in proper identification, authentication and documentation and in overcoming the ambiguity that arises out of different names of the same species by different tribes/ communities or localities. Further, it will also help in studying the molecular diversity as well as in addressing the related Intellectual Property Right and bio-piracy issues and Geographical Indication, if any.

Information on genetic diversity and relationships within and among crop species and their wild relatives is essential for the efficient utilization of plant genetic resource collections. Several molecular approaches have been employed to assess genetic diversity and

relationships, but isozyme or randomly amplified polymorphic DNA (RAPD) data can be generated faster and with less labor than other methods, such as RFLP (restriction fragment length polymorphism) and the use of microsatellites. However, isozyme studies are limited by the numbers of enzymes and loci that can be resolved, and reveal only genetic changes in coding regions of the genome that have resulted in an altered amino-acid sequence. In many cases, lack of allozyme polymorphism may further restrict its application in plant germplasm classification. In contrast, RAPD analysis enables the detection of informative genetic markers at a large number of loci in both coding and non-coding regions of the genome (Williams *et al.*, 1990). However, the dominant nature of RAPD markers requires that more individuals and loci be sampled, compared to co-dominant markers such as RFLP and isozymes (Lynch and Milligan, 1994), and the low homology between co-migrating fragments from different species may limit its application in phylogenetic studies at the interspecific level (Thormann *et al.*, 1994; Rieseberg, 1996). Thus, for comparative studies of crops and their wild relatives in plant germplasm collections, a complementary approach using both isozymes and RAPDs may be more appropriate for generating accurate estimates of genetic diversity and relationships than either method used alone.

Traditional vegetables are usually obtained from different sources as compared to exotic vegetables. While the majority of all exotic vegetables consumed were purchased from markets (extend of use 65.4%), the majority of traditional vegetables consumed is produced in their own field or collected from the bush. However, there are also marked differences across different households. A larger share of traditional vegetables produced in poor households be obtained through collection (extend of use 30%), while it is the middle class that consumes the largest share of traditional vegetables produced in their own fields. it is the wealthiest group that purchases the largest share of traditional vegetables from the market (extend of use 40% of all produce) (Weinberger and Swai, 2006).

A systematic survey was carried out to identify the common green leafy vegetables found in the markets and crop farms in ten towns in Edo State, Nigeria (Ekpoma,

Uromi, Okpella, Jattu, Sabongida- Ora, Agenabode, Okada, Ehor, Ekhenwa, and Ekiadolor) using structured questionnaires. In all 29 different green leafy vegetables were identified in ten open markets and fifteen fields located in the study area. The extent of use was ranged from 0.2 to 19.3% (Mensah *et al.*, 2008).

2.4 Nutritional contribution of indigenous leafy vegetables

2.4.1 Moisture content of indigenous leafy vegetables

Moisture generally refers to the presence of water, often in trace amounts. Water is an important part of all cells and fluids in the body. It carries nutrients to and waste products from cells in the body, aids in digestion and absorption of food and helps to regulate body temperature (Johnson, 1996). The maximum water content varies between individual vegetables because of structural differences and cultivation condition that influence structural differentiation and may also have a marked effect on water levels vegetables (Florkowski *et al.*, 2009). High moisture content in vegetables is indicative of its freshness as well as easy perishability (Adepoju and Oyediran, 2008). Higher moisture content in vegetables also suggests that the vegetable will not store for long without spoilage since a higher water activity could enhance microbial activity, bringing about food spoilage (Ejoh *et al.*, 2007). For vegetables to be kept for a long time before use, the moisture content has to be reduced to inhibit the autocatalytic enzymes (Ladan *et al.*, 1997). Removal of moisture results in increased concentration of nutrients (Morris *et al.*, 2004).

Water is the major component of the plant. It plays an important role in nutrient absorption and transportation, formation of new products, plant growth. Carbohydrates, the products of photosynthesis, are moved in water solution to storage organs. A major portion of the water absorbed by the plant evaporates through the stomata. This evaporation process is essential for absorbing oxygen for photosynthesis. In addition, water absorbs heat, cools the plant and prevents plant injury from high temperatures. The high moisture content of indigenous leafy vegetables give great impact on energy density (amount of energy in a given weight of food

(kcal/g)) as water adds substantial weight to the food without adding energy and this may give the consumers a better satiety without increase their energy intake (Ng *et al.*, 2012).

Baruah and Borah (2009) in their investigation reported that the moisture content of leafy vegetables varies from 2.53% to 89.00% (fresh weight basis). Wallace *et al.* (1998) in their investigation found that moisture content of green leafy vegetables varies from 74.41% to 91.43% (fresh weight basis). Mensah *et al.* (2008) in their investigation found that the moisture content in few green leafy vegetables of Nigeria were varies from 21.6% to 93.4%. Kwenin *et al.* (2011) reported that moisture content in few green leafy vegetables varies from 72.93 to 91.83%. Moisture content of the green leafy vegetables samples ranged between 73.0 and 95.3 g/100 g, with the highest being in *Coleus aromaticus* (95.3 g/100 g) *Delonix elata* had the lowest moisture content of 73 g/100 g on fresh weight basis (Gupta *et al.*, 2005).

2.4.2 Ash content of indigenous leafy vegetables

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food (Mc Clement, 2003). Higher ash content predicts the presence of an array of mineral elements as well as high molecular weight elements (Onot *et al.*, 2007). Ash is herbaceous biomass consists of inorganic constituents. Ash content can originate from the biomass itself. Ash is one of the components in the [proximate](#) analysis of biological materials, consisting mainly of salty, [inorganic](#) constituents, metal [salts](#) which are important for processes requiring [ions](#) such as Na⁺ (Sodium), K⁺ (Potassium), and Ca²⁺ (Calcium).

Baruah and Borah (2009) in their investigation reported that the ash content of leafy vegetables varies from 6.21% to 8.60% (dry weight basis). Wallace *et al.* (1998) in their investigation found that ash content in few of green leafy vegetables varies from 1.48% to 2.89% (dry weight basis). Ng *et al.* (2012) reported that the ash content of tropical wild vegetables varies from 10.7% to 17.6% (dry weight basis). Chibueze *et al.* (2011) in their investigation found that ash content in some leafy vegetables varies from 2.83% to 5.88% (dry weight basis). The ash content of wild vegetables ranged from 13-18 g/100 g (dry weight) was higher than *Brassica juncea* (10.7 g/100 g dry weight) indicating that consumption of these wild greens might contribute higher minerals content (Ng *et al.*, 2012). The ash content in fresh samples ranged from 9.4% in *T. accidentalis* to 24.6% in *T. triangulare* while in sun-dried samples the ash content ranged from 10.8% in *T. accidentalis* to 25.0% in *T. triangulare*. In the blanched samples the content ranged from 8.3% in *B. alba* to 20.9% in *T. triangulare* on dry weight basis (Adegunwa *et al.*, 2011). Ejoh *et al.* (2007) reported that *Vernonia amygdalina* has ash content of 7.7 g/100 g. A study by Nnamani *et al.* (2009) on *Zanthoxylum zanthoxyloides*, *Vitex doniana* and *Adenia cissampeliodes* stated that Ash content of the test vegetables ranged from 8.10 - 6.30%. *Cleome gynandra* has a total ash content of 2.1-3.0% (Chweya *et al.*, 1997).

2.4.3 Potassium content of indigenous leafy vegetables

Potassium is a key circulating electrolyte which is also involved in the regulation of ATP dependent channels along with sodium. These channels are the Na^+/K^+ -ATPases and their primary function is in the transmission of nerve impulses in the brain. Sodium and potassium maintains osmotic and water balance as well as membrane potentials. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended (Akubugwo *et al.*, 2007). Regular consumption of these leafy vegetables may assist in preventing hypertension and cardiovascular diseases, as dietary potassium is an important cation in regulating blood pressure and attenuating platelet reactivity, which is the major causative factor of vascular occlusion (He and Mac Gregor, 2008). Furthermore, consumption of wild vegetables with high potassium content enhances the bioavailability of calcium in body and promotes bone health by preventing the occurrences of calciuria.

The wild vegetables contained comparable potassium to other cruciferous vegetables (Miller-Cebert *et al.*, 2009) and the potassium content of *Etlingera elatior* is about two times higher than *Brassica juncea* (22 mg/g dry weight). In addition, the potassium content of *Etlingera elatior* was found greater than some wild leafy vegetables in South Africa which included *Sonchus asper*, *Solanum nigrum* and *Urtica urens* but not for *Chenopodium album* which demonstrated much higher potassium content (Afolayan *et al.*, 2009). Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 49028.6 mg/100 g and 61120.2/100 g of potassium on dry weight basis respectively. Kamga *et al.* (2013) reported that potassium content varies from 3080 mg to 5860 mg/100 g on dry weight basis in three leafy vegetables. Out of 13 green leafy vegetables analyzed, *Digera arvensis* contained the greatest amount of potassium (604 mg/100 g) and *Polygala eriopetala* the least amount (125 mg/100 g) (Gupta *et al.*, 2005). The potassium content in some green leafy vegetables ranges between 16.85 mg/100 g and 168.96 mg/100 g (Asaolu *et al.*, 2012). Mensah *et al.* (2008) reported potassium content of *Amaranthus cruentus*, *Cochlospermum vitifolium* and *Basella rubra* as 4.82, 3.83 and 5.8 mg/100 g dry weight basis respectively. *Cleome gynandra* has been reported to have potassium content of 410 mg/100 g (Chweya and Nameus, 1997).

2.4.4 Calcium content of indigenous leafy vegetables

Calcium is an important constituent of bones and teeth and is involved in regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of pro-thrombin to thrombin. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase and lipase. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability (Soetan *et al.*, 2010). Reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985). Calcium absorption requires calcium-binding proteins and is regulated by vitamin D, sunlight, parathyroid hormone and thyrocalcitonin. Thyrocalcitonin decreases plasma calcium and phosphate levels whereas parathyroid hormone increases them.

Sources of calcium include Beans, lentils, nuts, leafy vegetables, dairy products, small fishes including sardines, bones, etc. (Soetan *et al.*, 2010). Mensah *et al.* (2008) reported calcium content of *Amaranthus cruentu*, *Cochorus olitorius* and *Basella rubra* as 2.05, 1.26 and 2.32 mg/100 g dry weight basis respectively. Spider flower has a calcium content of 213-434 mg/100 g (Chweya and Nameus, 1997). A study by Mepba *et al.* (2007) indicated that blanching and cooking significantly ($P \leq 0.05$) decreased levels of calcium in the test vegetables.

The amount of calcium ranged between 3.9 and 11.9 mg/g, dry weight basis with the lowest in *Crassocephalum crepidioides* and the highest in *Limnophila aromaticoides*. The common vegetable, *Brassica juncea* has higher calcium content (12.9 mg/g, dry weight basis) as compared to other wild vegetables. However, the calcium level was found higher than some of the wild plants from Africa, for example, young shoot of *Borassus aethiopum* (0.2 mg/g dry weight basis), fruit of *Tamarindus indica* (1.9 mg/g dry weight basis) and *Momordica balsamina* (2.2 mg/g dry weight basis) (Glew *et al.*, 2005; Flyman and Afolayan, 2007). Calcium with the name of “super nutrient” has been proven clinically associated with reduced risk of various non-

communicable diseases such as osteoporosis (Nieves, 2005), cardiovascular diseases (Vaskonen, 2003) and it also helps to reduce colorectal cancer risk by promoting the apoptosis in human colorectal epithelium that reduce colorectal neoplasms (Flood *et al.*, 2005; Fedirko *et al.*, 2009). Few researches have promoted the intake of plant-based diet such as soybean and green leafy vegetable as a source of calcium instead of dairy or animal products. This is because animal protein could increase bone loss and risk of fracture through promoting calcium excretion and acid-base metabolism (Park *et al.*, 2011).

Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 18213.2 mg/100 g and 16890.0/100 g of calcium on dry weight basis respectively. Kamga *et al.* (2013) reported that calcium content varies from 1150 mg to 2690 mg/100 g on dry weight basis in three leafy vegetables. Caunii *et al.* (2010) reported that the calcium content of in four green leafy vegetables varies from 36 mg to 208 mg/100 g on dry weight basis. The analyzed green leafy vegetables were found to be rich sources of calcium. *Digera arvensis*, *Boerhaavia diffusa*, *Cucurbita maxima*, and *Amaranthus tricolor* had a high calcium content of 506, 330, 302 and 239 mg/100 g, respectively. *Trianthema portulacastrum* and *Polygala erioptera* were observed to have 52 and 41 mg/100 g, respectively on fresh weight basis (Gupta *et al.*, 2005). The calcium content in some green leafy vegetables ranges between 61.19 mg/100 g and 110.16 mg/100 g (Asaolu *et al.*, 2012).

2.4.5 Sodium content of indigenous leafy vegetables

Sodium is a mineral. In the body, it carries an electrical charge and a charged mineral is called an electrolyte. The body regulates the level of sodium in the body through numerous interacting processes because the concentration must remain in a narrow range. If sodium levels deviate too high or too low, it causes problems in the body. Sodium is important for fluid distribution, blood pressure, cellular work and electrical activity. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Okon and Akpanyung, 2005).

Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 68.0 mg/100 g and 116.9/100 g of sodium on dry weight basis respectively. Out of 13 green leafy vegetables, eight of them has sodium content in the range of 4.7–20.7 mg/100 g. *Celosia argentea* had a maximum amount of sodium (240.6 mg/100 g) and *Centella asiatica* had 107.8 mg/100 g on fresh weight basis (Gupta *et al.*, 2005). Achinewhu *et al.* (1995) have reported similar values for sodium in the indigenous herbs and leaves of Nigeria. The sodium content in some green leafy vegetables ranges between 15.01 mg/100 g and 88.0 mg/100 g (Asaolu *et al.*, 2012). Jain *et al.* (2010) reported that sodium content in *Oxalis corniculata* was 1.12% on dry weight basis. Imran *et al.* (2007) and Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain sodium 381 and 662 mg/100 g in Pakistan and Greece respectively.

2.4.6 Manganese content of indigenous leafy vegetables

Very low intakes of manganese are associated with poor bone formation. This phenomenon is thought to be due to altered formation of the protein matrix that keeps minerals like calcium in place. Manganese is a required co-factor for an enzyme called prolidase, which is in turn necessary to make collagen as a structural component of skin. It is also a co-factor for an enzyme called manganese superoxide dismutase (MnSOD), which is a potent antioxidant associated with protection against ultra violet damage. Manganese is needed to help multiple enzymes in a process called gluconeogenesis. This is the process by which we build non-carbohydrate food products, for example, digested fats into sugars to burn as fuel (Gunter *et al.*, 2013).

Manganese content of green leafy vegetables ranged from 1.8 to 10.2 mg/100 g (Singh *et al.*, 2001). Manganese content was below the detection limit in eight of the thirteen green leafy vegetables analysed. *Commelina benghalensis* had the least manganese content of 0.08 mg/100 g and *Trianthema portulacastrum* had the maximum content (0.43 mg/100 g) on fresh weight basis (Gupta *et al.*, 2005). The manganese content in some green leafy vegetables ranges between 2.54 mg/100 g and 10.06 mg/100 g (Asaolu *et al.*, 2012). Imran *et al.* (2007) and

Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain manganese 9 and 5.67mg/100 g in Pakistan and Greece respectively. Afolayan and Jimoh (2009) reported that manganese content of four green leafy vegetables ranged from 8.3 to 11.8 mg/100 g.

2.4.7 Zinc content of indigenous leafy vegetables

Zinc is found as a co-factor in over 300 different enzymes including antioxidant enzymes. Zinc has a role in the regulation blood glucose levels via insulin function. Zinc is an essential micronutrient for human growth and immune functions (Black, 2003).

Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 23.0 mg/100 g and 26.1 mg/100 g of zinc on dry weight basis respectively. Kamga *et al.* (2013) reported that zinc content varies from 0.021 mg to 0.102 mg/100 g on dry weight basis in three leafy vegetables. Zinc content of green leafy vegetables ranged from 2.4 to 6.0 mg/100 g (Singh *et al.*, 2001) on dry weight basis. Out of 13 green leafy vegetables *Centella asiatica* had 0.97 mg/100 g, the highest zinc content, followed by *Delonix elata* at 0.76 mg/100 g.

The remaining green leafy vegetables contained zinc levels in the 0.33–0.68 mg/100 g range on fresh weight basis (Gupta *et al.*, 2005). The level of Zn in green leafy vegetables ranged between 3.73 and 24.64 mg (Asaolu *et al.*, 2012). Similar values were reported by Asaolu and Asaolu (2010) and Ayoola *et al.* (2010). Imran *et al.* (2007) and Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain zinc 16 and 2.15 mg/100 g in Pakistan and Greece respectively. Afolayan *et al.* (2009) reported that zinc content of four green leafy vegetables ranged from 4.2 to 5.5 mg/ 100 g.

2.4.8 Iron content of indigenous leafy vegetables

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes C, C1, and A1 (Malhotra, 1998). Iron is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the

cytochromes (Chandra, 1990). Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis (Soetan *et al.*, 2010).

Adrenocortical hormones (glucocorticoids) play a role in regulating the level of plasma iron. During stress, when the hypothalamus, adenohypophysis, and adrenal cortex are activated, regardless of the source, the plasma iron decreases. Iron in ferrous form is more soluble and is readily absorbed than the ferric form. Phytic acid and oxalic acid decreases iron absorption by forming iron phytate and iron oxalate. The absorption of iron is inhibited by profuse diarrhoea, mal-absorption syndrome, achlorohydria, dissection of small intestine and partial or total gastrectomy.

The plasma iron content is determined by the extent of blood losses, role of erythropoiesis, rate of apoferritin synthesis, rate of iron absorption from intestines and rate of red blood cell destruction. Deficiency disease or symptoms include anaemia, (hypochromic, microcytic). Iron deficiency has been reported to have a role in brain development and in the pathophysiology of restless legs syndrome. Also, iron deficiency is associated with alterations in many metabolic processes that may impact brain functioning, among whom are neurotransmitter metabolism, protein synthesis and organogenesis. Early iron deficiency has also been reported to affect GABA metabolism in adult rats. Iron accumulation has been related to some neurologic disorders such as Alzheimer disease, Parkinson disease, type-1 neuro-degeneration with brain iron accumulation and other disorders (Barminas *et al.*, 1998).

Brain is quite sensitive to dietary iron depletion and uses a host of mechanisms to regulate iron flux homostatically. The pig is born with low iron stores and develops iron deficiency anaemia if not provided with supplementary iron. The factors causing the onset of anaemia in piglets are its relatively low iron stores at birth, its high growth rate early in life, and the low level of iron in sow milk. If the pig is given iron supplements at birth, the total red cell

mass or volume per unit of body weight increases from birth to three weeks of age (Soetan *et al.*, 2010).

Iron has a critical role in the transport of oxygen. Iron coordinates the oxygen molecule into heme of haemoglobin so that it can be transported from lungs to the tissues. Therefore, over 65% of body iron is found in haemoglobin. One-third of body iron is a storage form that can be mobilised during times of need. Non-heme sources of iron are found in plant and vegetable products and the absorption from these sources is generally lower and influenced to a greater extent by total diet composition. Vitamin C is the most significant enhancer of non-heme iron absorption, while plant phenolics such as tannins and phytates are some of the most potent inhibitors (Grace *et al.*, 2013).

Iron is an important trace element in the human body, it plays crucial roles in haemopoiesis, control of infection and cell mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Dioxin and Haris, 2004).

Mensah *et al.* (2008) reported iron content of *Amaranthus cruentus*, *Cochorus olitorius* and *Basella rubra* as 0.12, 0.04 and 0.04 mg/100 g dry weight basis respectively. *Cleome gynandra* has an iron content of 1-11 mg/100 g (Chweya and Nameus, 1997). Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 120.4 mg/100 g and 177.1/100 g of iron on dry weight basis respectively. Iron (Fe) is the most abundant trace mineral in wild leafy vegetables, ranged 3.6 mg/100 g - 33.1 mg/100 g dry weight basis. This result compares favorably with the underutilized vegetables reported by Flyman and Afolayan (2007). Kamga *et al.* (2013) reported that iron content varies from 3.34 mg to 38.79 mg/100 g on dry

weight basis in three leafy vegetables. Kwenin *et al.* (2011) reported that iron content in few green leafy vegetables varies from iron 1.00 mg/100 g to 40.50 mg/100 g on dry weight basis.

Total iron content of selected green leafy vegetables ranged from 22.3 to 84.4 mg/100 g on a dry matter basis. Iron content was found in bengal gram leaves (84.44 mg/100 g) and in carrots (7.7 mg/100 g) (Singh *et al.*, 2001). Iron content analyzed in Spinach (*Spinacea oleracea*), amaranth (*Amaranthus tricolor*), mint (*Mentha spicata*), coriander (*Coriandrum sativum*), bengal gram (*Cicer arietinum*) and cauliflower leaves (*Brassica oleracea*) and carrots (*Dacus carota*) was similar to that reported by Yadav in spinach (35.0 mg/100 g) (1992) and lower than that reported by Luthra and Sadana in the same vegetable (54.1 mg/100 g) (1995). The iron content of 13 green leafy vegetables analysed varied considerably.

Coleus aromaticus had a low iron content of 2.62 mg/100 g. Four greens, *Celosia argentea*, *Centella asiatica*, *Amaranthus tricolor* and *Digera arvensis*, were found to have exceptionally high iron content of 13.15, 14.86, 15.01 and 17.72 mg/100 g respectively on fresh weight basis. The rest varied from 4.16 to 9.86 mg/100 g on fresh weight basis. By including these iron rich green leafy vegetables in the diet, 40–50% of the daily requirements of iron from one serving can be easily fulfilled (Gupta *et al.*, 2005).

2.4.9 Copper content of indigenous leafy vegetables

Copper is an essential trace element (i.e., [micronutrient](#)) that is required for plant, animal, and human health. It is also required for the normal functioning of [aerobic](#) (oxygen-requiring) [microorganisms](#). Copper is incorporated into a variety of [proteins](#) and [metallo-enzymes](#) which perform essential metabolic functions; the micronutrient is necessary for the proper growth, development, and maintenance of bone, [connective tissue](#), brain, heart, and many other body organs.

Copper is involved in the formation of [red blood cells](#), the absorption and utilization of iron, the metabolism of [cholesterol](#) and [glucose](#), and the synthesis and release of life-sustaining [proteins](#) and [enzymes](#). These enzymes in turn produce cellular energy and regulate nerve transmission, blood clotting, and oxygen transport. Copper stimulates the [immune system](#) to fight [infections](#), to repair injured tissues, and to promote healing. Copper also helps to neutralize "[free-radicals](#)", which can cause severe damage to cells.

Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 9.1 mg/100 g and 11.2/100 g of copper on dry weight basis respectively. Copper content of green leafy vegetables ranged from 0.8 to 1.0 mg/100 g (Singh *et al.*, 2001). Out of 13 green leafy vegetables *Delonix elata*, *Centella asiatica*, *Boerhaavia diffusa* and *Cocculus hirsutus* had high amounts of copper, 0.27, 0.24, 0.22 and 0.22 mg/100 g, respectively.

Commelina benghalensis and *Amaranthus tricolor* had the least copper content of 0.09 mg/100 g. The rest of the greens contained copper in the range of 0.12–0.19 mg/100 g on fresh weight basis (Gupta *et al.*, 2005). The copper content in some green leafy vegetables ranges between 0.08 mg/100 g and 5.69 mg/100 g (Asaolu *et al.*, 2012). Imran *et al.* (2007) and Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain copper 54 and 1.71 mg/100 g in Pakistan and Greece respectively.

2.4.10 Arsenic content of indigenous leafy vegetables

Elements such as lead, chromium, cadmium and arsenic are likely to have cumulative side effects when large quantity of the leaves has been consumed. These elements could also affect the young nervous system of the child thereby adversely affecting the intellectual development and subsequent behaviour of the child (Grace *et al.*, 2013). The major arsenic species found in environment were arsenite, arsenate, arsenious acid, arsenic acid, monomethylarsenic acid (MMA), dimethylarsenic acid (DMA), arsenobetaïne (AB) and arsenocholine (AC). Inorganic arsenic is the predominant form in the soil and is toxic to the

plant. Methylated form of MMA and DMA is also present due to photooxidation and microbial biotransformation in the soil.

The vegetables are important food crops of Assam. But vegetables that are grown in arsenic contaminated soil, people unknowingly consume such arsenic contaminated crops and become exposed to arsenic toxicity. To prevent the toxicity several measures or techniques are suggested to minimize arsenic mobility in the soil and uptake by the plants. There are many competing ions, which may alleviate or reduce the arsenic accumulation by the plants. Phosphate is one of such competing ions, which is used as a fertilizer in irrigated field, which may compete with arsenic for sorption site and accumulate in vegetable for nutrition purpose. The unknowing ingestion of a toxic substance like arsenic may be hazardous to human health. Long time exposure of low doses of arsenic causes chronic toxicity.

Grace *et al.* (2013) reported that *Chenopodium album* and *Solanum nigrum* contains 1.8 mg/100 g and 1.9 mg/100 g of arsenic on dry weight basis respectively. Jahanara (2007) reported that the higher accumulation of total arsenic (mean $\pm$ S.D) was found in parble (3.24 ± 2.89 $\mu\text{g/g}$), papaya (2.44 ± 2.30 $\mu\text{g/g}$), bean (2.18 ± 1.95 $\mu\text{g/g}$), arum (3.89 ± 1.75 $\mu\text{g/g}$), arum loti (3.03 ± 2.86 $\mu\text{g/g}$) and carrot (2.40 ± 2.36 $\mu\text{g/g}$). Comparatively lower accumulation was found in kolmishak (0.47 ± 0.22 $\mu\text{g/g}$) and lalshak (0.85 ± 0.85 $\mu\text{g/g}$) in leafy vegetables.

2.4.11 Phosphorous content of indigenous leafy vegetables

Phosphorus is an essential macronutrient for plants and one of the three nutrients generally added to soils in fertilizers because of its vital role of energy transfer in living organisms and in plants. Adequate phosphorus availability stimulates early growth and hastens maturity in plants. Phosphorus is also needed for soil fertility. The phosphorus content of the plant is influenced markedly by the availability of phosphorus in the soil. A balance proportion of calcium and phosphorus is needed in the body. Phosphorus is essential component of bone mineral. Deficiency of phosphorus- calcium balance result in osteoporosis, arthritis, pyorrhea, rickets and tooth decay.

Kamga *et al.* (2013) reported that phosphorous content varies from 420 mg to 540 mg/100 g on dry weight basis in few green leafy vegetables. Kwenin *et al.* (2011) reported that phosphorus content in few green leafy vegetables varies from 74 mg/100 g to 81.90 mg/100 g. Caunii *et al.* (2010) reported that the phosphorous content of in four green leafy vegetables varies from 23 mg to 66 mg/100 g on dry weight basis.

The phosphorous content was found to be in the range of 16–63 mg/100 g for the green leafy vegetables on fresh weight basis (Gupta *et al.*, 2005) and these values were similar to those reported by Gopalan *et al.* (1996) for the common green leafy vegetables. The level of phosphorus in the leafy vegetables ranged between 26.19 mg/100 g and 36.91 mg/100 g on dry weight basis (Asaolu *et al.*, 2012). Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain 518 mg/100 g of phosphorous.

2.4.12 Crude fat content of indigenous leafy vegetables

Food fat plays an important role in the human diet. Fats are vital for a healthy body, provide it with energy, contribute to the absorption of fat-soluble vitamins, and act as structural elements of cell walls. As a carrier of flavors and aromatic substances, fat also plays an important part in cooking. On the other hand, no other nutrient has to combat as many prejudices as fat. It is linked to obesity, type 2 diabetes, cancer, and coronary heart disease ([Lichtenstein *et al.*, 1998](#)).

Dietary fats represent the most compact chemical energy available to man. They contain twice the caloric value of an equivalent weight of sugar. However, dietary fats should not be thought of solely as providers of unwanted calories as fats are as vital to cell structure and biological function as protein. Dietary fats provide the essential linoleic acid which seems to have both a structural and functional role in animal tissue. However, leafy vegetables are not noted for contributing significantly to the fat supply in foods.

Kwenin *et al.* (2011) reported that crude fat contents are 3.19%, 3.0%, 1.33% and 1.50% in *Xanthosoma sagittifolia*, *Amaranthus cruentus*, *Talinum triangulare* and *Moringa oleifera* respectively. All underutilized vegetables were found to be poor sources of fat with *Limnophila aromaticoides* has the highest fat content (3.3 g/100 g dry weight basis, only able to contribute 12% of the Dietary Reference Intakes (DRI) for fat (20-35 g/day) (Ng *et al.*, 2012).

The fat content in fresh samples ranged from 2.3% in *T. triangulare* to 4.8% in *B. alba*, while in the sun-dried samples the fat content ranged from 2.8% in *T. accidentalis* to 4.7% in *T. triangulare* and in blanched samples ranged from 2.7% in *V. amygdalina* to 6.7% in *T. triangulare* on dry weight basis (Adegunwa *et al.*, 2011). The percentage of fat content ranged from 3.51% - 14.02% dry weight basis in Bushbuck and *Amaranthus hybridus* respectively (Asaolu *et al.*, 2012). These values were fairly high when compared with values reported in some other leafy vegetable such as *Ocimum bassillium*, *Ocimum viride* and *Piper guineens* (Udosen, 1995).

2.4.13 Crude fibre content of indigenous leafy vegetables

Dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fibre consists of isolated, non-digestible carbohydrates which have beneficial physiological effects in humans. ‘Dietary fibre’, ‘unavailable carbohydrate’ or ‘roughage’ are the different terms used to define that part of the plant food which is not digested by the endogenous secretions of the human digestive system. Dietary fibre may be classified into three major groups according to structure and properties. The groupings are cellulose, non-cellulose and lignin (Komal and Kaur, 1992).

Increasing the fibre content of the diet increases the faecal energy excretion, principally in the form of fat and nitrogen. By virtue of its water holding capacity, fibre also helps in the formation of soft stools with bulk, which can be easily evacuated (Komal and Kaur, 1992). An increased intake of dietary fibre appears to be useful for the treatment of both obesity and diabetes mellitus. Fibre-rich food is usually satisfying without being calorically dense. Gel

forming fibres are particularly effective in reducing elevated cholesterol. The impaired glucose tolerance of manifest diabetes is also improved. Dietary fibre helps to prevent constipation, bowel problems and piles.

Dietary fibre or foods containing a high amount of dietary fibre are very low in caloric content. Dietary fibre yields only 2-3 calories /g. Thus a high fibre diet is recommended for weight reducing regimes (Komal and Kaur, 1992). Mensah *et al.* (2008) reported crude fibre content of *Amaranthus cruentus*, *Cochorus olitorius* and *Basella rubra* as 1.8, 8.5 and 0.6 g/100 g on dry weight basis respectively.

The wild vegetables viz. *Limnophila aromaticoides* (finger grass), *Ceratopteris thalictroides* (Indian fern), *Crassocephalum crepidioides* (fireweed), *Etlingera elatior* (torch ginger), and *Monochoria vaginalis* (pickerelweed) showed great fibre content as compared to *Brassicca juncea* (10.6 g/100 g dry weight basis), the commercialized vegetable with the highest in *Etlingera elatior* (19.8 g/100 g dry weight basis) (Ng *et al.*, 2012). Interestingly, the fiber content of *Etlingera elatior* is five times higher than *Momordica balsamina* (an underutilized vegetable) from Botswana (Flyman and Afolayan, 2007).

In addition, the average fibre content of the analyzed wild vegetables (15.84 g/100 g dry weight basis) is two times higher than the four common leafy vegetables (*Vernonia amygdalina*, *Solanum Africana*, *Amaranthus hybridus* and *Telfaria occidentalis*) found in Nigeria as reported by Aletor *et al.* (2002). Hence, these wild greens could potentially be a source of high fibre and low fat diet for those who concern about their weight or on weight management program. Kwenin *et al.* (2011) reported that crude fibre content in few green leafy vegetables varies from 1.00% to 10.40%, on dry weight basis. The crude fibre content ranged between 4.02% - 12.08% dry weight basis (Asaolu *et al.*, 2012). The crude fibre content ranged from 8.50- 20.90% in some Nigerian green leafy vegetables (Isong and Idiong, 1997).

2.4.14 Crude protein content of indigenous leafy vegetables

Proteins are essential organic compounds of high molecular weight found in all living tissues which synthesize them at one time or another. They are formed from much similar building units called amino acids. Proteins may be categorized based on factors such as solubility and shape. They are broadly divided in two groups namely: simply and conjugated. Simple proteins consist of only amino acids as building blocks while conjugated proteins contain amino acids but in addition, a non-protein or prosthetic group which may be glycoprotein, lipoprotein, chromoprotein (Osei, 2003).

Plant proteins may be less digestible because of intrinsic differences in the nature of the protein and the presence of other factors such as fibre, which may reduce protein digestibility by as much as 10%. Requirements are also greater during childhood for growth and development, during pregnancy or when breast-feeding in order to nourish a baby, or when the body needs to recover from malnutrition or trauma or after an operation. Protein deficiency is a serious cause of ill health and death in developing countries.

Symptoms of kwashiorkor include apathy, diarrhoea, inactivity, failure to grow, flaky skin, fatty liver, and edema of the belly and legs. Food processing has inevitable consequences on the nutritional value of foods. The macro- and micro-nutrients (carbohydrate, protein, fats, vitamins and minerals) contained within foods all show varying degrees of stability when foods are stored or processed and the degree of stability depends largely on the type and structure of the food/nutrient, food chemistry and the severity and duration of processing (Henry and Massey, 2001).

These leafy vegetables can play a significant role in providing cheap and affordable protein for rural communities. Pregnant and lactating mothers require 71 g of protein daily (FND, 2005). Assuming complete protein absorption, 100 g dry weight basis of nightshade, amaranth and jute mallow leaves would respectively contribute for about 49.5, 35.0 and 35.0% of the daily protein requirement of these pregnant and lactating mothers. It is obvious that

indigenous leafy vegetables are not a good source of protein but it provides almost 65% of the world supply of edible protein especially in developing countries. Therefore, in order to improve the low concentration and incompleteness of plant proteins, consumption of a mixture of plant proteins food are suggested to obtain well balanced of amino acids that is equivalent to animal protein.

Results of a study by Nnamani *et al.* (2009) on nutrient values of three underutilized indigenous leafy vegetables *Vitex doniana* Sweet, *Adenia cissamploides* Zepernick and *Zanthoxylum zanthoxyloides* Herms, indicated their protein contents as 8.74, 8.5 and 6.12%, respectively. On the other hand, Hassan *et al.* (2007) reported protein content of 14.30% dry weight.

The protein content of the wild vegetables ranged from 10.0 to 21.2 g/100 g on dry weight basis (Ng *et al.*, 2012). Levels of crude protein found in the three leafy vegetables viz. amaranth, nightshade and jute mallow were within the range of 20.48 - 41.66% reported by Ponka *et al.* (2005), with nightshade containing the highest amount. The level of crude protein in all jute mallow, night-shade and amaranth cultivars is far higher than $16.2 \pm 3.3\%$ reported by Ndlovu and Afolayan (2008) in *C. olitorius*, $24.9 \pm 0.02\%$ reported in *S. nigrum* by Akubugwo *et al.* (2007a) and 17.92% reported in *A. hybridus* by Akubugwo *et al.* (2007b). The protein content in the fresh vegetables ranged from 20.3% in *T. triangulare* to 35.7% in *T. accidentalis*, while in sun-dried sample the content ranged from 23.4% in *V. amygdalina* to 31.1% in *T. accidentalis*. In the blanched and sun-dried samples, the protein content ranged from 19.5% in *T. triangulare* to 23.4% in *T. accidentalis* on dry weight basis (Adegunwa *et al.*, 2011).

Protein content was highest in mint leaves 30.9% and lowest in carrots (9.8%) on a dry matter basis. Protein contents of coriander, bengal gram, spinach, cauliflower and amaranth leaves were 22.2, 26.2, 26.5, 29.9 and 26.2%, respectively (Singh *et al.*, 2001). Several workers reported a similar value for crude protein in amaranth (Gupta *et al.*, 1989; Gopalan *et al.*, 1996).

Protein content was lower than that reported by Yadav (1992) and Islam *et al.* (1987) in spinach leaves i.e., 31.4 and 32.9%, respectively.

However, Luthra and Sadana (1995) and Gupta and Wagle (1988) reported lower values in spinach i.e., 12.1 and 21.6%, respectively. The crude protein content ranged between 66.60% to 46.56% dry weight basis (Asaolu *et al.*, 2012). The crude protein was more than what was reported for some other leafy vegetables such as *Momordica balsamina* (11.29%), *Moringa oleifera* (20.72%), *Lesianthera africana* leaves (13.10%- 14.90%) and *Leptadenia hastata* (19.10%).

2.4.15 Total soluble protein content of indigenous leafy vegetables

Proteins are Macromolecules that act as alternate energy source when other energy sources are in short supply. They are the building block of any organism. Protein is an essential part of the dietary needs of humans. The reason is that it fulfills a variety of important functions in the body. It is necessary for growth, maintenance and repair of cells and for the production of enzymes and hormones. Furthermore, [proteins](#) are the main components of muscle tissue and are vital to the internal organs, bones, skin and the transmission of impulses through the nerves (Sheela, 2004).

Lin *et al.* (1986) reported that the leaf of four cultivars of sweet potato Tainong 65, HP-4, Chang-hua and Tainong 25 contains total soluble protein 6783.33 mg/100 g, 5800 mg/100 g, 5016.66 mg/100 g and 4250 mg/100 g, respectively.

Foda *et al.* (2013) reported that the total soluble proteins concentrations of hummayd (*Rumex vesicarius*) are 9.8 mg/g in leaves. In hummayd leaves, the concentration of soluble proteins was higher in leaves as compared with stems or roots contents. These results are in conformity with those of Alfawaz (2006) who found that the protein value of hummayd was 17.1–20.1 g/100 g.

2.4.16 Total free amino acid content of indigenous leafy vegetables

Amino acids build proteins, and proteins are life-sustaining macronutrients. Yet simply calling amino acids the building blocks of protein doesn't do justice to their value. While some amino acids only make proteins, others fill a variety of roles, from supporting metabolism to protecting heart.

Proteins are chains of amino acids linked together, bound together with peptide bonds and there are about 28 amino acids commonly referred to in human health. The liver manufactures about 80% of these amino acids, but the remaining 20% of such amino acids must be supplied directly by diet, and these amino acids are referred to as the essential amino acids (Brosnan, 2000).

Laitonjam *et al.* (2013) reported that total free amino acid content in the leaves of *Phlogacanthus pubinervius* and *Phlogacanthus jenkinsii* were 5312.50 and 4140.75 mg/100 g, respectively.

2.5 Carbohydrate content of indigenous leafy vegetables

2.5.1 Starch content of indigenous leafy vegetables

Starch constitutes a big source of calories in the diet. Starch belongs to the family of nutrients called carbohydrates, which contribute to over 50% of the energy consumed by most people. Starch used to be called a complex carbohydrate because it is made of a long chain of glucose, but this classification system is no longer used because the terms “complex” and “simple” carbs do not properly distinguish the impact of these nutrients in your body. Starch can be good for your body, but not all starches are created equal (Spiller, 2001).

Aberoumand (2011) reported that starch content of eight edible plants varies from 5.86 to 60.41%. Becker *et al.* (1981) and Saunders and Beciker (1984) reported that the starch content of pale-seeded leaves was reported to range from 48% for *A. cruentus* to about 62% for *A. hypochondriacus*.

2.5.2 Reducing sugar content of indigenous leafy vegetables

A reducing sugar is any sugar that either has an aldehyde group or is capable of forming one in solution through [isomerism](#). The aldehyde functional group allows the sugar to act as a [reducing agent](#), for example in the [Tollens' test](#) or [Benedict's reagent](#), or the [Maillard reaction](#), important in the browning of many foods. Reducing sugar in diet can result in weight loss, increased energy and healthier (Aberoumand, 2011).

Verma *et al.* (2012) reported that *Bauhinia variegata* buds and flowers contain reducing sugar 1.45% and 2.35% on dry weight basis respectively. Reducing sugar values for these *Vernonia calvoana* var. bitter, *V. amygdalina* var. bitter, *V. colorata* var. non bitter and *V. calvoana* var. non bitter ranged from 13.08% in *V. calvoana* var. non bitter to 15.79% in *V. calvoana* var. non bitter on dry weight basis (Ejoh *et al.*, 2007). Imran *et al.* (2007) reported that *Scandix pecten-veneris* contains reducing sugar 0.64%.

2.5.3 Total soluble sugar content of indigenous leafy vegetables

Sugars are found in the tissues of most plants, but are only present in sufficient concentrations for efficient extraction in [sugarcane](#) and [sugar beet](#). Sugar is the generalized name for sweet, short-chain, soluble carbohydrates, many of which are used in food. They are [carbohydrates](#), composed of carbon, hydrogen, and oxygen. There are various types of sugar derived from different sources. Simple sugars are called monosaccharide and include [glucose](#) (also known as dextrose), [fructose](#) and [galactose](#) (Saratha *et al.*, 2001).

The table or granulated sugar most customarily used as food is [sucrose](#), a [disaccharide](#). Other disaccharides include [maltose](#) and [lactose](#). Longer chains of sugars are called [oligosaccharides](#). Chemically-different substances may also have a sweet taste, but are not classified as sugars. Some are used as [lower-calorie](#) food substitutes for sugar described as [artificial sweeteners](#).

Verma *et al.* (2012) reported that *Bauhinia variegata* buds and flowers contain total soluble sugar 1.63% and 2.69% on dry weight basis respectively. Total soluble sugar values for these *Vernonia calvoana* var. bitter, *V. amygdalina* var. bitter, *V. colorata* var. non bitter and *V. calvoana* var. non bitter ranged from 2.41% in *V. calvoana* var. bitter to 4.83% in *V. calvoana* var. non bitter on dry weight basis (Ejoh *et al.*, 2007). Imran *et al.* (2007) reported that *Scandix pecten-veneris* contains total soluble sugar 0.76%.

Misra and Misra (2014) reported that among the 27 leafy vegetable studied, *Murraya koenigii* (187.6 mg/g) registered the highest total sugar content and the lowest value was shown by *Tridax procumbens* (18.0 mg/g). Among the leafy vegetable analyzed, *Tamarindus indicus* (181.3 mg/g), *Corchorus aestuans* (150.6 mg/g), *Premna latifolia* (135.2 mg/g), *Glinus oppositifolius* (129.9 mg/g), *Moringa oleifera* (125.5 mg/g), *Alternanthera sessilis* (115.5 mg/g) are with high total sugar content compared to *Bauhinia purpurea* (92.0 mg/g), *Cleome viscosa* (81.3 mg/g), *Boerhavia diffusa* (74.5 mg/g), *Caralluma adscendens* (71.2 mg/g), *Amaranthus spinosus* (69.3 mg/g), *Dendrocalamus strictus* (65.30 mg/g), *Melothria heterophylla* (64.4 mg/g), *Achyranthes aspera* (60.8 mg/g), *Celosia argentea* (58.5 mg/g), *Coccinia grandis* (58.16 mg/g), *Leucas aspera* (57.3 mg/g), *Bambusa bambos* (56.3 mg/g), *Senna tora* (54.7 mg/g), *Flacourtia indica* (54.6 mg/g). Much lower sugar content was shown by *Marselia minuta* (45.1 mg/g), *Amaranthus gangeticus* (35.2 mg/g), *Ipomoea aquatica* (31.0 mg/g), *Commelina benghalensis* (23.5 mg/g) and *Amaranthus viridis* (20.5 mg/g).

2.6. Vitamin content of indigenous leafy vegetables

2.6.1 Ascorbic acid or vitamin C content of indigenous leafy vegetables

High concentration of ascorbic acid in plant samples might associated with attractive free radical scavenging capacity and health benefit like anti-carcinogenic and anti-atherogenic (Lui *et al.*, 2008).

The various conventional food processing techniques caused a highly significant decrease in the vitamin C content of all the vegetables, the loss in vitamin C during processing agrees with the earlier report by (Achinewhu, 1983; Oboh, 2005; Akindahunsi and Oboh, 2004) that various conventional food processing methods would bring a loss in vitamin C content. This loss in vitamin C during processing could be attributed to the fact that vitamin C is soluble in water and at the same time not stable in high temperature (Nagy and Smooth, 1977).

Wild vegetables contained appreciable amounts of ascorbic acid (Vitamin C) with *Limnophila aromaticoides* (844.6 µg/g, fresh weight basis) contained the highest level whereas the lowest was found in *Etlingera* sp. (272.2 µg/g fresh weight basis) (Ng *et al.*, 2012). The consumption of every 100 g of *Limnophila aromaticoides* enable to fulfill 112% and 94% of the Dietary reference Intake (DRI) of ascorbic acid for both adult female (75 mg/day) and male (90 mg/day) respectively. Surprisingly, *Limnophila aromaticoides*, *Crassocephalum crepidioides* and *Ceratopteris thalictroides*, were found to have higher ascorbic acid than that found in broccoli (52.9 mg/100 g) (Singh *et al.*, 2007).

The vitamin C content ranged from 49.3 mg/100 g in *V. amygdalina* to 104.1 mg/100 g in *T. accidentalis* in fresh samples while the sun-dried samples ranged from 48.0 mg/100 g in *V. amygdalina* to 61.1 mg/100 g in *T. triangulare*. The blanched samples ranged from 31.2 mg/100 g in *V. amygdalina* to 67.8 mg/100 g in *T. accidentalis* on dry weight basis (Adegunwa *et al.*, 2011).

Ascorbic acid content in leafy vegetables varied from 4.9 to 110.4 mg/100 g (Singh *et al.*, 2001). Significant variations were observed in ascorbic acid content of green leafy vegetables and carrots. Almost similar values for ascorbic acid in amaranth and spinach leaves have been reported earlier. Ascorbic acid contents found in Spinach (*Spinacea oleracea*), amaranth (*Amaranthus tricolor*), mint (*Mentha spicata*), coriander (*Coriandrum sativum*), bengal gram (*Cicer arietinum*) and cauliflower leaves (*Brassica oleracea*) and carrots (*Dacus carota*) were higher than those reported by some workers in coriander (88.6 mg/100 g) and spinach (25.4

mg/100 g) (Tapadia *et al.*, 1995) and in amaranth leaves (91.0 mg/100 g) (Yadav and Sehgal, 1995).

Various reasons including differences in leaf maturity and differences in the ascorbic acid assay employed could account for the differences in ascorbic acid content of leafy vegetables and herbs (Oke, 1968; Stepanova *et al.*, 1973). Out of 13 green leafy vegetables, *Polygala eriopetra* and *Delonix elata* were found to be rich sources of ascorbic acid, having 85 and 295 mg/100 g, respectively. The remaining had ascorbic acid in the range of 11–49 mg/100 g with the exception of *Coleus aromaticus*, which was found to have 3 mg/100 g on fresh weight basis (Gupta *et al.*, 2005). Ascorbic acid (vitamin C) ranges from 356.11 mg/100 g to 215.63 mg/100 g in green leafy vegetables (Adeniyi *et al.*, 2012).

2.6.2 Riboflavin (Vitamin B2) content of indigenous leafy vegetables

Riboflavin is a B vitamin. It can be found in certain foods such as milk, meat, eggs, nuts, enriched flour, and green vegetables. Riboflavin is frequently used in combination with other B vitamins in vitamin B complex products. Vitamin B complex generally includes vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 ([niacin/niacinamide](#)), vitamin B5 (pantothenic acid), vitamin B6 ([pyridoxine](#)), [vitamin B12 \(cyanocobalamin\)](#) and [folic acid](#).

Riboflavin serves an antioxidant function due to its ability to activate antioxidant enzymes. Antioxidants protect cells from damage caused by harmful chemicals called reactive oxygen species, or ROS. ROS prove highly reactive in cells, interacting with proteins, cell membranes and even DNA, ultimately causing cellular damage. As an antioxidant, riboflavin helps neutralize ROS to prevent this damage, helping to preserve the health of your cells.

The riboflavin content was the highest in *Limnophila aromaticoides* (13.7 µg/g, fresh weight basis) followed by *Crassocephalum crepidioides* (12.4 µg/g, fresh weight basis) (Ng *et al.*, 2012). Therefore, daily consumption of these vegetables might help to alleviate riboflavin deficiency, which gives negative impact on the metabolism of other nutrients,

especially B-group vitamins, through flavin coenzyme activity. In addition, sufficient intake of riboflavin has proven to have protective effect against proximal colon cancer through preventing the aberrant of DNA methylation (de Vogel *et al.*, 2008). Riboflavin ranges from 0.18 mg/100 g to 0.06 mg/100 g in green leafy vegetables (Adeniyi *et al.*, 2012).

2.6.3 Thiamine (Vitamin B1) content of indigenous leafy vegetables

Thiamine derivatives and thiamine-dependent enzymes are present in all cells of the body, thus a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism (Okwu, 2004).

The amount of thiamine in *Limnophila aromaticoides* (16.6 µg/g, fresh weight basis), *Ceratopteris thalictroides* (9.0 µg/g, fresh weight basis) and *Etlingera elatior* (3.24 µg/g, fresh weight basis) (Ng *et al.*, 2012) are high compared to the uncommon vegetables studied by Raghuvanshi *et al.* (2001). Nevertheless, plants or vegetables usually contain great amount of heat-stable anti-thiamin compounds such as tannic acid and thiaminase I, which will affect the bioavailability of thiamine in plants (Lonsdale, 2006).

Out of 13 green leafy vegetables, *Delonix elata* was found to contain maximum amount of thiamine (0.33 mg/100 g), *Celosia argentea*, *Commelina benghalensis*, *Boerhaavia diffusa*, *Centella asiatica*, *Coleus aromaticus* and *Amaranthus tricolor* had less than 0.1 mg/100 g of thiamine whereas in rest of the greens the thiamine content varied from 0.1 to 0.2 mg/100 g on fresh weight basis (Gupta *et al.*, 2005). Raghuvanshi *et al.* (2001) have reported similar values for thiamine content in uncommon vegetables. Thiamine content ranges from 0.08 mg/100 g to 0.03 mg/100 g in green leafy vegetables (Adeniyi *et al.*, 2012).

2.6.4 Niacin content of indigenous leafy vegetables

Niacin is one of the most important nutrients in your diet, and its water soluble. That means that body excretes excess niacin in urine, rather than storing it. Therefore, we need to

eat a steady supply of niacin each day to ensure that we don't suffer from niacin deficiency (Lyimo *et al.*, 2003).

Niacin protects our heart by lowering blood levels of “bad” LDL cholesterol and preventing the accumulation of arterial plaque. Our digestive system, nervous system and brain need niacin to function. Niacin also helps our body make new DNA, and it helps your body use insulin efficiently to control blood sugar levels. Our body also needs niacin to process fats.

Adeniyi *et al.* (2012) reported that the niacin content ranges from 0.74 mg/100 g to 0.29 mg/100 g in green leafy vegetables.

2.7 Anti nutritional factors of indigenous leafy vegetables

Green leafy vegetables are important component of the dietary regime of humans because they provide the necessary vitamins, and minerals (Fasuyi, 2006). They however, also contain antinutrients which reduce the bioavailability of these nutrients (Akindahunsi and Salawu, 2005; Binita and Khetarpaul, 1997). Aletor and Adeogun (1995) however, reported that some antinutrients exhibit protective effects thus making them serve dual purpose. Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as ricket and osteomalacia (Ladeji *et al.*, 2004). The calcium crystals may also precipitate around renal tubules causing renal stones. Phytic acid combines with some essential elements such as Fe, Ca, Zn and P to form insoluble salts called the phytates which are not absorbed by the body therefore making these minerals bio-unavailable. Saponins are naturally oily glycosides occurring in wide variety of plants. When eaten, they are non-poisonous to warm blooded animals but are poisonous when injected into the blood stream (Applebaum *et al.*, 1969). Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. They combine with digestive enzymes thereby making them unavailable for digestion (Binita and Khetarpaul, 1997).

2.7.1 Saponin content of indigenous leafy vegetables

Saponins are nitrogen free glycosides each consisting of a sapogenin and a sugar. The sapogenin may be a steroid or a triterpene and the sugar is generally glucose, galactose, pentose or methyl pentose. They impart bitter taste and tend to foam in aqueous solution. Saponins are poorly absorbed and most of their effects are attributable to their hydrophilic/hydrophobic asymmetry and consequently their capacity to reduce interfacial tension (Champ, 2002). Gupta and Wagle (1988) reported 0.90 per cent saponin content in leaves of *Chenopodium album*.

This variation in result from other worker might be due to different varieties which lead to genetic variability, time of harvest, stage of harvest, analytical procedure applied and climatic conditions. Bhargava *et al.* (2009) revealed that saponin content in leaves of quinoa vary in different growing stages, low saponin content was found in branching stage and higher in blooming stage. From the nutritional or pharmacological point of view saponins could also have some value. They can increase membrane permeability thus enabling use for increased food intake at the intestinal level or even for drug assimilation (Gee *et al.*, 1993).

Sood *et al.* (2012) reported that saponin content in different cultivars of *Chenopodium* ranged from 0.027 to 0.867%. Saponin values for these *Vernonia calvoana* var. bitter, *V. amygdalina*, *V. colorata* and *V. calvoana* var. non bitter ranged from 0.83 in *V. calvoana* to 1.91 mg/100 g dry weight in *V. calvoana* var. non bitter (Ejoh *et al.*, 2007).

2.7.2 Tanin content of indigenous leafy vegetables

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their

inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Incidences of certain cancers, such as esophageal cancer, have been reported to be related to consumption of tannins-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic.

Tannins are basically polyphenolic compounds having complex mixture and are present in many plants. They form complexes with proteins, starches and digestive enzymes there by reducing the nutritional value of foods (Serrano *et al.*, 2009) and causing growth depression. They also interfere with protein absorption and reduce iron availability. Besides exhibiting negative effects tannin also provide some health benefits including antioxidant and radical scavenging properties, anti-carcinogenic, antibacterial and anti-enzymatic effects (Vattem *et al.*, 2005).

Tannin has antioxidants capacities that are much stronger than those of vitamin C and E (Adegunwa *et al.*, 2011). The tannin content in fresh vegetable used ranged from 1.8 mg/g in *T. triangulare* to 4.2 mg/g in *T. accidentalis*, while in sun-dried samples the content ranged from 1.8 mg/g in *B. alba* to 3.4 mg/g in *V. amygdalina*. In the blanched samples the tannin content ranged from 1.3 mg/g in *B. alba* to 3.4 mg/g in *T. accidentalis* on dry weight basis (Adegunwa *et al.*, 2011). The tannin values ranged between 61 and 205 mg/100 g with the exception of *Coleus aromaticus* (15 mg/100 g) and *Delonix elata* (1330 mg/100 g) on fresh weight basis (Gupta *et al.*, 2005).

2.7.3 Phytate content of indigenous leafy vegetables

Phytic acid (hexa phosphate of inositol) is a complex class of naturally occurring anti-nutritional factor present in plant foods and is a major phosphorus storage compound in green leafy vegetables. It affects the digestion and absorption of minerals. The phytate content in fresh samples ranged from 0.5 mg/g in both *T. accidentalis* and *T. triangulare* to 0.8 mg/g in *B. alba*, while in sun-dried samples the phytate content ranged from 0.6 mg/g in *B. alba* to 0.8 mg/g in both *T. accidentalis* and *V. amygdalina*. In the blanched samples the content

ranged from 0.6 mg/g in *B. alba* to 1.0 mg/g in both *T. accidentalis* and *T. triangulare* on dry weight basis (Adegunwa *et al.*, 2011). The phytic acid content of the analyzed green leafy vegetables was found to be in the range of 0.92–13.06 mg/100 g fresh vegetable on fresh weight basis (Gupta *et al.*, 2005).

These values are considerably lower than those reported by other workers in non-conventional vegetables (Gupta *et al.*, 1989; Aletor and Adeogun, 1995; Raghuvanshi *et al.*, 2001) indicating that the lower phytic acid content in the analyzed vegetables will provide a better bioavailability of minerals. Sood *et al.* (2012) reported that phytic acid content in different *Chenopodium* cultivars ranged from 238.33–268.33 mg/100 g. Yadav and Sehgal (2003) reported 234.50 mg/100 g phytic acid content in *bathua* (*C. album*) leaves. Khan *et al.* (2012) reported that *Scandix pecten-veneris* contains 0.025% phytate.

2.7.4 Oxalate content of indigenous leafy vegetables

Oxalates is a dicarboxylic acid and is found in the form of soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron in algae, fungi, lichens, ferns and higher plants. Insoluble oxalate is excreted in the feces while the soluble oxalate is absorbed by the body. Soluble oxalate forms strong chelates with dietary calcium, rendering it unavailable for absorption and assimilation.

Oxalic acid is one of the anti-nutritional factors, which are widely distributed in plant foods. Oxalic acid is known to interfere with calcium absorption by forming insoluble salts of calcium. Out of 13 green leafy vegetables, *Digera arvensis*, *Amaranthus tricolor*, *Boerhaavia diffusa*, *Trianthema portulacastrum* and *Celosia argentea* were found to have high total oxalate values of 1410, 1270, 1250, 1080 and 920 mg/100 g, respectively. The rest of the greens had lower total oxalate content, which was found to be in the range of 20–390 mg/100 g on fresh weight basis (Gupta *et al.*, 2005). Oxalic acid values of some of the unconventional leafy vegetables from the Konkan area of Maharashtra, India were also found to be in the same range (Shingade *et al.*, 1995).

Oxalic acid values for these *Vernonia calvoana* var. bitter, *V. amygdalina* var. bitter, *V. colorata* var. non bitter and *V. calvoana* var. non bitter ranged from 3.49 mg % in *V. calvoana* var. bitter to 7.56 mg % in *V. colorata* var. non bitter on dry weight basis (Ejoh *et al.*, 2007). Sood *et al.* (2012) reported that oxalate content in different cultivars of *Chenopodium* ranged from 518.42 mg/100 g to 394.19 mg/100 g. Khan *et al.* (2012) reported that *Scandix pecten-veneris* contains 0.225% oxalic acid.

2.8 Other significant phytochemicals of indigenous leafy vegetables

2.8.1 Total phenol content of indigenous leafy vegetables

Phenolics are nonnutritive secondary metabolites found in plants that promote significant health benefit and prevent various diseases. Phenols exhibit antioxidant potential (Awika *et al.*, 2003) due to their redox properties which allow them to act as reducing agents, hydrogen donors and single oxygen quenchers (Chang *et al.*, 2001).

The phenolic content of *Ceratopteris thalictroides* (Indian fern), *Crassocephalum crepidioides* (fireweed), *Etlingera elatior* (torch ginger), and *Monochoria vaginalis* (pickerelweed) were significantly higher in comparison to *Brassica juncea* (1.8 mg gallic acid equivalent/g, fresh weight basis). The phenolic content of both *Etlingera elatior* (4.1 mg gallic acid equivalent/g, fresh weight basis) and *Limnophila aromaticoides* (3.8 mg gallic acid equivalent/g, fresh weight basis) are two times higher than *Brassica juncea* (Ng *et al.*, 2012).

Some of the plant species found in Amazonian region such as *Bauhinia forficata* (16.6 mg gallic acid equivalent/g, fresh weight basis), *Davilla kunthii* (36.4 mg gallic acid equivalent/g, fresh weight basis) and *Byrsonima crassifolia* (45.5 mg gallic acid equivalent/g, fresh weight basis) have 10 fold higher phenolic contents (Silva *et al.*, 2007). Sood *et al.* (2012) reported that phenol content in different cultivars of *Chenopodium* ranged from 304.98 mg /100 g to 224.99 mg gallic acid equivalent/100 g. Kaur and Kapoor (2002) reported 253.5 mg gallic acid equivalent/100 g total phenol content in the leaves of *Chenopodium album*.

2.8.2 Total Alkaloid content of indigenous leafy vegetables

Alkaloids are a group of naturally occurring [chemical compounds](#) that contain mostly [basic nitrogen](#) atoms. This group also includes some related compounds with neutral and even weakly [acidic](#) properties. Some synthetic compounds of similar structure are also attributed to alkaloids. In addition to [carbon](#), [hydrogen](#) and [nitrogen](#), alkaloids may also

contain [oxygen](#), [sulfur](#) and more rarely other elements such as [chlorine](#), [bromine](#) and [phosphorus](#) (Manske, 1965).

Alkaloids are a special group of secondary compounds and are part of an organism's adoption mechanism to its living environment. They are not toxic when stored, but become toxic as a result of pH change. The defensive function of alkaloids is only secondary and connected to internal immune and regulation process. Animal responses to alkaloids are very diverse. Some animals can tolerate alkaloids relatively well, while others are harmed or even poisoned by them.

Sood *et al.* (2012) reported that alkaloid content in different cultivars of *Chenopodium* ranged from 0.69 to 1.67 mg/100 g. Adedapo *et al.* (2011) reported the alkaloid content in leaves of *C. album* as 1.8 mg/100 g. Jimoh *et al.* (2010) reported that *Amaranthus asper* leaves contained 0.3 mg/100 g (dry weight basis) alkaloid.

2.8.3 Total flavanoid content of indigenous leafy vegetables

Flavonoid is a group of polyphenolic compounds naturally present in most edible fruits and vegetable plants. They are secondary plant metabolite and are a natural antioxidant which helps to maintain the body's health and to protect against diseases. They also exhibit anti-nutritional properties due to their metal chelating properties. Flavonol and flavonone are flavonoid of particular importance because they have been found to possess antioxidant and free radical scavenging activity in vegetable (Amic *et al.*, 2003).

Furthermore, the type of flavonoids present in the plant samples could be another factor that determines their antioxidant capability. As Tsimogiannis and Oreopoulou (2006) found that antioxidant properties of flavonoids depend on their C-ring structure, flavonoids with full substitution C-ring like rutin and luteolin, which their ether bonded with three oxygen demonstrate better free radical scavenging capacity and higher reaction rate compare to flavonoids that lack of one or more C-ring structural elements.

The total flavonoid content of the vegetable samples varied considerably from 0.4 to 1.4 mg rutin equivalents (RE)/g fresh weight basis of sample (Ng *et al.*, 2012). The total flavonoid content of some medicinal plants (1.6-13.12 mg RE/g, dry weight basis) and vegetables (0.9-4.9 mg RE/g, dry weight basis) were reported in the previous study (Djeridane *et al.*, 2006). Sood *et al.* (2012) reported that total flavanoid content in different cultivars of *Chenopodium* ranged from 220.0 mg/100 g to 406.67 mg/100 g. Adedapo *et al.* (2011) found 182 mg/100 g flavonoid content in *C. album* leaves.

2.8.4 Total flavanol content of indigenous leafy vegetables

Phenolic compounds are secondary metabolites in plants. These compounds are one of the most widely occurring groups of phytochemicals that exhibit a wide range of physiological properties, such as antiallergenic, anti artherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Manach *et al.*, 2005; Middleton *et al.*, 2000).

Jimoh *et al.* (2010) reported that *Amaranthus asper* leaves contained 0.96 mg/g (dry weight basis) total flavanol. Bouaziz *et al.* (2009) reported that the highest flavonol amounts of 63.85, 53 and 41 mg quercetin equivalents (QuE)/100 g, on dry weight basis were obtained in ethyl acetate extract of *A. radiana*, *Cleome amblyocarpa* and *Anabasis oropediorum* respectively and 56.26, 40.8 and 40 mg QuE/100 g on dry weight basis in methanol extract of *P. angustifolia*, *Tricholaena teneriffae* and *E. glaucophyllum*, respectively Afolayan *et al.* (2009) reported that total flavonol (mg quercetin equivalents/g) content in *C. album*, *S. asper*, *S. nigrum* and *U. urens* are 0.98 ± 0.41 , 0.91 ± 0.26 , 0.71 ± 0.00 , 0.78 ± 0.08 , respectively.

2.9 Antioxidant activity of indigenous leafy vegetables

Native plant species are used for daily diet as vegetables, spices and condiments. Some of them are also employed for traditional medicine. There are several varieties of these leafy vegetables in the rural areas which are either wild or cultivated. High consumption of vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are considered to be related to the various antioxidants contained in them.

2.9.1 Free radical Scavenging Activity content of indigenous leafy vegetables

The antioxidant activity of the antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Oktay *et al.*, 2003). Plant extract with remarkable free radicals scavenging abilities possess excellent capabilities in preventing oxidative damages mediated by radicals within cellular system as well as in food system (Valko *et al.*, 2007).

Most of the methanolic extract of wild vegetables studied show appreciable free radical scavenging activities in concentration-dependent manner, where scavenging activity increased with an increase in the concentration of each individual plant extract. Two mg/ml of *Etlingera elatior* extract able to scavenge almost 80% of the DPPH free radicals in contrast, the scavenging activity of 2 mg/ml of *Monochoria vaginalis* extract is 8 times lower than *Etlingera elatior* extract, where only 10% of the radicals had been scavenged.

For *Crassocephalum crepidioides* extract at 2 mg/ml, the scavenging ability towards free radicals was 35.4%, but it increased to 93.47% when the concentration of the extract was 10 mg/ml. Its scavenging activity surpassed other wild vegetables and comparable with the synthetic antioxidant agent, BHA (93.63%).

The superior scavenging activity of this methanolic extract could be also due to its high ascorbic acid content since ascorbic acid has been reported as an excellent DPPH radical-scavenger compared to other polyphenols and synthetic antioxidant agent like BHT (Lui *et al.*, 2008). Kiruthiga *et al.* (2014) reported that the methanolic leaves and callus extracts of *C. pallida* exhibited highest antioxidant activity of 94.47 ± 0.54 and 96.5 ± 0.51 at 50 mg/ml concentration respectively.

2.9.2 Super oxide dismutase activity of indigenous leafy vegetables

Super oxide dismutase (SOD) out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is [spin-forbidden](#). In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as [nitric oxide](#) (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutates to O_2 and hydrogen peroxide (H_2O_2) quite rapidly ($\sim 10^5 M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets. Super oxide radicals are inactivated by the enzyme superoxide dismutase (SOD), the only enzyme known to use a free radical as a substrate. The radical scavenging activity of SOD is effective only when it is followed by increase in activity of catalase and other peroxidases (Ramasarma, 1990).

Superoxide dismutase activity was found to be significantly higher in *Cassia fistula* ($2.29 \pm 0.325 \text{ min}^{-1} \text{ mg}^{-1}\text{protein}$) (Kumar *et al.*, 2012). The highest SOD activity was found in the *Cucumis melon* varieties such as Galia C8, Midyat and Semame varieties (377.67 , 357.00 and $350.00 \mu\text{Mol min}^{-1} \text{ mg}^{-1}$, on fresh weight basis, respectively) on the 8th day callus culture (Sebnem *et al.*, 2012). Superoxide dismutase activity of *C. pallida* fresh leaves (*in vivo*) and callus (*in vitro*) exhibited 0.78 ± 0.036 and $0.826 \pm 0.025 \text{ min}^{-1} \text{ mg}^{-1}\text{protein}$ respectively (Kiruthiga *et al.*, 2014).

2.9.3 Peroxidase activity of indigenous leafy vegetables

Plants protect cell and sub-cellular systems from the cytotoxic effects of the active oxygen radicals using antioxidant enzymes such as peroxidase. Antioxidant defense is important in the removal of free radicals, providing the maximal protection of biological sites such as thiol groups which are part of active sites in some metabolizing enzymes. A good antioxidant should specifically quench free radicals, chelate redox metals, interact with other antioxidants within an antioxidant network, have a positive effect on gene expression, be readily absorbed, have a concentration in tissues and bio-fluids at a physiologically relevant level, act in both aqueous and/or membrane domains (Valko *et al.*, 2007).

A number of peroxidases are found in plants, where they may help minimize damage caused by stress factors or insect pests. When plants are subjected to stress such as drought or high temperatures or to attack by pests, this tends to result in the release of reactive [oxygen](#) species (ROS). These are forms of oxygen, or compounds of this element, including hydrogen peroxide, in which the oxygen is very reactive, and can damage or kill cells. It is thought that peroxidases remove ROS, helping prevent damage (Schenk *et al.*, 2000).

One unusual use of these enzymes occurs in a group of insects known as bombardier beetles. They have a chamber containing a mixture of hydrogen peroxide and chemicals called hydroquinones. When threatened, they mix these with peroxidases, which catalyze a redox reaction in which a lot of heat is released, with the resulting liquid ejected explosively at 212°F (100°C). It is a very effective way of deterring predators (Apel and Hirt, 2004).

Peroxidases are referring to heme containing enzymes which are able to oxidise organic and inorganic compounds using hydrogen peroxide as co-substrate. The non-specificity of peroxidase makes the enzyme suitable to a broad range of electron donor substrates (Halliwell, 2006). Kiruthiga *et al.* (2014) reported that peroxidase activity in *Crotalaria pallid* is $0.229 \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$.

2.9.4 Catalase activity of indigenous leafy vegetables

Plants protect cell and sub-cellular systems from the cytotoxic effects of the active oxygen radicals using antioxidant enzymes such as catalase. Antioxidant enzymes, including catalase, form the first line of defence against free radicals; therefore their regulation depends mainly upon the oxidant status of the cell. However, there are other factors involved in their regulation, including the enzyme-modulating action of various hormones such as growth hormone, prolactin and melatonin. Melatonin is a derivative of the amino acid tryptophan that acts as a neurohormone in mammals, but is also synthesized by many other species, including plants, algae and bacteria. Melatonin has been shown to markedly protect both membrane lipids and nuclear DNA from oxidative damage. Melatonin can directly neutralise several ROS, including hydrogen peroxide. It can also stimulate various antioxidant enzymes, including catalase, either by increasing their activity or by stimulating gene expression for these enzymes. The decrease in melatonin levels observed with age correlates with an increase in neurogenerative disorders such as Parkinson's disease, Alzheimer's disease, Huntington's disease and stroke, all of which may involve oxidative stress. In general, the production of ROS increases with aging and is associated with DNA damage to the tissues.

Catalase is a common antioxidant enzyme which is produced naturally in almost all living organisms. The reactions in catalyses are important to life, for example, it helps the body to break down hydrogen peroxide (a powerful and harmful oxidizing agent) into oxygen and water and thus it prevents the accumulation of carbon dioxide bubbles in the blood. Catalase is a very potent enzyme; one molecule of catalase can decompose millions of hydrogen peroxide molecules into oxygen and water. It also uses hydrogen peroxide to oxidize potentially harmful toxins in the body including formaldehyde, formic acid, alcohol, and phenol.

Kiruthiga *et al.* (2014) reported that catalase activity in *Crotalaria pallid* is 0.434 min⁻¹ mg⁻¹protein. Umesh *et al.* (2012) reported that catalase activity was in *Cassia fistula* 1.196 min⁻¹ mg⁻¹protein.

2.10 Molecular characterization

2.10.1 Polymorphism of *Amaranthus* spp. (Hatkhatura), *Alternanthera* spp. (Matikandori), *Chenopodium* spp. (Jilmil) and *Enhydra* spp. (Helosi sak) land races revealed by RAPD

Conventional plant breeding is based on the use of genetic variation and selection of the desired genotypes what requires the screening of relatively large populations and also requires long time (Gajdošová *et al.*, 2008). Genetic variations in *Amaranthus* cultivars were studied by using electrophoretic differences for six isozyme markers namely, esterase (Est.), peroxidase (Per.), phosphoglucoisomerase (Pgi), 6-phosphogluconate dehydrogenase (6-Pgd), malate dehydrogenase (Mdh) and phosphoglucomutase (Pgm) (Okeno, 1992).

A high level of genetic similarity between *A. hypochondrius* and *A. caudatus* were reported which supported earlier RAPD observations of Transue *et al.* (1994) and Chan and Sun (1997) and Mandal and Das (2002). On the basis of genetic divergence 61 genotypes of grain amaranth were grouped into ten clusters (Kalpande *et al.*, 2007). The Genetic diversity and relationships among 6 *Amaranthus* species from 8 phytogeographic regions of the Indo-Gangetic plains were analyzed using a random amplified polymorphic DNA (RAPD) marker. Variation existed in the genetic diversity of different populations. The SCAR marker formed was potentially useful for germplasm conservation and identification of amaranth ecotype (Ray and Roy, 2009). Thirty-nine strains of vegetable amaranth (*Amaranthus tricolor*) were evaluated for eight morphological and seven quality traits for two test seasons to study the extent of genetic divergence among the strains. Multivariate analysis showed that the first four principal components contributed 67.55% of the variability. Cluster analysis grouped the strains into six clusters that displayed a wide range of diversity for most of the traits. Thus Cluster analysis was proved to be an effective method in grouping strains that may facilitate effective management and utilization in crop-breeding programmes (Shukla *et al.*, 2009).

The application of traditional selection methods (hybridization, polyploidy, etc.) is complicated by the mixed pollination system and the extremely small size of the reproductive system. Thus, it is topical to apply methods that allow us to identify genotypes with economically valuable characteristics reliably and rapidly, study population processes, determine phylogenetic relationships between various species of the genus *Amaranthus* as well as control the insertion of new genes into breeding material. The application of various DNA markers based on PCR (RAPD, SSR, ISSR, AFLP, etc.) allows one to solve different tasks (Altukhov and Salmenkova, 2002; Kalendar' and Glazko, 2002). RAPD analysis has several disadvantages, such as sensitivity to reaction conditions, lack of specificity, and the dominant nature and small number of allelic variants. However, it has several advantages over other marker systems, such as speed and simplicity and the use of any decamer primer, which can significantly reduce analysis costs, since the same primers can be used for various species and genera of living organisms (Malyshev and Kartel, 1997). Moreover, the RAPD method allows us to analyze multiple loci simultaneously, and it can be beneficial and useful for the study of the genetic structure of populations and identification of relationships between species (Kochieva, 1999). Observed unique RAPD fragments can be used to develop more specific SCAR markers. It has been successfully realized for the genus *Amaranthus* (Ray and Roy, 2009).

The genetic variability and phylogenetic relationships between wild and cultivated species was studied by RAPD analysis in (Ray and Roy, 2009; Chan and Sun, 1997; Mandal and Das, 2002; Ranade *et al.*, 1997; Stefunova, 2008; Faseela and Salikutty, 2007). In these works, RAPD analysis was used to investigate intra and interspecific genetic variability, as well as plant origins. The authors of these papers notice a high level of genetic similarity between grain amaranth species *A. caudatus*, *A. cruentus*, *A. hybridus* and *A. hypochondriacus*. (Stefunova, 2008) emphasizes the high level of intraspecific polymorphism of these species basing on the results of RAPD and ISSR analyses. However, it should be noted that at present genetic data on amaranth are still insufficient. Up to now, only one genetic map of amaranth has been constructed using SSR loci (Mallory *et al.*, 2008). The problem concerning the relationships

between grain amaranth species and their putative previous crops remains open owing to the presence of conflicting data (Ray and Roy, 2009; Chan and Sun, 1997; Mandal and Das, 2002; Ranade *et al.*, 1997; Xu and Sun, 2001). There are some discrepancies in the taxonomy of the plant (Ranade *et al.*, 1997).

A patent has been filed for transferring the gene that codes for protein in *Amaranthus* to other cereals like rice and wheat (Raina and Dutta, 2009). Studies show that RAPD analysis could be used to efficiently classify grain amaranth accessions by species (Transuae *et al.*, 1994). *Amaranth* landraces belonging to three species namely *Amaranthus tricolor*, *A. dubius* and *A. hypochondriacus* were studied for assessing genetic diversity and interspecific relationship using RAPD markers. A very high level of diversity within landrace collections were found which were mainly attributed to interspecific diversity. RAPD markers specific to each species were identified which could be utilized in future as SCARs. *A. dubius* and *A. hypochondriacus* were observed to be genetically closer than *A. tricolor* (Sun, 1997).

Genetic diversity and relationships among 24 cultivated and wild *Amaranthus* accessions were examined using the total low-Cot DNA and five individual repetitive sequences as probes. The results based on low-Cot DNA were comparable with previous RAPD and isozyme studies of the same set of species/accessions of *Amaranthus*, indicating that low-Cot DNA sequences are suitable probes for a fingerprinting analysis of plant germplasm diversity and for determining phylogenetic relationships (Lanoue *et al.*, 1996). The Genetic diversity and relationships of 23 cultivated and wild *Amaranthus* species were examined using both isozyme and RAPD markers. The evolutionary relationships between grain amaranths and their putative ancestors were examined and both the RAPD and isozyme data sets supported a monophyletic origin of grain amaranths, with *A. hybridus* as the common ancestor (Chan and Sun, 1997).

Studies reported on the genetic diversity of 68 Chinese landraces and 4 Canadian lines by RAPD markers. The results showed that Greater genetic diversity was found within

winter *B. juncea* than within springs. High similarity was revealed among Hubei, Jiangsu and Guizhou landraces. Small genetic distances were observed between Henan and Shaanxi landraces (Ocho Sanchez *et al.*, 2009). The varietal identification of 18 *Amaranthus* genotypes through 10 RAPD markers were studied and results illustrated the relationship among the 18 amaranth genotypes (Kalpande *et al.*, 2007). The genetic diversity and phylogenetic relationships among six popular *Amaranthus* species were studied from different regions using RAPD markers and showed that there is a slightly inter- and intra- species polymorphism (Popa *et al.*, 2010). The infraspecific classification in *Amaranthus spinosus* was studied based on morphological and molecular data. *Amaranthus spinosus* was classified into 8 variants (Arik *et al.*, 2013). Studies were successful in the investigation of the relationships of four *A. hypochondriacus* varieties (Barba De La Rosa *et al.*, 2009). *Amaranth* genetic resource in Czech Gene bank were characterized using Amaranth glutelins and showed that they were the best tool for the amaranth species identification (Drzewiekie *et al.*, 2003). The genetic variability of an amaranth collection was studied by RAPD analysis. A high level of polymorphism of the studied samples was detected. It was about 85% on average. Unique fragments characteristic of only definite accessions and 18 monomorphic loci proper for all amaranth accessions were detected using several primers. The amaranth accessions were classified into two clusters according to the species affiliation (Lymanskaya, 2002). Different species (*Amaranthus hypochondriacus* and *A. tricolor*) of amaranthus collected from different agro-ecological zones of Pakistan were compared for their phenotypic and nutritional traits. A UPGMA cluster, grouped the 13 amaranthus genotypes into two major clusters, I and II, differentiating the ornamental amaranthus cultivars from edible. However, comparative view of the cluster showed that the *Amaranthus hypochondriacus* were closest to the China variety than to the *Amaranthus tricolor* according to their morphological characters (Naeemullah *et al.*, 2012).

RAPD fingerprints are very useful and promising for genotype identification (Caetans Anolles *et al.*, 1991), population and pedigree analysis, phylogenetic studies and genetic mapping. Hu and Quiros (1991) could identify the various cultivars of *Brassica* by

employing RAPD fingerprinting technique. Caetan- Anolles *et al.* (1991) employed RAPD technique to determine the genotypic relationship among ten populations of *Micrioseris elegans* collected through out California. Through the technique of RAPD, Kresovich *et al.* (1992) characterized the genetic diversity and relationship of *Brassica oleraceae*, both including forage and vegetable crops. Huff *et al.* (1993) explored the technique of RAPD to study populations of buffalo grass and revealed a great genetic diversity between two regional ecotypes. Ko *et al.* (1994) identified and differentiated eleven apple cultivars by using RAPD markers. RAPD technique technology is a rapid, precise and sensitive technique for genomic analysis and is much faster than traditional RFLP (Stiles *et al.*, 1993). Yang and Quiros (1993) employed the technique of RAPD to identify and classify the celery cultivars. 23 cultivars were screened and classified into three groups based on the marker difference.

Stiles *et al.* (1993) applied RAPD technique for evaluating genetic relationship among 10 papaya cultivars. Seven Hawaiian types grouped into one cluster and the other three formed a different group. Connolly *et al.* (1994) generated RAPD markers for six clonal cultivars of sweet potato and were applied to produce genetic fingerprints and to estimate genetic distance between these cultivars. The genetic relationship between 37 rice varieties were analyzed and the polymorphism generated was sufficient enough to distinguish between the individual varieties (Ko *et al.*, 1994). Rani *et al.* (1995) used RAPD to access genetic fidelity of 23 micro propagated plants of a single clone of *Populus deltoids* and found six plants have undergone genetic changes while the others were true to the type giving all monomorphic bands.

Virk *et al.* (1995) applied RAPD for the study of genetic diversity within rice germplasm collections. Wachira (1996) with the help of RAPD marker probed the genetic diversity in Kenyan tea germplasm and tried to trace its origin. Cheng and Chang (1997) studied the ecotypic variation of *Imperata cylindrical*, an aggressive weed using RAPD to work out the phylogenetic relationship among the six populations collected from different ecotypes of Taiwan.

Relationship among 37 North American octoploid strawberry was studied by evaluating 44 morphological traits and 36 RAPD markers (Harrison *et al.*, 1997). Huang *et al.* (1998) analyzed the genetic variation among 12 populations of the American chest nut using RAPD and found that *C. dendata* of both the populations and species level has narrow genetic diversity as compared to other species in the genus. With the help of RAPD and AFLP markers Yee *et al.* (1997) screened the genetic diversity among selected azuki accessions of *Vigna angularis*. Results of this study suggest that the level of genetic diversity among selected azuki accessions will be sufficient for developing an intra-specific mapping population. Santos *et al.* (1994) conducted RAPD analysis to detect genetic diversity in 5 tea accessions and genetic stability in their progeny. RAPD revealed that inheritance of traits was stable in these tea germplasm. The genetic relationship between ten rice varieties was probed with RAPD marker and each of the varieties could be distinguished from others (Nandarajan *et al.*, 1999). RAPD markers were characterized for 25 specimens of *Usnea fitipendula* to evaluate the genetic diversity of populations reinvading uninhabited regions in Northrhine-West phalia due to decreasing SO₂ levels and UPGMA analysis showed that the reinvading populations are distributed over the phenogram and are not genetically closed related (Heun *et al.*, 1994).

Research into in vitro cultures has been done on both grain and vegetable amaranths. Callus and cell suspension cultures of several grain and vegetable amaranths have been obtained. Moreover, it was found that various explants of these species can be easily grown in cell culture in standard basal media formulations supplemented with a few chemicals like naphthalenacetic acid and 2,4-dicholorophenoxyacetic acid (Flores, 1990; Flores *et al.*, 1982). In vitro culture amaranths may prove useful in the further improvement of amaranth. Several chromosomal studies have been made on amaranths (Kirpicznikov, 1969; Desai, 1971; Behera and Patnaik, 1974; Madhusoodanan and Pal, 1981; Sammour *et al.*, 1993; Greizerstein and Poggio, 1994). Most of the species investigated so far (about 40) have a diploid number of chromosomes equal to 32 or 34, the basic number (x) is 16 or 17 (Madhusoodanan and Pal, 1981). Other than some tetraploid species such as *A. caturtus* and *A. dubius*, the basic

complement of chromosome is either 32 or 34. Although in some cases, it was reported that a species had 16 pairs of chromosomes in one study but 17 pairs of chromosomes in another. The discrepancy may be real, but the chance is that the specimen was misidentified. Karyological studies of grain amaranths have shown that species with the higher chromosome number possess the lowest total DNA content. Combined with the information on karyotypes, it was suggested that cytogenetic analyses can be used to differentiate species of grain amaranth (Greizerstein and Poggio, 1994).

Moreover, cultivated amaranths have a relatively lower chiasma frequency compared to the wild species, and this phenomenon was attributed to repeated cycles of hybridization and selection in cultivation which produced an inherent heterozygosity in the cultivated amaranths (Madhusoodanan and Pal, 1981). Cultivated species also had more chiasmata per bivalent than semi-wild species like *A. spinosus*, and the cultivated species were characterized by bigger chromosomes and pollen grains (Sreelathakumary and Peter, 1993). Natural polyploidy occurs in wild amaranth. *Amaranthus dubius* is an example of naturally occurred tetraploid. It is not known whether it is an autotetraploid or an allotetraploid (Behera and Patnaik, 1975). The tetraploid appears generally shorter and more robust than diploid species of amaranths. The tetraploid seeds were bigger but had lower fertility of about 83%. On the other hand, spontaneous polyploidy was not known to occur in grain amaranths, but colchicine was employed to induce the formation of tetraploids and amphiploids. Induced polyploids from *A. dubius* and one of the grain amaranths, *A. hypochondriacus*, have been obtained through colchicine treatment of the seeds and seedlings. Octoploid of *A. dubius* was more difficult to form and had a higher degree of pollen and seed sterility when compared with the tetraploid form of *A. hypochondriacus* (Behera and Patnaik, 1975). Other studies have shown successful artificial inducement of polyploidy, and the resulting polyploid had preferable agronomic characteristics. Tetraploids of vegetable amaranths, such as *A. edulis* and *A. caudatus*, have also been produced through colchicine treatment, the tetraploid of *A. edulis* was shorter, stockier and more robust than the diploid, with little loss in fertility. Nutritive values were unaffected by

autopolyploidy, and in case of the tetraploid of *A. caudatus*, the protein content of its grains increased by 60% (Mohindeen and Irulappan, 1993). It is still an unknown whether the polyploids can be maintained and still preserve the favorable characteristics in future generations. Interspecific hybrid is formed as a result of cross-pollination of two different species. Natural interspecific hybridization occurs frequently in amaranths, eight naturally occurring interspecific hybrids have been reported in vegetable amaranth species and they show varying degrees of sterility.

In the case of natural hybridization between *A. edulis* and *A. hybridus*, complete chromosome pairings were observed, but the pollen fertility was less than 50% (Sreelathakumary and Peter, 1993). Interspecific hybrid of *A. lividus* and *A. tricolor* has an even higher degree of hybridization defects, about 90% of pollen sterility, because of the occurrence of two or more interchanges of several chromosomes and subsequent meiotic abnormalities (Sreelathakumary and Peter, 1993). Electrophoretic variations of total seed proteins revealed that *A. cruentus* and *A. hypochondriacus* are closely related to *A. hybridus* (Sammour *et al.*, 1993). Sammour concluded that *A. cruentus* and *A. hypochondriacus* are subspecies of *A. hybridus*. In an investigation of the morphological and genetic variation in Indian amaranths, high levels of morphological polymorphisms were found with nearly monomorphism of the allozyme loci. The lack of positive correlation between morphological polymorphism and genetic variation may be due to the relatively recent introduction and rapid spread of grain amaranths in India, and it is concluded that the grain amaranths maintain enzyme monomorphism in spite of artificial and natural selection for morphological diversity (Jain *et al.*, 1980). A more complete picture of phylogenetic relationships of the species of *Amaranthus* is given by Lanoue *et al.* (1996). The phylogenetic relationships of 28 species of *Amaranthus* were studied by restriction site analysis of PCR-amplified chloroplast and nuclear DNA. Genetic variations found by restriction site analysis were used to construct the phylogenetic tree of the 28 species. The tree suggested that *A.*

dubius and/or *A. hybridus* are possible progenitors of *A. cruentus*, and *A. quitensis* is a possible progenitor of *A. caudatus*.

Isozyme analysis has gained wide applications in many fields of study, including systematics, population genetics, genomic organization, plant breeding, sociobiology and basic genetics. Apart from isozyme electrophoresis, many novel techniques of molecular analyses have been developed since the invention of the polymerase chain reaction (PCR) in 1987. Random amplified polymorphic DNA (RAPD) is a powerful PCR-based technique (Williams *et al.*, 1990). Genetic analysis using RAPD as markers can be applied to any organisms one wants to study, even if they have not been studied at the molecular level before. Extensive uses of this technique bring about a revolution in genetic analysis of plants and animals or even micro-organisms. RAPD can be used in fingerprinting, genome analysis, gene mapping, pedigree assessment, population genetics, phylogenetic analysis, and germplasm evaluation (Demeke *et al.*, 1992; Hendrick, 1992; Dweikat *et al.*, 1993; Williams *et al.*, 1990; Buren *et al.*, 1994; Caetan-Anollés *et al.*, 1991; Aboelwafa and Shimoda, 1995; Graham and McNicol, 1995; Rieseberg and Gerber, 1995).

Some studies showed that RAPD assay has better separation of clusters of accessions than the isozyme method as a result of greater sensitivity of RAPD in detecting genetic polymorphism within closely related accessions (Heun *et al.*, 1994). In addition, RAPD can be as effective in assessing genetic relationships within accessions as RFLP, if either one is used alone (Santos *et al.*, 1994). Bulk RAPD analyses have also been carried out successfully on other species for the purposes of cultivar identification and classification, such as in alfalfa (Yu and Pauls, 1993), celery (Yang and Quiros, 1993), perennial ryegrass (Sweeney and Danneberger, 1994), rapeseed (Mailer *et al.*, 1994) and sorghum (Vierling *et al.*, 1994).

CHAPTER III

MATERIALS AND METHODS

The present investigation was conducted in the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat-785013 during the period 2010-2014. Details of materials and methodologies followed in conducting the experiments have been discussed here under.

3.1 Materials

3.1.1 Indigenous leafy vegetables

The Plant species used for the investigation are presented in the following table along with their scientific name and family.

Sl. No.	Local name	English name	Botanical name*	Family
1	Brahmi	Bacopa	<i>Bacopa monnieri</i> (L.) Wettst.	Scrophulariaceae
2	Dorun	Dhudani	<i>Leucas longifolia</i> Benth.	Lamiaceae
3	Helosi sak	Water cress	<i>Enhydra fluctuans</i> DC.	Asteraceae
4	Mandhania	Long coriander	<i>Eryngium foetidum</i> L.	Apiaceae
5	Hatikhutora	Spiny amaranth	<i>Amaranthus spinosus</i> L.	Amaranthaceae
6	Dhekia	Bracken fern	<i>Pteridium aquilinum</i> (L.) Kuhn.	Dennstaedtiaceae
7	Khutora	Slender amaranth	<i>Amaranthus viridis</i> (L.)	Amaranthaceae
8	Saru Manimoni	Indian pennywort	<i>Hydrocotyl rotundifolia</i> Lam.	Apiaceae
9	Matikanduri	Sessile Joywood	<i>Alternanthera sessilis</i> (L.) R. Br. Ex DC.	Amaranthaceae
10	Bhedailata	Skunk vine	<i>Paederia foetida</i> L.	Rubiaceae
11	Keheraj	False Daisy	<i>Eclipta alba</i> L.	Asteraceae
12	Masundori	Coriander scented/Stinkgrass	<i>Houttuynia cordata</i> Thunb	Saururaceae

Sl. No.	Local name	English name	Botanical name	Family
13	Pirali paleng	Water leaf	<i>Talinum triangularae</i> (Jacq.) Wild.	Portulacaceae
14	Madhuholang	Chinese Knotweed	<i>Polygonum chinense</i> L.	Polygonaceae
15	Saru tengesi	Creeping woodsorrel	<i>Oxalis corniculata</i> L.	Oxalidaceae
16	Bonjaluk	Francais	<i>Oldenlandia diffusa</i> (Wild.) Roxb.	Rubiaceae
17	Laffa	Common mallow	<i>Malva sylvestris</i> L.	Malvaceae
18	Baburi	Crown daisy	<i>Chrysopogon coronarium</i> L.	Poaceae
19	Methi	Fenugreek	<i>Trigonella foenum-graecum</i> L.	Fabaceae
20	Puroi (red)	Malabar spinach	<i>Basella rubra</i> L.	Basellaceae
21	Jilmil	Fathen	<i>Chenopodium album</i> L.	Chenopodiaceae
22	Morolia sak	Common chickweed	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae
23	Puroi (green)	Malabar spinach	<i>Basella alba</i> L.	Basellaceae`
24	Bor Manimoni	Indian pennywort	<i>Centalla asiatica</i> (L.) Urb.	Mackinlayaceae
25	Bor tangeshi	Common woodsorrel	<i>Oxalllis acetosella</i> L.	Oxalidaceae

*Only genus plus species names are mentioned in subsequent sections of the thesis.

The above plant materials were selected for the present investigation on the basis of survey carried out in the various places of Jorhat district and on the basis of their popularity (extend of uses) by the people of different places of district. The plant species were collected and grown in earthen pots in the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat-785013 during the period 2010-2014 are used as experimental materials.

3.1.2 Photograph of selected plants



Bacopa monnieri



Leucas longifolia



Enhydra fluctuans



Erynginum foetidum



Amaranthus spinosus



Pteridium aquilinum



Amaranthus viridis



Hydrocotyl rotundifolia



Alternanthera sessilis



Paederia foetida



Eclipta alba



Houttuynia cordata



Talinum triangularae



Polygonum chinense



Oxalis corniculata



Oldenlandia corymbosa



Malva sylvestris



Chrysopagon coronarium



Trigonella foenum-graecum



Basella rubra (red)



Chenopodium album



Stellaria media



***Basella rubra* (green)**



Centalla asiatica



Oxallis acetosella

3.2 Soil collection

The soils were collected from Instructional cum Research Farm of Assam Agricultural University, Jorhat for pot experiments. The soils were mixed with farmyard manure (FYM) in the ratio of 1:1.

3.3 Pot culture

The experimental plant materials were grown separately in earthen pot.

3.4 Soil analysis

The soil sample was analyzed to check the suitability of soil used for pot experiments.

3.4.1 Determination of soil pH

Soil pH was determined by Potentiometer Method in 1: 2.5 soil water ratio using Systronic μ -pH System 361 (Jackson, 1973).

3.4.2 Determination of soil organic carbon

Organic carbon of the soil was determined by Walkey and Black Wet Digestion Method (Walkey and Black, 1934).

3.4.3 Determination of available nitrogen in soil

Available nitrogen in the soil sample was determined by Alkaline Potassium Permanganate (KMnO_4) method (Subbiah and Asija, 1956).

3.4.4 Determination of available phosphorus in soil

Available phosphorus in the soil was determined by Bray's 1 method (Bray and Kurtz, 1945) using UV-Vis spectrophotometer, VARIAN, Model 50 Bio.

3.4.5 Determination of available potassium in Soil

Available potassium in the soil was determined by Ammonium Acetate extraction (Jackson, 1973) using Flame Photometer, Systronic, Model-130.

3.5 Plant analysis

3.5.1 Plant sample collection

The edible tender plant parts were collected from the selected pot and washed under tap water and finally with distilled water and allowed to dry in an oven at 45°C for 24 hours. After cooling, the plant materials were powdered. The powdered samples were taken for phytochemical analysis.

3.5.2 Determination of moisture content

Moisture content was determined by the method of AOAC (1970). 10g of the sample is weighted in aluminum moisture boxes and dried in an oven at 100°C (±2°C) for 16 hours, cooled in a desiccators and reweighed. The experiments have been conducted in triplicate and the mean was recorded.

Calculation:

Moisture content on fresh weight basis (g/100g sample)

$$= [\text{Initial weight (g)} - \text{Final weight (g)}] \times 100 / \text{Initial weight of sample (g)}$$

3.5.3 Determination of ash content and preparation of ash solution

The ash content was determined as described in the AOAC method (1970). 5 g powdered and dry sample was taken in a silica crucible, charred in low bunsen flame and finally ignited at 600°C for 6 hours in the muffle furnace.

$$\text{Ash content (g/100g sample)} = \text{Weight of ash (g)} \times 100 / \text{Weight of sample (dry basis)}$$

Preparation of ash solution: From this ash, mineral solution was prepared according to the method described in AOAC (1970). For this, ash was dissolved in HCl (1:1) on a water bath at 100°C and this solution was evaporated to dryness. After that 4 ml HCl and 2 ml distilled water were added, warmed and the acid soluble portion obtained after filtration was made up to 100 ml in a volumetric flask with distilled water. This solution was used for the estimation of mineral viz. Calcium, Phosphorous, Potassium, Sodium, Iron etc.

3.5.4 Determination of crude fat

The crude fat content was determined by the method described by Thimmaiah (1999). Where ether is continuously distilled, condensed and then allowed to pass through the sample to extract ether soluble materials. When the process is completed the ether is distilled, collected in another container, remaining crude fat is dried, weighed and percent oil is calculated.

3.5.5 Determination of crude fiber

The crude fiber content was determined by the method described by Maynard (1970) where 2g of ground material were extracted with ether or petroleum ether to remove fat (Initial boiling temperature 35-38°C and final temperature 52°C). After extraction with ether 2g of dried material was boiled with 200ml of sulphuric acid for 30 min with bumping chips. Filtered through muslin cloth and washed with boiling water until washings were no longer acidic. Boiled with 200ml of sodium hydroxide solution for 30 min. Filtered through muslin cloth again and washed with 25 ml of boiling 1.25% H₂SO₄, thrice by 50ml water and 25 ml alcohol. Removed the residue and transferred to the ashing dish (pre-weighed dish W₁). Dried the residue for 2 hrs. at 130 ± 2°C and cooled the dish in a desiccators and weighed (W₂). Ignited for 30 min at 600 ± 15°C. Cooled in desiccators and reweighed (W₃).

Calculation

$$\% \text{ Crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1) \times 100}{\text{Initial weight of the sample}}$$

3.5.6 Determination of crude protein content

The Nitrogen content was determined by Micro-Kjeldahl method (AOAC, 1965). 200 mg of dried sample were taken and transferred it to a Kjeldahl digestion tube and 1 g of CuSO₄.5H₂O - K₂SO₄ mixture and 5 ml of concentrated H₂SO₄ were added. Digested the sample in the digestion unit till the solution becomes colorless. Cooled and placed the digestion tube in Kel plus Unit and added 40ml of 40% NaOH . 100 ml conical flask containing 25 ml of 4% boric acid solution was taken and placed the flask at another end of Kel plus unit by dipping plastic tube in the boric acid solution. Distilled the digest for 6 min and collect liberated ammonia in boric acid. The colour change is an indication of ammonia absorbed and titrates against 0.1 N HCl. Then percentage of nitrogen is calculated. Crude protein was calculated by multiplying the total nitrogen value by a factor of 6.25 and ex-pressed in percentage.

$$\text{Crude protein (g \%)} = \text{N\%} \times 6.25$$

3.5.7 Determination of mineral content

The ash solution was prepared as described in 3.5.3 and from this solution different mineral was determined.

3.5.7.1 Determination of Potassium, Sodium and Calcium content

The mineral solution was used for the estimation of potassium, sodium and calcium content by flame photometry method (Baruah and Borah, 1998).

3.5.8 Determination of phosphorous content

The phosphorous content of the indigenous leafy vegetables was determined by the method of Fiske and Sukhrow (1925). An aliquot (eg. 0.1 or 0.2 ml) of ash solution were taken in a test tube and added to it 1ml of ammonium molybdate- sulphuric acid solution, 1 ml (0.5%) of hydroquinone solution and 1 ml (20%) of sodium sulphite solution in this order. Mixed thoroughly after each addition. Made up the volume to 10 ml with water, mixed properly and allowed to stand for 30 minutes. Measured the absorbance at 660 nm. Analysis was done against a standard prepared from potassium dihydrogen phosphate (KH_2PO_4).

3.5.9 Determination of iron content

The iron content of the indigenous leafy vegetables was determined by the method of Wong (1928). To an aliquot (6.5 ml) of the ash solution was added to make final volume of 6.5 ml and added to this 1 ml of 30% sulphuric acid, 1 ml of 7% potassium persulphate solution and 1.5 ml of 40% potassium thiocyanate solution in this order. Mixed the contents of the tube thoroughly and measured the intensity of the red color at 540 nm. Analysis was done against a standard prepared from ferrous ammonium sulphate.

3.5.10 Determination of nitrogen content

The Nitrogen content was determined by Micro-Kjeldahl method as described in 3.5.6.

The percentage of nitrogen calculated by the following formula:

$$\% \text{ N} = \frac{(\text{Sample titre} - \text{Blank titre}) \times \text{Normality of HCl} \times \text{Volume made of the digest} \times 14 \times 100}{\text{Aliquot of the digest taken} \times \text{mg sample}}$$

3.5.11 Determination of copper, zinc, manganese and arsenic content

The ash solution as described in 3.5.3 was used for the estimation of copper, zinc, manganese and arsenic by Atomic Absorption Spectrophotometric method.

3.5.12 Determination of total free amino acid

The total free amino acid content was determined by method described by Lee and Takahashi (1966). 0.1 g powdered plant sample was extracted with 80% ethanol, homogenate and then centrifuge at 10000 rpm for 10 min., extraction was done for three times. Pooled the

supernatant, made the volume to 20 ml with 80% ethanol and 2 ml of sample was taken in a clean dry test tube. Then 1.32 ml ninhydrin reagent followed by 0.53 ml citrate buffer was added, mixed and added 3.16 ml glycerol. After shaking heated for 30 min. in boiling water, cooled under tap water and then cooled in ice water, reading was taken at 570 nm. Analysis was done against a standard prepared from glycine.

3.5.13 Determination of soluble protein

The soluble protein content was determined by dye binding method described by Bradford (1976). For estimation of soluble protein, 0.01, 0.02, 0.04, 0.1 ml of standard protein solution were taken into a series of test tubes, made up volume in each tube to 0.1 ml with an appropriate buffer, 0.1 ml of buffer alone serves as the blank. 5 ml of protein reagent was added and mixed thoroughly by inversion. The unknown sample was done as above and absorbance was measured at 595 nm after 2 min and before 1 hr. against a reagent blank. Analysis was done against a standard prepared from bovine serum albumin.

3.5.14 Determination of starch content

Starch content was determined by the method, described by Clegg (1956). 100 mg powdered plant sample was extracted with 80% hot ethanol, centrifuged and retained the residue. Washed the residue with 80% hot ethanol for several times. This residue was dried over a water bath. To this residue 5 ml of distilled water and 6.5 ml 52% perchloric acid was added, mixed the content in shaker for 5 min., centrifuged and collected the supernatant. The extraction was repeated using 5 ml fresh perchloric acid and shaking was done in shaker for 10 min., centrifuged and collected the supernatant. Re-extracted the pellet by using 5 ml fresh perchloric acid and shaking was done for 30 min. Then, the supernatant was pooled, made the volume up to 100 ml with distilled water. Pipetted out 0.1 or 0.2 ml of the supernatant, made the volume to 1 ml with distilled water. The standard curve was made by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard prepared from glucose, made the volume to 1 ml in each tube with distilled water. Then, 4 ml of anthrone reagent was added to each tube, heated for 10 min. in a boiling water bath. Cooling was done rapidly and read the intensity of green to dark green color at 630 nm. The glucose content was calculated by using standard curve and the value was multiplied by a factor 0.9 as 0.9 g of starch yields 1 g of glucose on hydrolysis.

3.5.15 Determination of total soluble sugar content

Total soluble sugar content was determined by the method described by Clegg (1956). 150 mg of dried powdered plant sample was taken in a test tube and a few drops of ethanol and 2 ml distilled water were added, stirred for 5 minutes with a glass rod. 10 ml of hot boiling 80% ethanol was added and stirred for 5-10 minutes, transferred the supernatant to a volumetric flask without disturbing the residue in the tube, another 10 ml of hot 80% ethanol was added to the residue, stirred for 5 minutes and then filtered. This process was repeated for 4-5 times, pooled the extracts together and filtered through Whatman no. 41 filter paper and evaporated ethanol on water bath. 10 ml of water was added and dissolved the sugars and this solution can be used for the estimation of total soluble sugar, reducing sugar and non-reducing sugar. For estimation of total soluble sugar, pipetted out 0.1 or 0.2 ml of the supernatant, made the volume to 1 ml with distilled water. The standard curve was made by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard prepared from glucose, made the volume to 1 ml in each tube with distilled water. Then, 4 ml of anthrone reagent was added to each tube, heated for 10 min. in a boiling water bath. Cooling was done rapidly and read the intensity of green to dark green color at 630 nm.

3.5.16 Determination of reducing sugar content

Reducing sugar content was determined by the method described by Miller (1972). For estimation of reducing sugar, pipetted out 0.5 to 3.0 ml alcohol free supernatant into test tube, made the volume to 3ml with distilled water. The standard curve was made by taking 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard prepared from glucose, made the volume to 3 ml in each tube with distilled water. Then 3 ml of DNS reagent was added to each tube, mixed properly, heated for 5 min. in a boiling water bath. Cooling was done at room temperature and read the intensity at 510 nm.

3.5.17 Determination of ascorbic acid or vitamin C content

Ascorbic acid or vitamin C content was determined by the method described by AOAC (1970).

Extraction of ascorbic acid: 5 g fresh sample was grounded in 4% oxalic acid, made up to a known volume (100 ml) and centrifuged. This supernatant was used for estimation of ascorbic acid.

Procedure

5 ml of the working standard solution prepared from ascorbic acid was pipetted out into a 100 ml conical flask, added 10 ml of 4% oxalic acid and titrated against the dye (V_1 ml). End point was the appearance of pink color which persists for a few minutes.

Pipetted out 5 ml of ascorbic acid extract, 10 ml of 4% oxalic acid was added and titrated against the dye (V_2 ml). The amount of ascorbic acid was calculated by the following formula.

$$\text{Amount of ascorbic acid mg/100 g (Fresh weight basis)} = \frac{X \text{ mg} \times V_2 \times Z}{V_1 \times Y \text{ ml} \times \text{wt. of the sample}} \times 100$$

Where,

X = mg of standard ascorbic acid

V_1 = Titre value of standard ascorbic acid against dye

V_2 = Titre value of sample against dye

Y = Amount of aliquot (ml) taken for estimation

Z = Total volume made up of extracted sample

3.5.18 Determination of thiamine or vitamin B₁ content

Thiamine or vitamin B₁ content was determined by the method described by Sadasivam and Manickam (1992). For estimation of thiamine, 5 g finely powdered plant sample was taken into a 250 ml conical flask in duplicate, added 100 ml of 0.1 N H₂SO₄ slowly without shaking, stoppered the flask and left it to stand overnight. Next morning it was shaken vigorously and filtered through whatman No.1 filter paper. Pipetted out 10 ml of the extract in duplicate into 100 ml separating funnels and pipetted out 10 ml of working standard (in 4-5 replicates). Then, 3 ml of 15% NaOH was added into each separating funnel ensuring that the solution is alkaline. Immediately four drops (0.2 ml) of potassium ferricyanide reagent was added and shake the contents gently for 30 sec, 15 ml of isobutanol was added rapidly and shaking was done

thoroughly for 60 sec. Allowed it to stand till the two phases were separated out completely. The lower aqueous layer was discarded and removed the traces of water in the organic layer by adding solid anhydrous sodium sulphate, clear extract was collected from the top into a clean dry test tube. A set of sample blanks was prepared by pipetting out 10 ml of the extract by following the above procedure but omitting addition of potassium ferricyanide and a blank was prepared for the standard separately. Reading was taken in a fluorimeter and the thiamine content was calculated by the following formula.

$$\mu\text{g thiamine content in 100g sample} = \frac{0.25 \times 10 \times (x - x^1) \times 100 \times 10}{(a - a^1) \times 10 \times 5}$$

Where,

a = reading of standard = 100

a¹ = reading of standard blank = 0

x = reading of standard sample

x¹ = reading of standard sample blank

3.5.19 Determination of riboflavin content

Riboflavin content was determined by the method described by AACC (1976). For estimation of riboflavin, 2-5 g of powdered plant sample was taken into a 250 ml volumetric flask, 75 ml 0.1N H₂SO₄ was added and mixed. The flask was immersed in boiling water bath for 30 min; the flask was shaken in every 5 min., cooled at room temperature. 5 ml 2.5M sodium acetate solution was added, mixed, allowed to stand for at least one hour. It is then transferred to a 100 ml volumetric flask, made up the volume with distilled water and filtered through Whatman No.2 or No. 4, discarding the first 10–15 ml.

Preparation of standard

Dried riboflavin over phosphorous pentoxide (P₂O₅) in desiccator for 24 hr. Dissolved 50 mg in 1.5 lit water containing 2.4 ml glacial acetic acid in a 2 liter flask. Warmed to aid solution. Cooled and made up the volume. Stored under toluene in amber bottle in a refrigerator (25µg/ml).

Working standard

Diluted 20 ml riboflavin solution to 50 ml with water. From it, taken 10 ml riboflavin solution and diluted to 100 ml with water . Prepared fresh daily. Protected from light (1 ml = 1 µg riboflavin).

Oxidation of impurities was done as follows: In two test tubes of 1 inch diameter, with stirring rods, marked A and B, conduct oxidation as follows:

	Low-Blank Tube A	Sample Tube B	High-Blank Tube A	Tube B
Sample solution (ml)	10	10	10	10
Standard solution	1	-	1	-
Water (ml)	1	2	-	1
4% potassium permanganate (ml)	0.5	0.5	1	1
Time lapse (min)	2	2	4	4
3% H ₂ O ₂	0.5	0.5	1	1

Set fluorometer so that glass standard or sodium fluorescein solution gives suitable reading as directed for instrument. Determined fluorescence of solutions A and B. Do not exposed the solution for more than 10 seconds. To solution B in cuvette, added 20 mg sodium hydrosulfite, stirred, and determined blank fluorescence, C. Do not used readings C after colloidal sulfur begins to form.

Reading was taken in a flourimeter and the riboflavin content was calculated by the following formula.

$$\text{Riboflavin } \mu\text{g}/100\text{g} = \frac{B - C}{A - B} \times \frac{R}{S} \times \frac{V}{V_1} \times 100$$

Where,

A = fluorometer reading of sample plus riboflavin standard

B = fluorometer reading of sample plus water

C = fluorometer reading after addition of sodium hydrosulfite

R = standard riboflavin added, $\mu\text{g}/V_1$ of sample solution

V = original volume of sample solution (ml)

V_1 = volume of sample solution taken for measurement (ml)

S = sample weight (g)

3.5.20 Determination of niacin content

Niacin content was determined by the method, described by Sadasivam and Manickam (1992). The plant samples were extracted as follows-

The powdered sample (5 g) was ground in 4N sulphuric acid (30 ml) and steamed it for 30 min., cooled and made up to 50 ml with distilled water. Filtered through Whatman No.1 filter paper. 60% basic lead acetate was added to the filtrate (5 ml to 25 ml of filtrate) and pH was adjusted to 9.5 with a pH meter. To the supernatant 2 ml conc. H_2SO_4 was added and allowed to stand for 1 hr. and centrifuged. The supernatant was collected, 5 ml 40% ZnSO_4 was added and adjusted PH to 8.4 with 10N NaOH. Centrifuged and collected the supernatant. Adjusted pH to 7.0. After that pipette out 0.1 ml to 0.5 ml of the standard niacin solution into a series of test tubes pipette, two or three different volumes of samples solution was taken in other test tubes. Made up the volume to 6 ml with distilled water in all tubes. After that 3 ml of cyanogens bromide was added using a burette and shake the contents in the tube well. After 10 min, 1 ml of 4% aniline was added to each tube, after 5 min, yellow colour was developed and reading was taken at 420 nm against a reagent blank. Analysis was done against a standard prepared from niacin.

3.5.21 Determination of phytic acid content

Phytic acid content was determined by the method described by Wheeler and Ferrel (1971). For estimation of phytic acid, a finely ground (40 mesh) 100 mg sample taken into a 125 ml Erlenmeyer flask. Extracted in 50 ml 3% TCA for 30 min. with mechanical shaking or with occasional swirling by hand for 45 min., centrifuged the suspension and transferred a 10 ml aliquot of the supernatant to a 40 ml conical centrifuge tube. 4 ml of FeCl_3 solution was added to the aliquot by blowing rapidly from the pipette, heated the contents in a boiling water bath for 45 min., centrifuged (10 to 15 min) and carefully decanted the clear supernatant and the precipitate

was washed twice by dispersing well in 20 to 25 ml 3% TCA, heated in boiling water for 5 to 10 min and centrifuged. Repeated the washing with water, dispersed the precipitate in a few ml of water and 3 ml 1.5 N NaOH was added with mixing, made volume 30 ml with water and heated in boiling water for 30 min., filtered through a Whatman No. 2, washed the precipitate with 60-70 ml hot water and discarded the filtrate. Dissolved the precipitate from the paper with 40 ml hot 3.2 N HNO₃ into a 100 ml volumetric flask, washed paper with several portions of water, collecting the washings in the same flask, cooled the flask and contents to room temperature and diluted to volume with water. Transferred a 5 ml aliquot to another 100 ml volumetric flask and diluted to approximately 70 ml, 20 ml of 1.5M Potassium thiocyanate (KSCN) was added, and read the colour immediately (within 1 min.) at 480 nm. Analysis was done against a standard prepared from Fe (NO₃)₃.

3.5.22 Determination of oxalate content

The oxalate content is determined by the method given by National Institution of Nutrition (NIN, 1983). To 1 g of well ground sample, 50 ml of 2N HCl was added and the mixture was shaken well for about 2 hr. in a mechanical shaker. It is then centrifuged and filtered. The mixture was then transferred to the same beaker and weighed. It was then boiled for about 15 minutes and cooled. The mixture is then adjusted to previous weight with distilled water. Made up to 50 ml with 2N HCl, shaken well and filtered.

To 25 ml of the filtrate, 5 ml of phosphorus - tungstate reagent was added. The solution is then stirred well and kept overnight. The next day it was centrifuged and filtered, to 25 ml of the filtrate, 2 to 3 drops of methyl red was added along with 5 ml of calcium chloride buffer. Then the mixture was stirred well and kept overnight. Next day, the mixture was filtered through Whatman No. 42 filter paper and washed free from chloride using distilled water (Silver nitrate test). The precipitate along with filter paper was transferred to the same beaker and some distilled water was added followed by 5 ml of 2N H₂SO₄. The mixture is then heated to 80°C over a burner and titrated against the N/100 potassium permanganate solution. The results are calculated by the following formula

1 ml of N/100 KMnO₄ = 0.45 mg of oxalic acid

$$\frac{\text{Mg of Oxalic acid/100g sample}}{\text{= Titer Value}} \times \text{N of KMnO}_4 \times \frac{0.45}{0.01} \times \frac{\text{dilution}}{\text{factor}} \times \frac{100}{\text{wt of the sample}}$$

3.5.23 Determination of tannin Content

The tannin content is determined by the method given by Schanderl (1970). For estimation of tannin, 0.5 g of powdered material was weighed and transferred to 250 ml conical flask and added 75 ml of distilled water. Then the flask was heated gently and boiled for 30min. After that centrifugation was done at 2000 rpm for 20 min and the supernatant was collected in 100ml volumetric flask and volume was make up to 100 ml. Transferred 1ml of the sample extract to a 100 ml volumetric flask containing 75 ml water and 5 ml of Folin Denis reagent, 10 ml o f sodium carbonates solution was added and diluted to 100 ml with water. Shaken well and read the absorbance at 700 nm after 30 min. Analysis was done against a standard prepared from tannic acid.

3.5.24 Determination of total phenol content

The phenol content was determined by the method given by Bray and Thorpe (1954). Weighed exactly 1.0 g of sample and grind it with a mortar in 10 times volume of 80% ethanol. Then centrifugation was done and the supernatant was pooled. The supernatant was evaporated to dryness. Then the residue was dissolved in a known volume of distilled water (5 ml) and pipette out different aliquots (0.2 to 2 ml) into test tubes. The volume of each tube made up to 3 ml with distilled water and 0.5 ml of Folin-ciocalteau reagent was added. After 3 minutes, 2 ml of 20% sodium carbonate solution was added to each tube. After thorough mixing, the test tubes were placed in boiling water for exactly one minute, cooled and measured the absorbance at 650 nm against a reagent blank. Analysis was done against a standard prepared from catechol.

3.5.25 Determination of total alkaloid content

The total alkaloid content was determined by the method given by Obadoni and Ochuko (2001). 5 g sample were weighed into 250 ml beaker and 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 hr. This was filtered and the extract was concentrated using a water bath to one quarter of original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitation was collected by filtration and weighed.

3.5.26 Determination of saponin content

The saponin content was determined by the method given by Obadoni and Ochuko (2001). The samples were ground. 20 g of each plant samples were dispersed in 200 ml of 20% ethanol. The suspension was heated over a hot water bath for 4 hr. with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight. The saponin content was calculated in percentage.

3.5.27 Determination of free radical scavenging activity of different indigenous leafy vegetables grown in pot

Antioxidant activity was measured by using DPPH (2,2 diphenyl-1-picryl-hydrazyl) Method (Vani *et al.*, 1997). Two gram of fresh sample was extracted with 20 ml of methanol (99.5%). The extraction was done twice each for 2 hrs. in shaking machine. The supernatant was filtered using whatman No. 1 filter paper after centrifuging the suspension at 10,000 rpm for 15 min. Till analysis, filtrated stored at -20°C. 100 µl of aliquot of sample extract was taken in a test tube and added 2.9 ml of DPPH Solution (0.05 mM solution of 2,2 diphenyl-1-picryl-hydrazyl prepared in 99.5% methanol) after added this solution vortex vigorously. The test tube was incubated in dark for half an hour. The dis-colouration of DPPH was measured against blank at 517 nm. Methanol and DPPH methanolic solution was used as blank.

3.5.28 Determination of total flavonoid content

Preparation of extract

0.5 g dried powdered plant sample was weighed and mixed with 5 ml of 80% ethanol in 100 ml of conical flask and put on a shaker at 200 rpm for 24 hrs. After 24 hrs., the extracts were filtered through Whatman no. 42 filter paper. The volume of the filtrate was adjusted to 5 ml with 80% ethanol and was used for further analysis.

The total flavonoid was determined according to Woisky and Salatino (1998) with slight modification. 0.5 ml of extracts of each sample was taken in a test tube followed by addition of 1.5 ml 95% ethanol, 0.1ml of 10% aluminium chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation for 30 min at room temperature, the absorbance was measured at 415 nm in a spectrophotometer.

Quercetin was used to make the calibration curve. 10 mg of quercetin was weighed and dissolved in 100 ml of 80% ethanol, whose final concentration was 100 µg/ml. This was used as working standard for preparing standard curve.

From 100 µg/ml quercetin solution 0.2, 0.4, 0.6, 0.8 and 1 ml of quercetin were taken in 5 test tubes corresponding to concentration of 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and 100 µg/ml respectively. To each of the tubes volume was made up to 3 ml by adding distilled water, followed by addition of 0.1 ml 10% aluminium chloride, 0.1 ml of 1M of potassium acetate and 1.5 ml 95% ethanol respectively. After incubation at room temperature for 30 min. absorbance was measured at 415 nm in a spectrophotometer. The blank was prepared in the same way by taking water in place of quercetin.

3.5.29 Determination of total flavonol content

Plants were dried in the shade to ambient temperature until total dehydration. Dried aerial parts of plants were blended into fine powder and stored in the dark at a dry place. The powdered aerial part of plants were extracted in a 1/10 (w/v) of methanol, and water respectively for 12 hrs., under a continuous reflux set-up in a soxhlet extractor (hot) or at room temperature (cold) for 24 hrs. The extracts were concentrated by rotary evaporation at 40°C under vacuum to dryness and the yield of extraction was determined. All the dried extracts were preserved in the refrigerator until further use. Plant extracts were dissolved in (20/80, v/v) ethanol/water.

The content of flavonol was determined as described by Miliauskas *et al.* (2004). The quercetin calibration curve was prepared by mixing 2 ml of (0.5, 0.4, 0.3, 0.2, 0.166, 0.1, 0.05, 0.025, and 0.0166 mg/ml) quercetin ethanolic solution with 2 ml of (20 g/l) aluminium trichloride solution and 6 ml of (50 g/l) sodium acetate solution. The absorption at 440 nm was read after 2.5 hrs. at 20°C. The same procedure was carried out with 2 ml of plant extract (10 g/l) instead of quercetin solution. All determinations should carry out in three replications.

3.5.30 Determination of super oxide dismutase activity

The superoxide dismutase activity is estimated by the method of Giannopolitis and Ries (1977) with little modifications.

Extraction of enzyme: Fresh leaf tissue was collected from plants, 200 mg of leaf tissue was weighed and ground to a fine powder in pre-cooled mortar and pestle, homogenized in 1.2 mL of 0.2 M potassium phosphate buffer (pH 7.8 with 0.1 mM EDTA). The samples were centrifuged at 15,000 g for 20 min at 4°C. The supernatant was removed, the pellet re-suspended in 0.8 mL of the same buffer, and the suspension centrifuged for another 15 min at 15,000 g. The combined supernatants were stored on ice and used to determine SOD activity.

The reaction mixture contains 0, 0.2, 0.3, 0.4, 0.5 and 1.0 ml of enzyme extract in separate sets with addition 1 ml of each of methionine, NBT, EDTA and Na₂CO₃ and the total volume of 5 ml is made with buffer in each set, adjusting pH at 10.2. Then 1 ml of riboflavin was added to each set in the last. The tubes were shaken and placed 30 cm from light blank consisting of two 10-15 W fluorescent lamps. The reaction was allowed to run for 15 min. and then stopped by switching off the light. The tubes were immediately covered with a black cloth. Then the absorbance was recorded at 560 nm. The enzyme activity was expressed as unit⁻¹ mg⁻¹protein min⁻¹.

3.5.31 Determination of peroxidase activity

The peroxidase activity was determined by the method given by Bergmeyer (1974). The enzymes were extracted as -10 g of fresh plant sample in 50 ml of 50 mM Tris buffer (pH 7.5) was extracted by grinding in a pestle and mortar, filtered the homogenate through the cheese cloth and centrifuged at 10000 g at 4°C for 10 minutes. The supernatant used as enzyme source. For estimation enzyme activity, 3.6 ml of 50 mM sodium acetate buffer (pH 5.4) was taken, 0.1 ml of enzyme extract was added to it, 0.1 ml of freshly prepared *O*-dianisidine solution and 0.2 ml of distilled water was added. The assay mixture was brought to 30°C, placed the cuvette in spectrophotometer at 460 nm, use it as blank, the assay mixture was taken without water in the cuvette along with 3.6 ml of 50 mM sodium acetate buffer (pH 5.4), 0.1 ml of enzyme extract, 0.1 ml of freshly prepared *O*-dianisidine solution and 0.2 ml of distilled water. 0.2 ml of H₂O₂ added and mixed, immediately starts the stopwatch, read the initial absorbance and then at every 30 seconds interval up to 3 minutes, read the change in absorbance per minute

and expressed enzyme activity in terms of rate of increased absorbance per unit time per mg protein.

3.5.32 Determination of catalase activity

Catalase activity can be measured according to the method given in Analytical Biochemistry (Sinha, 1972).

200 mg plant sample was homogenized with 10 ml of phosphate buffer 0.1 M, pH 7.0 and centrifuged at 10,000 rpm at 40°C for 30 min. Collected the supernatant and store at low temperature (4°C). Used the supernatant for enzyme assay and estimate the enzyme activity as given below:

Reaction mixture

Test	Blank	Reagents
1.25 ml	-	H ₂ O ₂
0.50 ml	0.50 ml	Enzyme extract
3.25 ml	4.50 ml	Phosphate buffer
Total = 5.0 ml	Total = 5.00 ml	

Reaction mixture was taken in conical flask and mixed rapidly at 37°C. After 3 min. withdrew 2.0 ml reaction mixture and added 2.0 ml potassium dichromate acetic acid reagent. Kept on boiling water bath for 10 min and recorded O.D. at 570 nm against blank after cooling.

Express result as enzyme unit per gram fresh or dry weight or per mg protein basis where one unit of catalase activity defined as change in O.D by 0.1 unit/min.

3.5.33 Molecular characterization

For molecular characterization RAPD analysis was done using 20 random decamer primers by the method of Williams *et al.* (1990). DNA was extracted from fresh tender leaves of few indigenous leafy vegetables by the method of Murray and Thomson (1980).

3.5.33.1 Extraction of DNA

500 mg of young tender plant leaf sample was taken and placed inside a eppendorf tube and ground with micro pestle with 500 µml of CTAB buffer. Incubated at 65°C

for 45 min in water bath and to this homogenate, added equal volume of Phenol: Chloroform: Isoamyl alcohol (50:48:2). Mixed gently by inverting it and centrifuged at 10,000 rpm for 10 min. at 4°C. Supernatant was separated from it and to this supernatant added equal volume of Chloroform: Isoamyl alcohol (96:4), mixed gently by inverting it then centrifuged at 10000 rpm for 10 min. at 4°C. Upper aqueous layer was taken to fresh eppendrof tube and to this supernatant was added (double volume of supernatant) chilled isopropanol and (1/10 volume of supernatant) 3M Na-acetate. Kept at -20°C for overnight precipitation and centrifuged at 10,000 rpm for 5 min., removed the liquid portion, washed the solid DNA pellet with 70% ethanol, dried in vacuum. Finally dissolved in minimum quantity of T.E. buffer (60 µl) and treated with 1 µl RNAase, incubated at 37°C for 45 min and stored at 4°C for analysis. DNA sample was ready and OD of the sample was taken in Nano drop machine, dilute the sample at concentration of 50 ng/µl (total volume 60 µl). Sample is ready for RAPD analysis.

3.5.33.2 Randomly amplified polymorphic DNA (RAPD) analysis

For RAPD analysis 30 random decamer primers supplied by Bangalore Gene were used. The primers and their sequences are shown in Table 1.

Table 1. Sequence of decamer primers

Sl. No.	Primers	Sequence
1	OPA-01	(5'CAGGCCCTTC3')
2	OPA-02	(5'TGCCGAGCTG3')
3	OPA-03	(5'AGTCAGCCAC3')
4	OPA-04	(5'AATCGGGCTG3')
5	OPA-05	(5'AGGGGTCTTG3')
6	OPA-06	(5'GGTCCCTGAC3')
7	OPA-07	(5'GAAACGGGTG3')
8	OPA-08	(5'GTGACGTAGG3')
9	OPA-09	(5'GGGTAACGCC3')
10	OPA-10	(5'GTGATCGCAG3')
11	OPB-01	(5'GTTTCGCTCC3')
12	OPB-02	(5'TGATCCCTGG3')
13	OPB-03	(5'CATCCCCCTG3')
14	OPB-04	(5'GGACTGGAGT')
15	OPB-05	(5'TGCGCCCTTC3')
16	OPB-06	(5'TGCTCTGCCC3')

17	OPB-07	(5'GGTGACGCAG3')
18	OPB-08	(5'GTCCACACGG3')
19	OPB-09	(5'TGGGGGACTC3')
20	OPB-10	(5'CTGCTGGGAC3')
21	OPC-01	(5'TTCGAGCAAG3')
22	OPC-02	(5'GTGAGGGCTC3')
23	OPC-03	(5'GGGGGTCACG3')
24	OPC-04	(5'CCGCATCGAC3')
25	OPC-05	(5'GATGACCCCC3')
26	OPC-06	(5'GAACGGACTC3')
27	OPC-07	(5'GTCCCGACGA3')
28	OPC-08	(5'TGGACCGGTG3')
29	OPC-09	(5'CTCACCGTCC3')
30	OPC-10	(5'TGTCTGGGTG3')

3.5.33.3 Polymerase Chain Reactions

DNA amplification reactions were done in a volume of 10 µl reaction mixture containing sterile distilled water - 5.5 µl, Taq buffer A-1 µl, DNTPs-0.5 µl, Primer-0.5 µl, Taq polymerase-0.5 µl and 2 µl DNA sample. The reagents were mixed thoroughly by slight vortex followed by spinning in microfuge. The tubes are then placed in thermal cycler for cyclic amplification. The conditions for amplification were as follows:

94°C for 1 min (initial denaturation)

94°C for 5 min (denaturation after every cycle)

30°C (Jilmil), 36°C (Hatkhotura), 38°C (Matikandori), 42°C (Helosisak) [primer annealing]

72°C for 1 min (extension of annealed primer)

72°C for 10 min (final extension)

The total reaction was carried out for 35 cycles. The PCR products were run in Agarose gel electrophoresis with known DNA ladder (mol.wt.1kb) on 1.5% Agarose gel. Data analysis was done by entering the presence (1) and absence (0) of an amplified fragment for each variety of primer combination using the editor of the NTSYS programme and a dendrogram was

created. The PCR machine used was supplied by Applied Biosystem. Percentage polymorphism was calculated by using the formula:

$$\text{Polymorphism (\%)} = \frac{\text{Total number of bands} - \text{number of monomorphic bands}}{\text{Total number of bands}} \times 100$$

3.5.33.4 Statistical analysis

The data obtained was subjected to statistical analysis using Completely Randomized Design (Sumithiana, 1961).

CHAPTER IV

RESULTS AND DISCUSSION

Results obtained on the studies entitled “Biochemical and Molecular Characterization of Indigenous Leafy Vegetables of Jorhat district of Assam” are presented and discussed in this chapter.

4.1 Survey for exploration of indigenous leafy vegetables

A suevey was conducted through out the Jorhat district and the following plant materials were collected from different location of Jorhat district and identified by taxonomist. During the survey 50 (fifty) numbers of persons were contacted and asked about the consumption of the following indigenous leafy vegetables. Out of all the surveyed indigenous leafy vegetables first 25 green leafy vegetables were found to be consumed by majority of the people. The results of survey are presented in the Table 2.

Table 2. Exploration of indigenous leafy vegetables

Sl. No.	Local name	English name	Botanical name	Family	Part used
1	Brahmi	Bacopa	<i>Bacopa monnieri</i> (L.) Wettst.	Scruphulariaceae	Tender leaf and stem as vegetable
2	Dorun	Dhudani	<i>Leucas longifolia</i> Benth.	Lamiaceae	Tender leaf and stem as vegetable

3	Helosi sak	Water cress	<i>Enhydra fluctuans</i> DC.	Asteraceae	Tender leaf and stem as vegetable
4	Mandhania	Long coriander	<i>Eryngium foetidum</i> L.	Apiaceae	Tender leaf, Mature leaf as spice, vegetable
5	Hatikhutora	Spiny amaranth	<i>Amaranthus spinosus</i> L.	Amaranthaceae	Tender leaf along with tender stem as vegetable
6	Dhekia	Bracken fern	<i>Pteridium aquilinum</i> (L.) Kuhn.	Dennstaedtiaceae	Fiddlehead greens
7	Khotor	Slender amaranth	<i>Amaranthus viridis</i> L.	Amaranthaceae	Tender leaf along with tender stem as vegetable

Sl. No.	Local name	English name	Botanical name	Family	Part used
8	Saru Manimoni	Indian pennywort	<i>Hydrocotyl rotundifolia</i> Lam.	Apiaceae	Tender leaf along with tender stem as vegetable
9	Matikandori	Sessile Joywood	<i>Alternanthera sessilis</i> (L.) R.Br.ex DC.	Amaranthaceae	Tender leaf along with tender stem as vegetable
10	Bhedailata	Skunk vine	<i>Paederia foetida</i> L.	Rubiaceae	Tender leaf along with tender stem as vegetable
11	Keheraj	False Daisy	<i>Eclipta alba</i> L.	Asteraceae	Tender leaf along with tender stem as vegetable
12	Masundori	Coriander scented	<i>Houttuynia cordata</i> Thunb.	Saururaceae	Tender leaf along with tender stem as vegetable
13	Pirali paleng	Water leaf	<i>Talinum triangularae</i> (Jacq.) Wild.	Portulacaceae	Tender leaf along with tender stem as vegetable
14	Madhuholang	Chinese Knotweed	<i>Polygonum chinense</i> L.	Polygonaceae	Tender leaf along with tender stem as vegetable
15	SaruTengesi	Creeping woodsorrel	<i>Oxalis corniculata</i> L.	Oxalidaceae	Tender leaf along with tender stem as vegetable
16	Bonjaluk	Francais	<i>Oldenlandia diffusa</i> (Wild.) Roxb.	Rubiaceae	Tender leaf along with tender stem as vegetable
17	Laffa	Common	<i>Malva sylvestris</i> L.	Malvaceae	Tender leaf along with tender stem

18	Baburi	mallow Crown daisy	<i>Chrysopogon coronarium</i> L.	Poaceae	as vegetable Tender leaf along with tender stem as vegetable
19	Methi	Fenugreek	<i>Trigonella foenum-graecum</i> L.	Fabaceae	Tender leaf along with tender stem as vegetable

Sl. No.	Local name	English name	Botanical name	Family	Part used
20	Puroi(red)	Malabar spinach	<i>Basella rubra</i> L.	Basellaceae	Tender leaf along with tender stem as vegetable
21	Jilmil	Fathen	<i>Chenopodium album</i> L.	Chenopodiaceae	Tender leaf along with tender stem as vegetable
22	Morolia sak	Common chickweed	<i>Stellaria media</i> (L.) Vill.	Caryophyllaceae	Tender leaf along with tender stem as vegetable
23	Puroi (green)	Malabar spinach	<i>Basella alba</i> L.	Basellaceae`	Tender leaf along with tender stem
24	Bor Manimoni	Indian pennywort	<i>Centalla asiatica</i> (L.)Urb.	Mackinlayaceae	Tender leaf along with tender stem as vegetable
25	Bor tangeshi	Common woodsorrel	<i>Oxalllis acetosella</i> L.	Oxalidaceae	Tender leaf along with tender stem as vegetable
26	Malbhog Khotura	Purselane	<i>Portulaca oleraceae</i> L.	Portulacaceae	Tender leaf along with tender stem as vegetable
27	Bharali Bhukoya	Graceful pouzolzbush	<i>Ponzolzia indica</i> L.	Urticaceae	Tender leaf as vegetable
28	Laijabori	Chickweed	<i>Drymaria cordata</i> Wild Ex.SCHULT	Caryophyllaceae	Tender leaf as vegetable
29	Bhutmoola	African Spider Flower	<i>Gynandropis gynandra</i> (L) Briq.	Capparidaceae	Tender leaf as vegetables
30	Nephaphu	East Indian Glory Bower	<i>Clerodendrum colebrookianum</i> L.	Chenopodiaceae	Tender leaf as vegetables
31	Sh-ukloti	Pathchouli	<i>Pogostemon benghalensis</i> (Burm.f.) Kuntze	Lamiaceae	Tender leaf

The most important positive aspect of wild vegetables is that nearly all of them are known to possess therapeutic properties and are being used by native people as a remedy for a variety of human and cattle diseases (Ibrar *et al.*, 2003). Plants of 23 different families were collected and identified with their folk uses. The indigenous knowledge system, traditional technologies and resource management practices of different ethnic communities of north-eastern India provide ample opportunities for agricultural diversification as well as intensification (Sarma *et al.*, 2006). The present study has thrown light on wild plants used as components of agriculture sector and contributes its economic development. The sustainable harvesting of plants having both medicinal and economic value has a great potential. In fact, there is no local awareness about proper collection of various species. Thus, there is a need to create awareness of the importance of these plants among local people and to provide them guidance and training in collection and processing to enhance their income. However, this study revealed some useful information to phytochemists, taxonomists etc. for screening of individual species and rapid assessing of some useful vegetables.

Traditional vegetables are usually obtained from different sources as compared to exotic vegetables. While the majority of all exotic vegetables consumed were purchased from markets (extend of use 65.4%), the majority of traditional vegetables consumed is produced in their own field or collected from the bush. However, there were also marked differences across different households. A larger share of traditional vegetables produced in poor households be obtained through collection (extend of use 30%), while it is the middle class that consumes the largest share of traditional vegetables produced in their own fields, it is the wealthiest group that purchases the largest share of traditional vegetables from the market (extend of use 40% of all produce) (Weinberger and Swai, 2006).

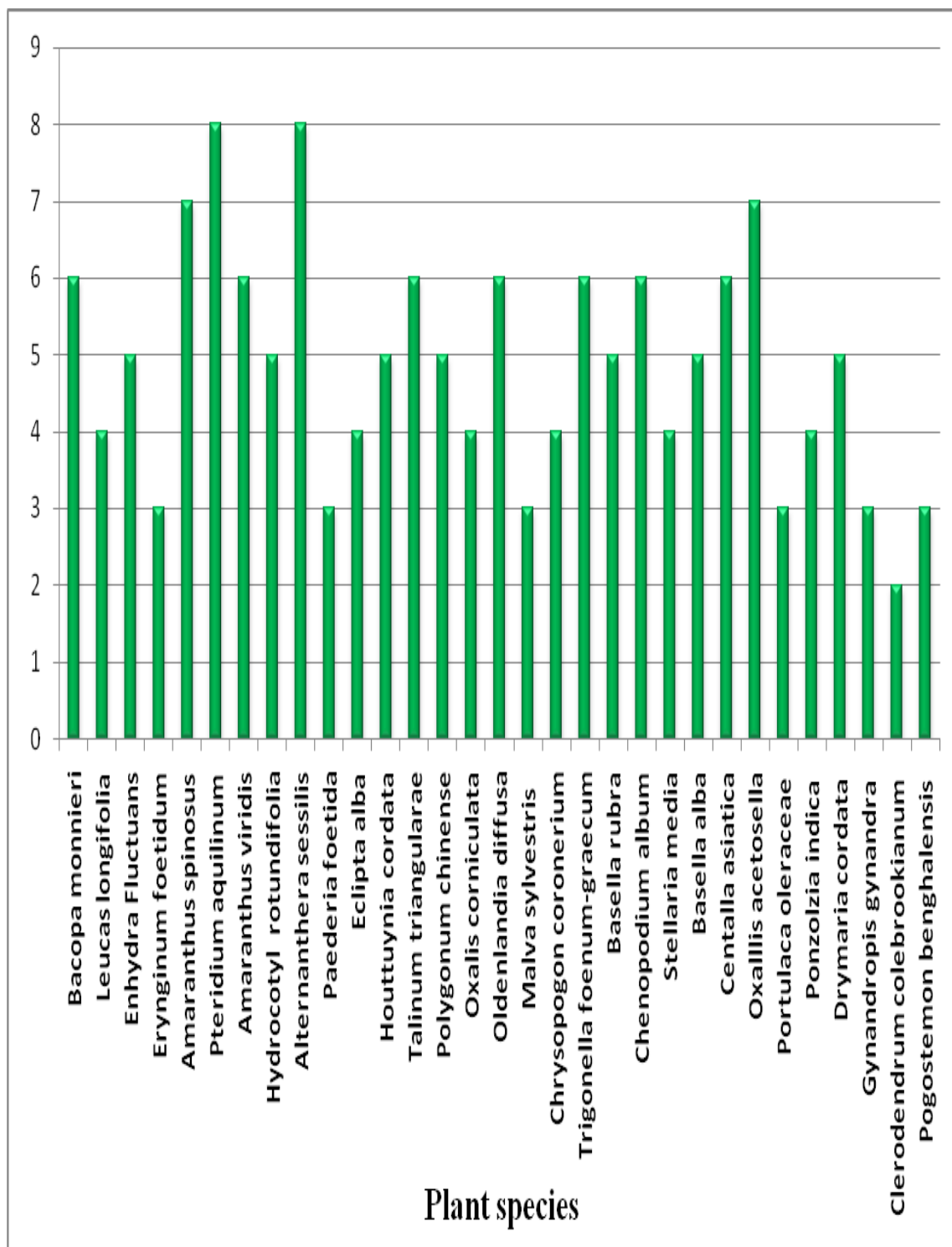


Fig. 1. Number of locations for collection of indigenous leafy vegetables of Jorhat District of Assam

4.2 Indigeneous Traditional Knowledge (ITK) of indigenous leafy vegetables

During the survey 50 (fifty) numbers of persons from representative localities were contacted and asked about their traditional beliefs for consumption of the following indigenous leafy vegetables. These vegetables are available throughout, all the localities of the district.

Table 3. Indigeneous Traditional Knowledge of indigenous leafy vegetables

Sl. No.	Local name	Botanical name	Traditional knowledge
1	Brahmi	<i>Bacopa monnieri</i> (L.) Wettst.	Improve memory, digestion, eye power, cure itching
2	Dorun	<i>Leucas longifolia</i> Benth.	Improve digestion, cure tonsil, cure pharangitis, cure flow disease, clear bowel
3	Helosi sak	<i>Enhydra Fluctuans</i> DC.	Cure jaundice, clear bowel
4	Mandhania	<i>Erynginum foetidum</i> L.	Improve digestion, flatulance, cure diabetes
5	Hatikhutora	<i>Amaranthus spinosus</i> L.	Cure jaundice, stomach pain, clear bowel
6	Dhekia	<i>Pteridium aquilinum</i> (L.) Kuhn.	Improve bone formation, health, clear bowel
7	Khutora	<i>Amaranthus viridis</i> L.	Clear bowel, improve digestion
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i> Lam.	Improve digestion, cure jaundice, cure liver ailments, cure diarrhoea
9	Matikandori	<i>Alternanthera sessilis</i> (L.) R.Br.ex DC.	Increase blood, wound healing, cooling brain
10	Bhedailata	<i>Paederia foetida</i> L.	Improve digestion, cure diarrhea, cure ulcers, clear bowel
11	Keheraj	<i>Eclipta alba</i> L.	Cure jaundice, cure teeth diseases
12	Masundori	<i>Houttuynia cordata</i> Thunb.	Improve digestion, cure diarrhea, cure dysentary
13	Pirali paleng	<i>Talinum triangularae</i> (Jacq.) Wild.	Clear bowel, source of vitamin
14	Madhuholang	<i>Polygonum chinense</i> L.	Cure poxes, clear bowel, source of vitamin
15	SaruTengesi	<i>Oxalis corniculata</i> L.	Purify blood, cure dysentery, clear bowel
16	Bonjaluk	<i>Oldenlandia diffusa</i> (Wild.) Roxb.	Cure liver diseases, cure flatulence, cure jaundice, cure eye swelling
17	Laffa	<i>Malva sylvestris</i> L.	Clear bowel, create problem in throat

Sl. No.	Local name	Botanical name	Traditional knowledge
18	Baburi	<i>Chrysopogon coronarium</i> L.	Clear bowel, source of vitamin
19	Methi	<i>Trigonella foenum-graecum</i> L.	Kill worm, cure diabetes, improve digestion
20	Puroi (red)	<i>Basella rubra</i> L.	Cure low pressure, cure burning
21	Jilmil	<i>Chenopodium album</i> L.	Increase blood, clear bowel
22	Morolia sak	<i>Stellaria media</i> (L.) Vill.	Clear bowel, cure dysentery
23	Puroi (green)	<i>Basella alba</i> L.	Cure low pressure, cure burning
24	Bor Manimoni	<i>Centalla asiatica</i> (L.) Urb.	Cure jaundice, cure appendix, purify blood
25	Bor tangeshi	<i>Oxallis acetosella</i> L.	Cure allergy, clear bowel, purify blood
26	Malbhog Khotura	<i>Portulaca oleraceae</i> L.	Clear bowel, purify blood, improve digestion
27	Bharali Bhukoya	<i>Ponzolzia indica</i> L.	Cure ulcers, cure skin diseases
28	Laijabori	<i>Drymaria cordata</i> Wild Ex.SCHULT	Clear bowel, increase blood
29	Bhutmoola	<i>Gynandropis gynandra</i> (L) Briq.	Cure dysentery, improve digestion
30	Nephaphu	<i>Clerodendrum colebrookianum</i> L.	Improve digestion, cure dysentery
31	Sh-ukloti	<i>Pogostemon benghalensis</i> (Burm.f.) Kuntze	Cure injury, create thirsty tendency

Jorhat district is the repository of wild edible plants and hence, indispensable to the local communities. Many households collect minor products and sell them in local markets for cash income and livelihoods. The present study documented use of 31 wild edible plants belonging to 23 families (Table 3). Traditional knowledge of wild food plants is passed orally from parents to children through words of mouth. Children learn names of wild foods at home collected by their parents. They learn to identify the plant and parts collected by accompanying their parents to forests. Later, utilizing their experience, children collect plant foods by themselves; parents/elders correct them for any error or deficiency. Girls usually acquire knowledge of cooking from mothers and grandmothers while the males learn cooking from varied sources as their activities are more diversified and mostly outside the house (Sarma *et al.*, 2008; Pegu *et al.*, 2013).

4.3 Chemical analysis of soil used for growing plants in pots

The soil sample before planting of leafy vegetables were analysed and the results are presented in the Table 4. The pH value was found 6.2 which are ideal for the growth of microbes. Similar results were reported by Karmakar (1985). According to Karmakar (1985) in alluvial and flood plain soils of northern bank of the Brahmaputra valley of Assam, the pH varied from 5.1 to 6.3. The organic carbon value was found 0.834% which is ideal source for plant nutrients which are in agreement with the report of Chakraborty *et al.* (1984). According to Chakraborty *et al.* (1984) in Brahmaputra Valley, average organic matter content in new alluvial, old alluvial and upland soils were 0.9, 1.02 and 2.70% respectively. The available nitrogen (kg ha⁻¹) value was found 492.1 which are in agreement with the report of Baruah *et al.* (2012). As per the report of Baruah *et al.* (2012), the soil available nitrogen was found within the medium range (250–500 kg/ha) in majority of the soil samples collected from various tea belt of Golaghat district except samples from Rungajaun tea estate belt in dry season and Balijan tea estate belts in wet season. The range was 350.41–502.35 kg/ha and 274.10–561.31 kg/ha during dry and wet season, respectively. Samples of Balijan tea estate were found changing medium category (250–500 kg/ha) to high category (>500 kg/ha) in available nitrogen from dry season to wet season. The available P₂O₅ (kg ha⁻¹) value was found 29.8. Similar results are reported by Das *et al.* (2013). According to Das *et al.* (2013) available P₂O₅ of soil of Assam is 26.8 kg/ha. The available K₂O (kg ha⁻¹) value was found 185.9 which are almost similar with values (173.9 kg ha⁻¹) obtained by Das *et al.* (2013). According to Das *et al.* (2013) available K₂O of soil of Assam is 173.9 kg/ha. This may be due to faryard manure added to the soil in the ratio of 1:1. The chemical analysis of soil used for growing experimental plants revealed that the soil truly represents the average soils of the district and suitable for growing the traditional leafy vegetables considered under the experiment.

Table 4. Few chemical parameters of soil

Parameters	Value
pH	6.2
Organic carbon (%)	0.834
Available nitrogen (kg ha ⁻¹)	492.1

Available P ₂ O ₅ (kg ha ⁻¹)	29.8
Available K ₂ O (kg ha ⁻¹)	185.9

4.4 Nutritional composition of different indigenous leafy vegetables

The data of moisture content, ash content, crude fibre content, crude fat content, crude protein content, total free amino acid content and soluble protein content of different leafy vegetables are presented in the Table 5.

4.4.1 Moisture content

Moisture generally refers to the presence of water, often in trace amounts. Water is an important part of all cells and fluids in the body. It carries nutrients to and waste products from cells in the body, aids in digestion and absorption of food and helps to regulate body temperature (Johnson, 1996). The maximum water content varies between individual vegetables because of structural differences and cultivation condition that influence structural differentiation and may also have a marked effect on water levels vegetables (Florkowski *et al.*, 2009). High moisture content in vegetables is indicative of its freshness as well as easy perishability (Adepoju and Oyediran, 2008).

Water is the major component of the plant. It plays an important role in nutrient absorption and transportation, formation of new products, plant growth. Carbohydrates, the products of photosynthesis, are moved in water solution to storage organs. A major portion of the water absorbed by the plant evaporates through the stomata. This evaporation process is essential for absorbing oxygen for photosynthesis. In addition, water absorbs heat, cools the plant and prevents plant injury from high temperatures. The high moisture content of indigenous leafy vegetables give great impact on energy density (amount of energy in a given weight of food (kcal/g) as water adds substantial weight to the food without adding energy and this may give the consumers a better satiety without increase their energy intake (Ng *et al.*, 2012).

The results of the present investigation revealed that Puroi (green) (92.77%) had the highest moisture content followed by Pirali Paleng (92.47%) and Bonjaluk (76.40%) had the lowest moisture content. This might be due to genetical, soil moisture and environmental conditions of the plant species. There is a significant difference among the plant species. This result was comparable with the values obtained by Gupta *et al.* (73.0 to 95.3%) (2005); Wallace *et al.* (74.41% to 91.43%) (1998); Baruah and Borah (2.53% to 89.00%) (2009); and Kwenin *et al.* (21.6% to 93.4%) (2011).

4.4.2 Ash content

Ash is the inorganic residue remaining after the water and organic matter have been removed by heating in the presence of oxidizing agents, which provides a measure of the total amount of minerals within a food (Mc Clement, 2003). Higher ash content predicts the presence of an array of mineral elements as well as high molecular weight elements (Onot *et al.*, 2007). Ash is herbaceous biomass consists of inorganic constituents. Ash content can originate from the biomass itself. Ash is one of the components in the [proximate](#) analysis of biological materials, consisting mainly of salty, [inorganic](#) constituents, metal [salts](#) which are important for processes requiring [ions](#) such as Na⁺ (Sodium), K⁺ (Potassium), and Ca²⁺ (Calcium).

The data of the present investigation revealed that Puroi (green) (14.70%) had the highest ash content followed by Khotura and Jilmil (14.60%) and Bhedailata (7.80%) had the lowest ash content. This might be due to genetical, soil fertility and environmental conditions of the plant species. There is a significant difference among the plant species. Similar results were obtained by Baruah and Borah (6.21% to 8.60%) (2009); Ng *et al.* (10.7% to 17.6%) (2012); Adegunwa *et al.* (8.3% to 25.0%) (2011).

4.4.3 Crude fibre content

Dietary crude fibre is the edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary crude fibre includes polysaccharides, oligosaccharides, lignin, and associated plants substances. It promotes beneficial physiological effects including laxation, and/or blood cholesterol attenuation, and/or blood glucose attenuation.

Dietary fibre consists of non-digestible carbohydrates and lignin that are intrinsic and intact in plants. Functional fibre consists of isolated, non-digestible carbohydrates which have beneficial physiological effects in humans. 'Dietary fibre', 'unavailable carbohydrate' or 'roughage' are the different terms used to define that part of the plant food which is not digested by the endogenous secretions of the human digestive system. Dietary fibre may be classified into three major groups according to structure and properties. The groupings are cellulose, non-cellulose and lignin (Komal and Kaur, 1992).

Table 5. Nutritional composition on dry weight basis of indigenous leafy vegetables (g %)

Sl. No.	Local name	Botanical Name	Moisture (FWB)	Ash	Crude fibre	Crude fat	Crude protein	Total free amino acid (mg%)	Soluble protein
1	Brahmi	<i>Bacopa monnieri</i>	83.59	8.70	9.13	3.10	24.67	167.34	1.85
2	Doron	<i>Leucas longifolia</i>	84.6	12.5	13.10	2.80	26.26	220.11	1.23
3	Helosi sak	<i>Enhydra fluctuans</i>	85.57	13.57	12.70	3.10	33.27	239.20	2.27
4	Mandhanian	<i>Eryngium foetidum</i>	86.60	11.50	9.17	3.60	31.63	312.74	1.10
5	Hatikhotora	<i>Amaranthus spinosus</i>	86.45	13.50	5.80	3.13	36.76	383.47	2.40
6	Dhekia	<i>Pteridium aquilinum</i>	87.14	11.27	9.10	2.30	39.38	992.00	2.03
7	Khotora	<i>Amaranthus viridis</i>	89.95	14.60	7.20	2.80	30.65	868.21	2.76
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	89.55	12.87	15.10	2.13	24.54	970.72	1.90
9	Matikandori	<i>Alternanthera sessilis</i>	83.46	13.80	9.50	5.20	27.16	170.61	2.21
10	Bhedailata	<i>Paederia foetida</i>	79.63	7.80	20.40	2.10	16.64	79.37	1.47
11	Keheraj	<i>Eclipta alba</i>	91.09	13.80	16.10	2.13	23.64	168.08	1.86
12	Masundori	<i>Houttuynia cordata</i>	88.25	12.67	17.60	4.80	17.6	145.20	2.35
13	Pirali paleng	<i>Talinum triangularae</i>	92.47	13.20	9.10	4.70	29.77	234.98	4.13
14	Madhuholang	<i>Polygonum chinense</i>	91.14	9.77	12.20	3.20	34.15	250.65	3.51
15	Saru Tengesi	<i>Oxalis corniculata</i>	86.10	10.47	16.40	6.20	19.27	168.68	2.20
16	Bonjaluk	<i>Oldenlandia diffusa</i>	76.40	9.40	26.10	4.10	17.63	137.18	2.99
17	Laffa	<i>Malva sylvestris</i>	88.46	11.10	11.10	6.20	31.60	305.45	2.23
18	Baburi	<i>Chrysopogon coronarium</i>	91.27	8.70	6.20	2.70	31.60	329.06	2.69
19	Methi	<i>Trigonella foenum-graecum</i>	88.44	11.60	10.13	3.20	35.85	403.40	2.52
20	Puroi (red)	<i>Basella rubra</i>	92.19	13.70	5.50	7.10	18.40	173.78	2.92
21	Jilmil	<i>Chenopodium album</i>	86.63	14.60	4.40	3.90	36.76	365.52	2.78
22	Morolia	<i>Stellaria media</i>	85.39	13.30	4.67	2.30	20.47	200.27	2.43
23	Puroi (green)	<i>Basella alba</i>	92.77	14.70	2.50	4.20	28.10	223.35	2.62
24	Bor	<i>Centalla asiatica</i>	83.54	12.70	8.90	1.13	15.77	129.27	2.40

Manimoni									
25	Bor Tangeshi	<i>Oxalllis acetosella</i>	89.08	13.50	10.50	4.33	31.54	332.43	3.26
SED(±)			0.41	0.09	0.10	0.09	1.89	0.25	0.12
CD at 5 %			0.72*	0.15*	0.15*	0.20*	3.23*	0.42*	0.21*

The data of the present investigation revealed that Bonjaluk (26.10%) had the highest crude fibre content followed by Bhedailata (20.40%) and Puroi (green) (2.50%) had the lowest crude fibre content. This might be due to genetical, soil fertility, growth condition and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Ng *et al.* (2012) in *Etlingera elatior* (19.8 g/100 g, dry weight basis); Isong and Idiong (1997) found crude fibre content ranged from 8.50-20.90% in some Nigerian green leafy vegetables and Asaolu *et al.* (2012) found crude fibre content ranged between 4.02- 12.08% on dry weight basis.

4.4.4 Crude fat content

Food fat plays an important role in the human diet. Fats are vital for a healthy body, provide it with energy, contribute to the absorption of fat-soluble vitamins, and act as structural elements of cell walls. As a carrier of flavors and aromatic substances, fat also plays an important part in cooking. On the other hand, no other nutrient has to combat as many prejudices as fat. It is linked to obesity, type 2 diabetes, cancer, and coronary heart disease ([Lichtenstein et al., 1998](#)).

Dietary fats represent the most compact chemical energy available to man. They contain twice the caloric value of an equivalent weight of sugar. However dietary fats should not be thought of solely as providers of unwanted calories as fats are as vital to cell structure and biological function as protein. Dietary fats provide the essential linoleic acid which seems to have both a structural and functional role in animal tissue. However, leafy vegetables are not noted for contributing significantly to the fat supply in foods (Kummerow, 2007).

The data of the present investigation revealed that Puroi (red) (7.10%) had the highest crude fat content followed by Saru Tengesi (6.20%) and Bor Manimoni (1.13%) had the lowest crude fat content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Asaolu *et al.* (2012) in Bushbuck and *Amaranthus hybridus* contains 3.51 - 14.02% crude fat on dry weight basis and Kwenin *et*

al. (2011) reported that crude fat contents are 3.19%, 3.0%, 1.33% and 1.50% in *Xanthosoma sagittifolia*, *Amaranthus cruentus*, *Talinum triangulare* and *Moringa oleifera* respectively.

4.4.5 Crude protein content

Proteins are essential organic compounds of high molecular weight found in all living tissues which synthesize them at one time or another. They are formed from much similar building units called amino acids. Proteins may be categorized based on factors such as solubility and shape. They are broadly divided in two groups namely: simply and conjugated. Simple proteins consist of only amino acids as building blocks whiles conjugated proteins contain amino acids but in addition, a non-protein or prosthetic group which may be glycoprotein, lipoprotein, chromoprotein (Osei, 2003).

Protein helps in building and maintaining all tissues in the body, forms an important part of enzymes, fluids and hormones of the body. It helps form antibodies to fight infection and supplies energy (Johnson, 1996). How much protein is needed in a person's daily diet is determined in large part by overall energy intake, as well as by the body's need for nitrogen and essential amino acids. Physical activity and exertion as well as enhanced muscular mass increase the need for protein.

The data of the present investigation revealed that Dhekia (39.38%) had the highest crude protein content followed by Jilmil (36.76%) and Bor Manimoni (15.77%) had the lowest crude protein content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Ponka *et al.* (2005) in the three leafy vegetables viz. amaranth, nightshade and jute mallow were within the range of 20.48 - 41.66%; Asaolu *et al.* (2012) found crude protein content ranged between 66.60% to 46.56% on dry weight basis and Singh *et al.* (2001) found crude protein contents of coriander, bengal gram, spinach, cauliflower and amaranth leaves were 22.2, 26.2, 26.5, 29.9 and 26.2%, respectively.

4.4.6 Total free amino acid content

Amino acids build proteins and proteins are life-sustaining macronutrients. Yet simply calling amino acids the building blocks of protein doesn't do justice to their value. While some amino acids only make proteins, others fill a variety of roles, from supporting metabolism to protecting heart.

Protein is needed by every living organism, and next to water, makes up the largest portion of our body weight since it is contained in muscles, organs, hair, etc. The protein used in making up the body is not directly derived from diet, but the dietary protein is broken down into amino acids, and the body then re-constitutes these amino acids into the specific proteins needed. Enzymes and hormones regulating body functions are also proteins. And amino acids are used in most body processes from regulating the way the body works to how the brain functions - They activate and utilize vitamins and other nutrients (Sakami and Harrington, 1963).

The data of the present investigation revealed that Dhekia (992.00 mg %, *i.e.* mg per 100g dry wt. basis) had the highest total free amino acid content followed by Saru Manimoni (970.72 mg %) and Bor Manimoni (129.27 mg %) had the lowest total free amino acid content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are lower as compared to the results obtained by Laitonjam *et al.* (2013) in the leaves of *Phlogacanthus pubinervius* and *Phlogacanthus jenkinsii* were 5312.50 and 4140.75 mg/100 g, respectively.

4.4.7 Soluble protein content

Proteins are macromolecules that act as alternate energy source when other energy sources are in short supply. They are the building block of any organism. Protein is an essential part of the dietary needs of humans. The reason is that it fulfills a variety of important functions in the body. It is necessary for growth, maintenance and repair of cells and for the production of enzymes and hormones. Furthermore, [proteins](#) are the main components of muscle tissue and are vital to the internal organs, bones, skin and the transmission of impulses through the nerves.

The body's need for protein can be met with a wide variety of foods, from [animal sources](#) alone, from plant and animal sources or from specific plant-protein combinations. High

quality protein foods such as milk, eggs, meat, poultry and fish are the best sources of protein and will supply all the amino acids, a body will need for protein synthesis in a single food. But, fortunately these are not the only sources of dietary protein. Nuts, legumes, and grains such as wheat, rice and maize, potatoes and leafy vegetables provide lesser quality protein that is an important source of all amino acids. But they do have an important advantage over animal proteins – as plants they do not contain cholesterol at all and when they do have fat, it is in the form of unsaturated oil which is healthier than animal fat.

The data of the present investigation revealed that Pirali paleng (4.13%) had the highest soluble protein content followed by Madhuholeng (3.51%) and Mandhania (1.10%) had the lowest soluble protein content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Lin *et al.* (1986) in the leaf of four cultivars of sweet potato Tainong 65, HP-4, Chang-hua and Tainong 25 contains total soluble protein 6.79 g/100 g, 5.8 g/100 g, 5.02 g/100 g and 4.25 g/100 g respectively and Alfawaz (2006) found that the soluble protein value of hummayd was 17.1-20.1 g/100 g.

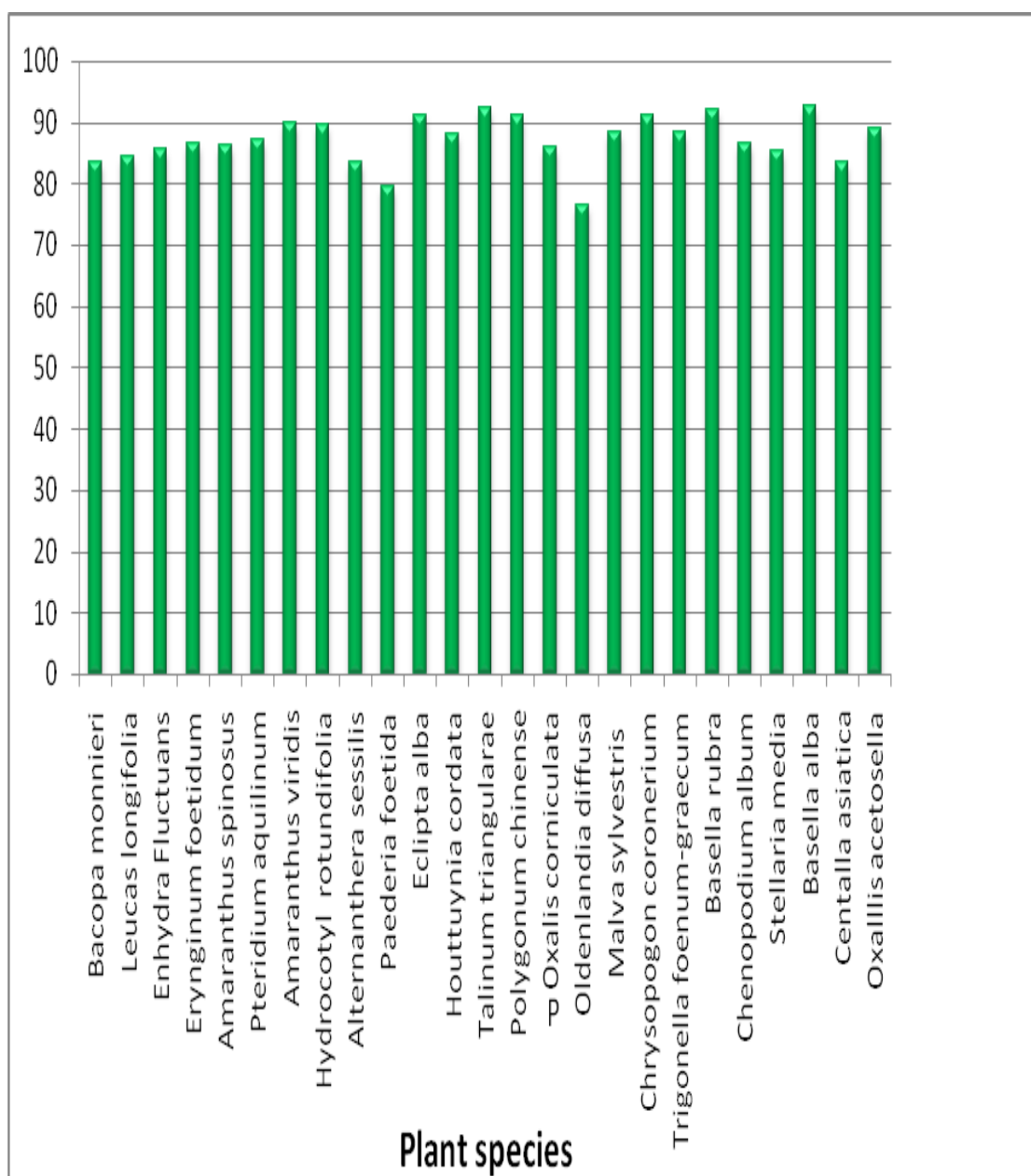


Fig. 2. Moisture content (gram percentage) of indigenous leafy vegetables

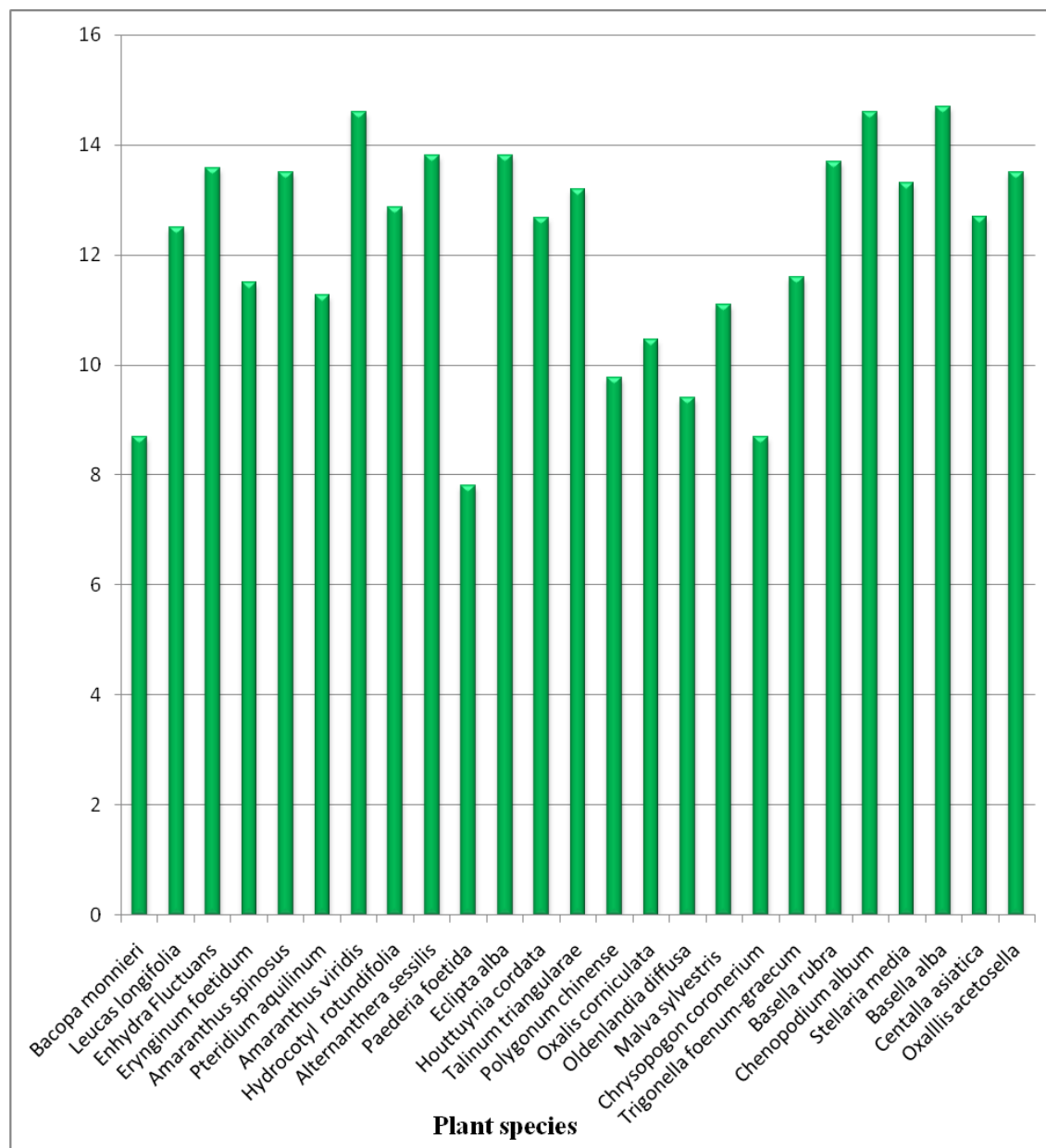


Fig. 3. Ash content (gram percentage) of indigenous leafy vegetables

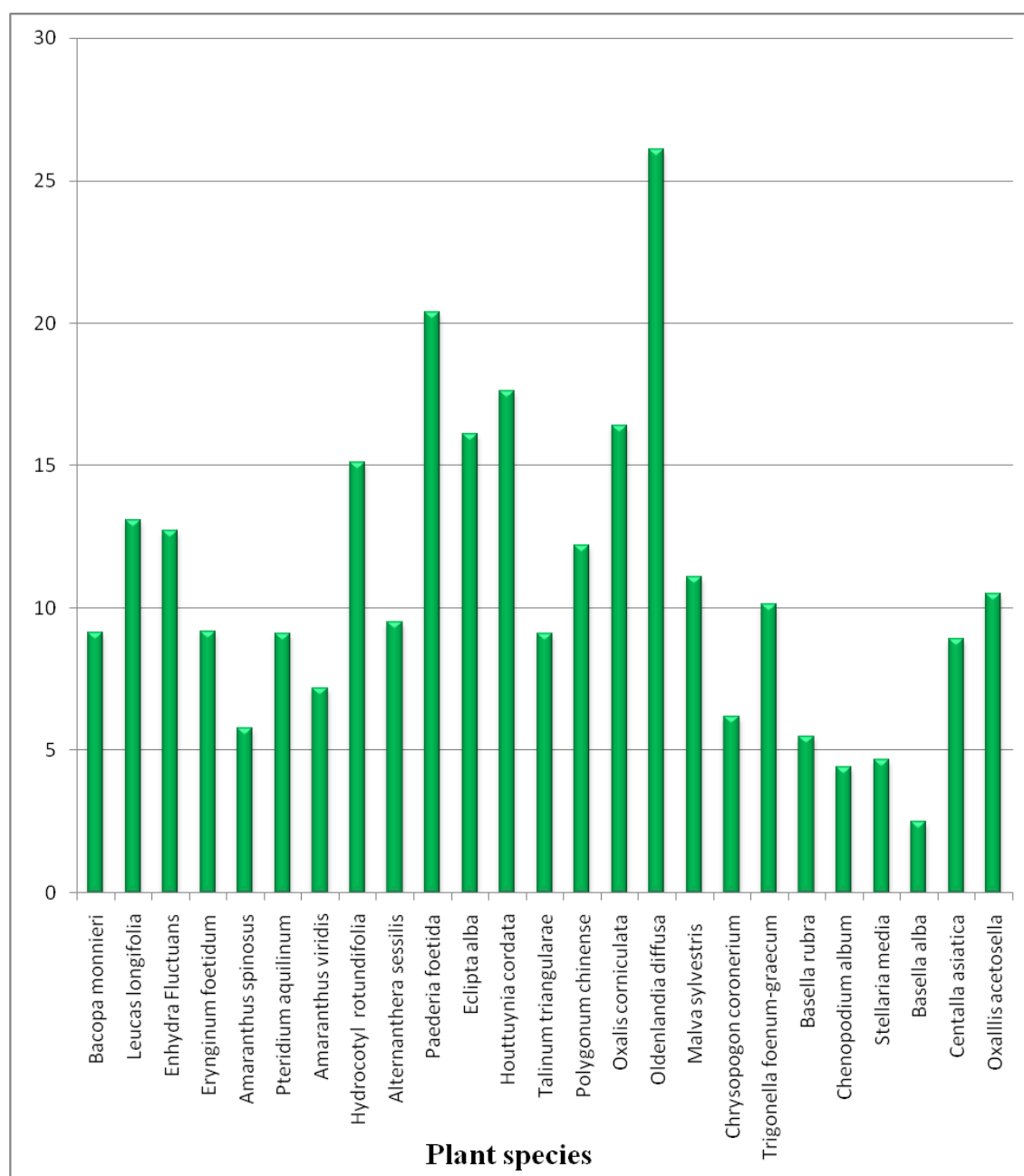


Fig. 4. Crude fibre content (gram percentage) of indigenous leafy vegetables

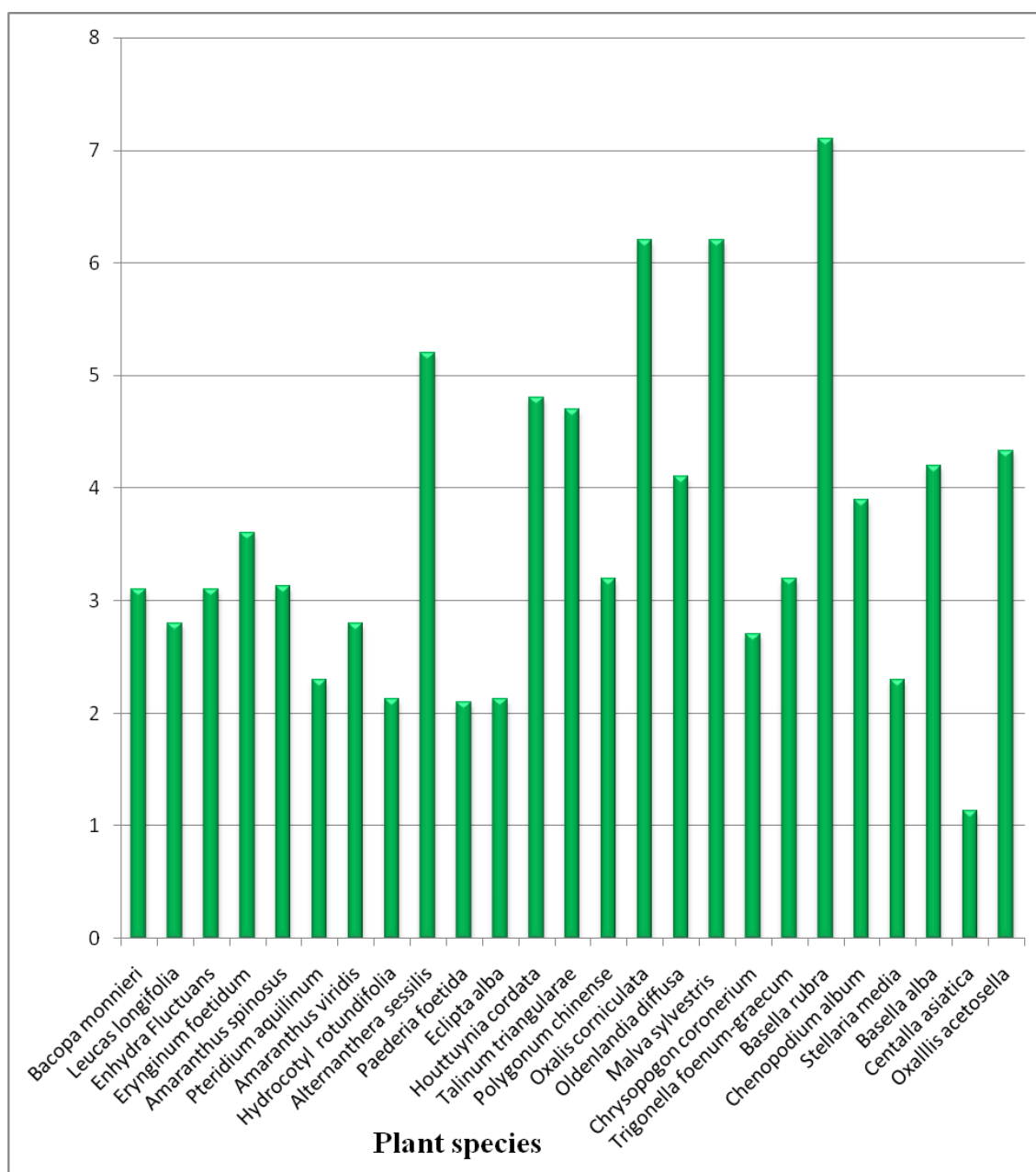


Fig. 5. Crude fat content (gram percentage) of indigenous leafy vegetables

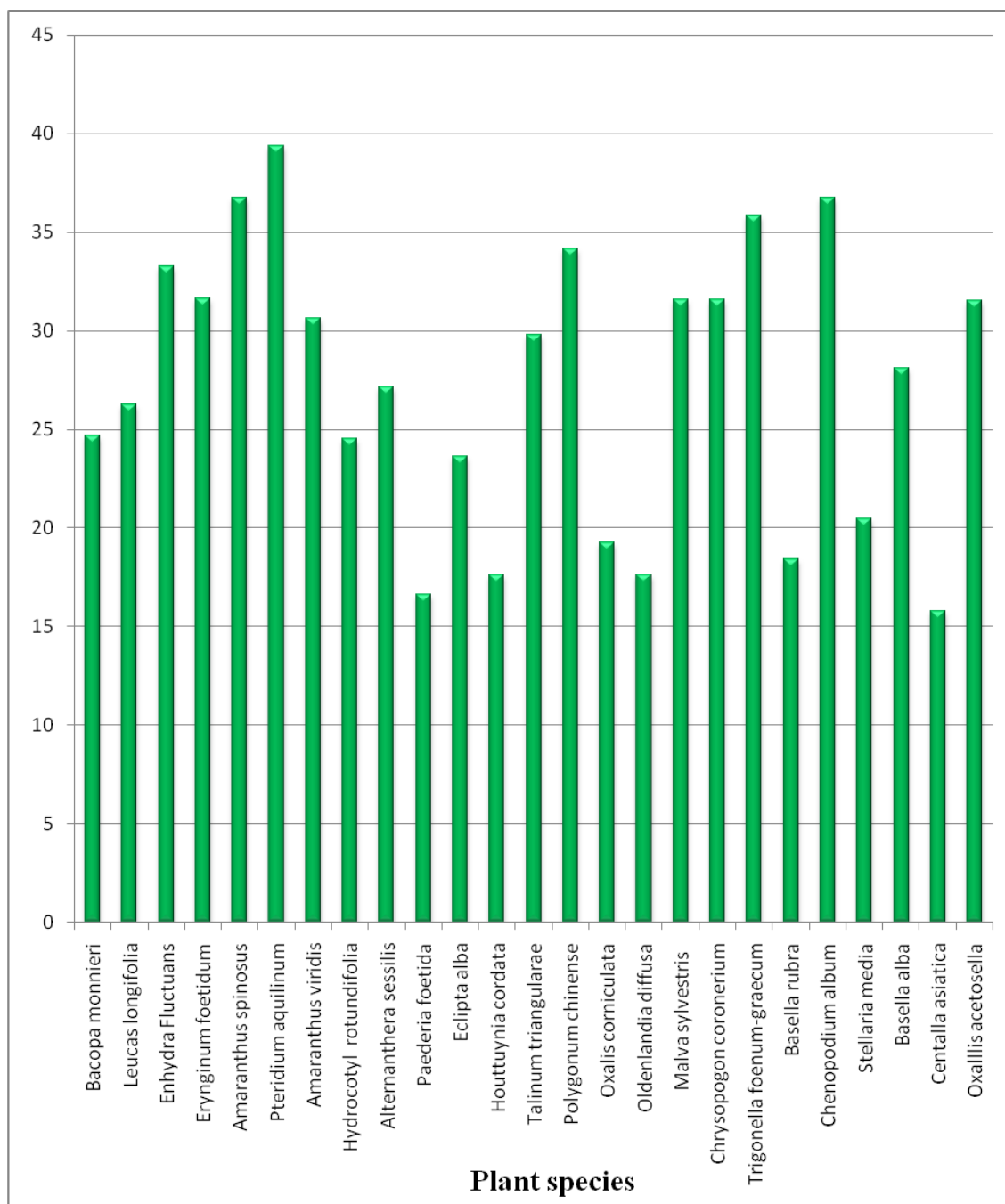


Fig. 6. Crude protein content (gram percentage) of indigenous leafy vegetables

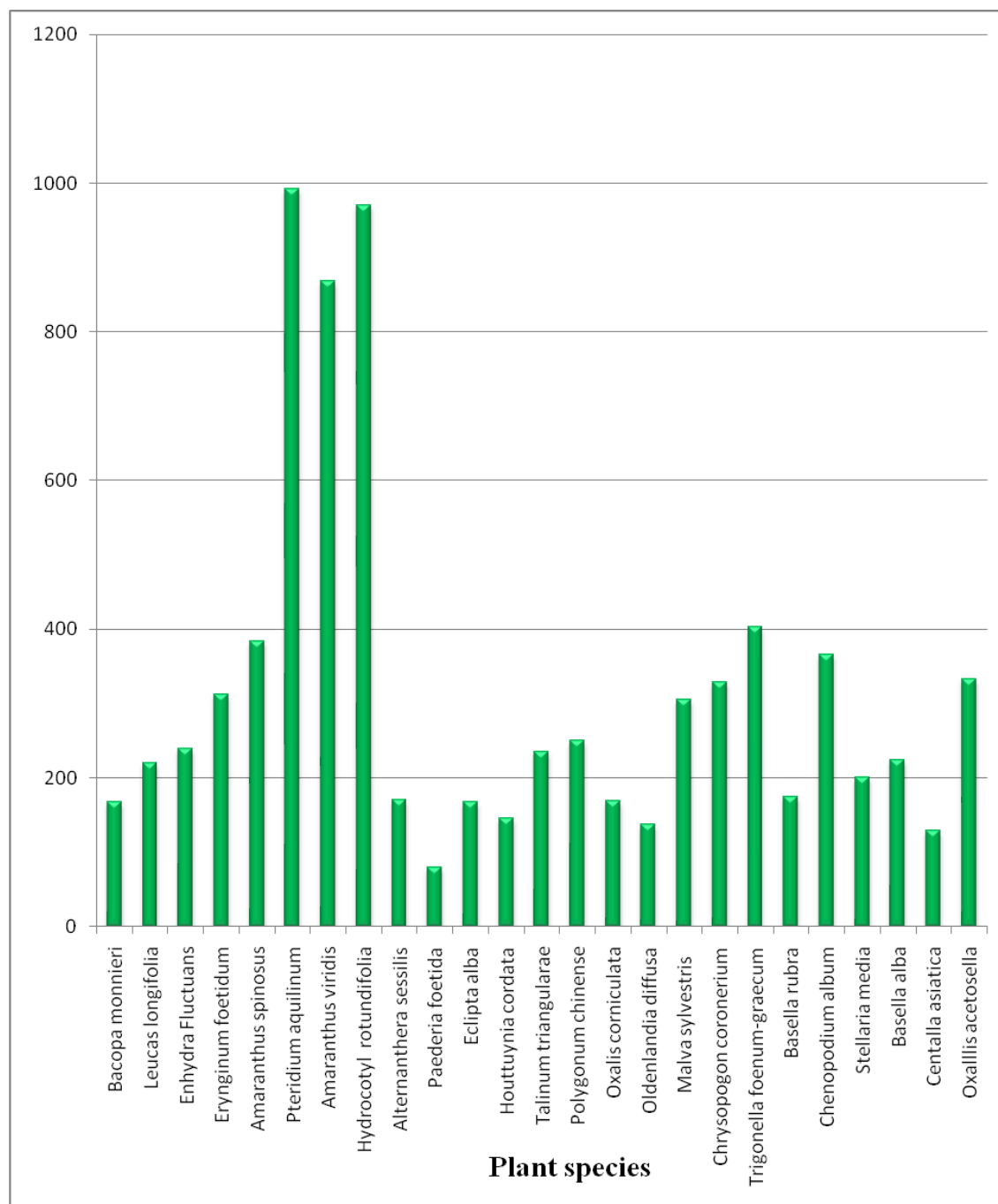


Fig. 7. Total free amino acid content (milli gram percentage) of indigenous leafy vegetables

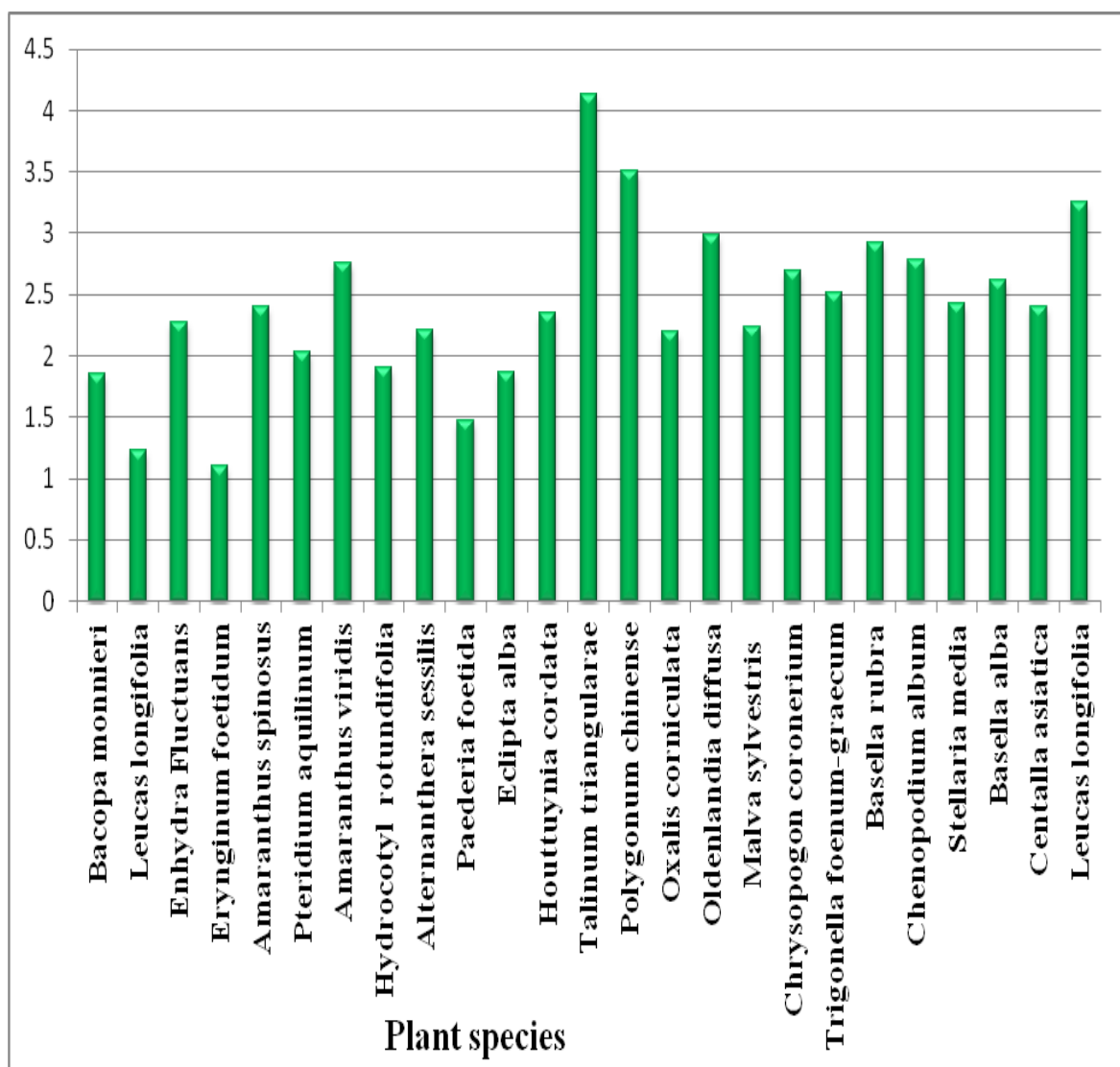


Fig. 8. Soluble protein content (gram percentage) of indigenous leafy vegetables

4.5 Mineral content

4.5.1 Macro nutrient content of indigenous leafy vegetables

The data of phosphorous content, potassium content, sodium content and calcium content of different leafy vegetables are presented in the Table 6.

4.5.1.1 Phosphorous content

Phosphorus is a part of every cell of the body and is vitally concerned with many metabolic processes, including those involving the buffers in body fluids (Hays and Swenson, 1985). It functions as a constituent of bones, teeth, adenosine triphosphate (ATP), phosphorylated metabolic intermediates and nucleic acids. It aids the buffering system (phosphate buffers, functions in the formation of high energy compounds), and is involved in the synthesis of phospholipids and phosphoproteins. Practically, every form of energy exchange inside living cells involves the forming or breaking of high-energy bonds that link oxides of phosphorus to carbon or to carbon-nitrogen compounds.

The data of the present investigation revealed that Madhuholang (603.00 mg %) had the highest phosphorous content followed by Saru Tengesi (142.00 mg %) and Bor Tangeshi (38.00 mg %) had the lowest phosphorous content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Kamga *et al.* (2013) which varies from 420 mg to 540 mg/100 g on dry weight basis in few green leafy vegetables; Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain 518 mg/100 g of phosphorous and Caunii *et al.* (2010) reported that the phosphorous content of in four green leafy vegetables varies from 23 mg to 66 mg/100 g on dry weight basis.

4.5.1.2 Potassium content

Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na^+/K^+ -ATPase. Potassium is also required during glycogenesis. It also helps in the transfer of phosphate from ATP to pyruvic acid

and probably has a role in many other basic cellular enzymatic reactions. Its metabolism is regulated by aldosterone. Hyperkalaemia is increased level in serum potassium and this occurs in Addison's disease, advanced chronic renal failure, shock and dehydration (Soetan *et al.*, 2010). Toxicity disease or symptoms include dilatation of the heart, cardiac arrest, small bowel ulcers. Hypokalaemia is low level of serum potassium and this occurs in diarrhoea, metabolic alkalosis and familial periodic paralysis. Deficiency disease or symptoms occurs secondary to illness, functional and structural abnormalities including impaired neuromuscular functions of skeletal, smooth, and cardiac muscle, muscular weakness, paralysis, mental confusion (Others are cardiac arrhythmias, impaired carbohydrate tolerance, altered electrocardiogram in calves. Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, and also causes alterations of gastric secretions and intestinal motility.

Potassium is a key circulating electrolyte which is also involved in the regulation of ATP dependent channels along with sodium. Sodium and potassium maintains osmotic and water balance as well as membrane potentials. The Na/K ratio in the body is of great concern for prevention of high blood pressure. Na/K ratio less than one is recommended (Akubugwo *et al.*, 2007). Regular consumption of these leafy vegetables may assist in preventing hypertension and cardiovascular diseases, as dietary potassium is an important cation in regulating blood pressure and attenuating platelet reactivity, which is the major causative factor of vascular occlusion (He and Mac Gregor, 2008). Furthermore, consumption of wild vegetables with high potassium content enhances the bioavailability of calcium in body and promotes bone health by preventing the occurrences of calciuria.

The data of the present investigation revealed that pirali paleng (9.58%) had the highest potassium content followed by helosi sak (6.06%) and Puroi (red) (0.64%) had the lowest potassium content. The results are slightly higher than obtained by other authors. This might be due to genetical, soil fertility and environmental conditions of the plant species. There is a significant difference among the plant species. These results are comparable with results obtained by Grace *et al.* (4.90 to 6.11%) (2013) and Kamga *et al.* (3.08 to 5.86%) (2013).

Table 6. Macro nutrient content (on dry weight basis) of indigenous leafy vegetables (g %)

Sl. No.	Local name	Botanical name	P (mg%)	K	Na (mg%)	Ca
1	Brahmi	<i>Bacopa monnieri</i>	91.00	1.09	231.00	0.20
2	Doron	<i>Leucas longifolia</i>	119.00	3.87	132.67	1.20
3	Helosi sak	<i>Enhydra fluctuans</i>	92.00	6.06	233.00	0.90
4	Mandhania	<i>Erynginum foetidum</i>	119.67	4.54	156.33	0.80
5	Hatikhotora	<i>Amaranthus spinosus</i>	129.00	5.28	132.00	1.30
6	Dhekia	<i>Pteridium aquilinum</i>	130.67	4.39	92.00	0.30
7	Khotora	<i>Amaranthus viridis</i>	100.00	3.69	126.33	1.20
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	95.00	4.02	116.33	1.50
9	Matikandori	<i>Alternanthera sessilis</i>	143.33	6.34	155.00	0.70
10	Bhedailata	<i>Paederia foetida</i>	102.67	0.70	52.00	2.10
11	Keheraj	<i>Eclipta alba</i>	78.00	3.58	105.00	1.00
12	Masundori	<i>Houttuynia cordata</i>	109.67	3.56	116.67	1.00
13	Pirali paleng	<i>Talinum triangularae</i>	86.00	9.58	186.00	0.40
14	Madhuholang	<i>Polygonum chinense</i>	603.00	3.32	92.33	1.20
15	SaruTengesi	<i>Oxalis corniculata</i>	142.00	3.76	136.00	0.70
16	Bonjaluk	<i>Oldenlandia diffusa</i>	134.00	1.27	101.33	1.20
17	Laffa	<i>Malva sylvestris</i>	120.67	3.66	102.00	1.50
18	Baburi	<i>Chrysopogon coronarium</i>	104.33	4.93	186.33	0.80
19	Methi	<i>Trigonella foenum-graecum</i>	62.67	2.50	186.00	1.10
20	Puroi (red)	<i>Basella rubra</i>	115.00	0.64	172.00	1.50
21	Jilmil	<i>Chenopodium album</i>	129.00	5.62	153.33	1.70
22	Morolia	<i>Stellaria media</i>	108.33	2.26	92.00	1.40
23	Puroi (green)	<i>Basella alba</i>	108.00	0.86	194.67	1.50
24	Bor Manimoni	<i>Centalla asiatica</i>	95.00	1.66	82.33	1.00
25	Bor Tangesi	<i>Oxallis acetosella</i>	38.00	2.24	92.67	0.80
SED(±)			1.32	0.10	1.65	0.41
CD at 5 %			2.27*	0.027*	2.82*	0.70*

4.5.1.3 Sodium content

Sodium is a mineral. In the body, it carries an electrical charge and a charged mineral is called an electrolyte. The body regulates the level of sodium in the body through numerous interacting processes because the concentration must remain in a narrow range. If sodium levels deviate too high or too low, it causes problems in the body. Sodium is important for fluid distribution, blood pressure, cellular work and electrical activity. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Okon and Akpanyung, 2005).

Sodium atoms carry a strong positive electrical charge. They dissolve in the body fluids, which are mostly water. Water does not carry a charge, but it is a polar molecule. This means that it has a positive and a negative side. Water follows sodium in the body. If the concentration of sodium increases in a tissue, the fluid there increases as well. In this way, concentrations of sodium control the distribution of fluids in the body. This is important for ensuring that cells and tissues do not swell or shrink.

The data of the present investigation revealed that Helosi sak (233.00 mg %) had the highest sodium content followed by Brahmi (231.00 mg %) and Bhedailata (52.00 mg %) had the lowest sodium content. This might be due to genetical, soil fertility and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Gupta *et al.* (2005) in *Celosia argentea* (240.6 mg %) and *Centella asiatica* had (107.8 mg %) and Zeghichi *et al.* (2003) in *S. pecten-veneris* (381 mg % and 662 mg %) in Pakistan and Greece respectively.

4.5.1.4 Calcium content

Calcium is an important constituent of bones and teeth and is involved in regulation of nerve and muscle function. In blood coagulation, calcium activates the conversion of pro-thrombin to thrombin. It plays a vital role in enzyme activation. Calcium activates large number of enzymes such as adenosine triphosphatase (ATPase), succinic dehydrogenase and lipase. It is also required for membrane permeability, involved in muscle contraction, normal transmission of nerve impulses and in neuromuscular excitability (Soetan *et al.*, 2010). Reduced extracellular blood calcium increases the irritability of nerve tissue, and very low levels may

cause spontaneous discharges of nerve impulses leading to tetany and convulsions (Hays and Swenson, 1985). Calcium absorption requires calcium-binding proteins and is regulated by vitamin D, sunlight, parathyroid hormone and thyrocalcitonin. Thyrocalcitonin decreases plasma calcium and phosphate levels whereas parathyroid hormone increases them.

In children, calcium deficiency causes rickets due to insufficient calcification by calcium phosphate of the bones in growing children. The bones therefore remain soft and deformed by the body weight. In adults, it causes osteomalacia, a generalized demineralization of bones. It may also contribute to osteoporosis, a metabolic disorder resulting in decalcification of bone with a high incidence of fracture, that is, a condition where calcium is withdrawn from the bones and the bones become weak and porous and then breaks (Hays and Swenson, 1985; Malhotra, 1998; Murray *et al.*, 2000). Calcium deficiency also affects the dentition of both children and adult. Toxicity symptoms occur with excess absorption due to hypervitaminosis D or hypercalcaemia due to hyperparathyroidism, or idiopathic hypercalcaemia.

Excess calcium depresses cardiac activity and leads to respiratory and cardiac failure; it may cause the heart to stop in systole, although, normally, calcium ions increase the strength and duration of cardiac muscle contraction. Excess calcium and phosphorus are excreted by the kidney. Ca and P excreted in faeces are largely the unabsorbed dietary minerals; some comes from the digestive juices, including bile (Hays and Swenson, 1985). Growing, pregnant and especially lactating humans and animals require liberal amounts of calcium and phosphorus. Parturient paresis, or milk fever, in cows is associated with calcium metabolism. This illness usually occurs with the onset of profuse lactation and the most common abnormality is acute hypocalcaemia with decline in blood calcium level from normal. Serum magnesium levels may be elevated or depressed, low levels being accompanied by tetany and high levels by a flaccid paralysis.

The data of the present investigation revealed that Bhedailata (2.10%) had the highest calcium content followed by Jilmil (1.70%) and Brahmi (0.20%) had lowest calcium content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Grace *et al.* (2013) in *Chenopodium album* and *Solanum nigrum* 18.21 g/100 g and 16.89 g /100 g of calcium respectively and Kamga *et al.* (2013) reported that calcium content varies from 1.15 g to 2.69 g/100 g in three leafy vegetables.

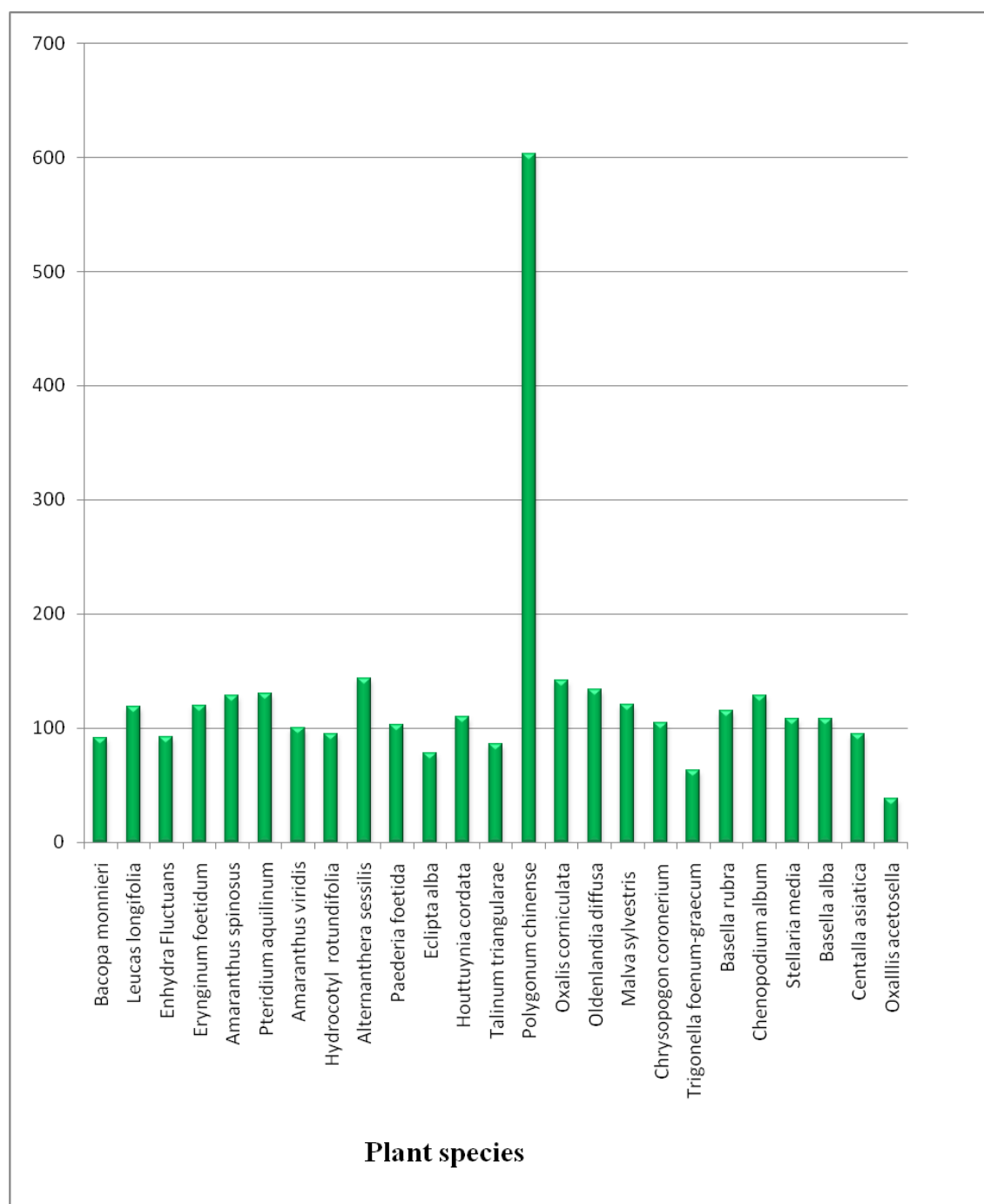


Fig. 9. Phosphorous content (milli gram percentage) of indigenous leafy vegetables

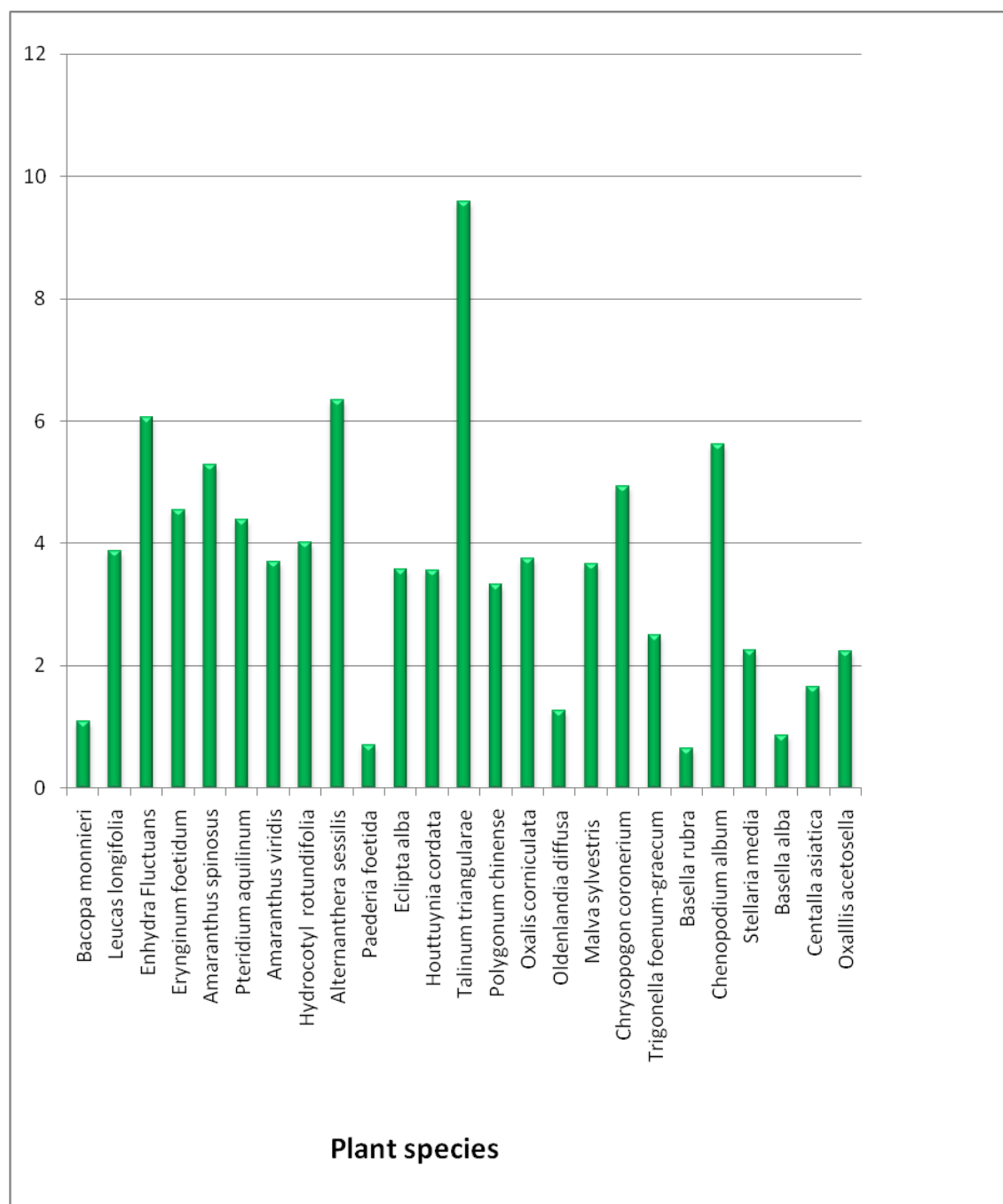


Fig. 10. Potassium content (gram percentage) of indigenous leafy vegetables

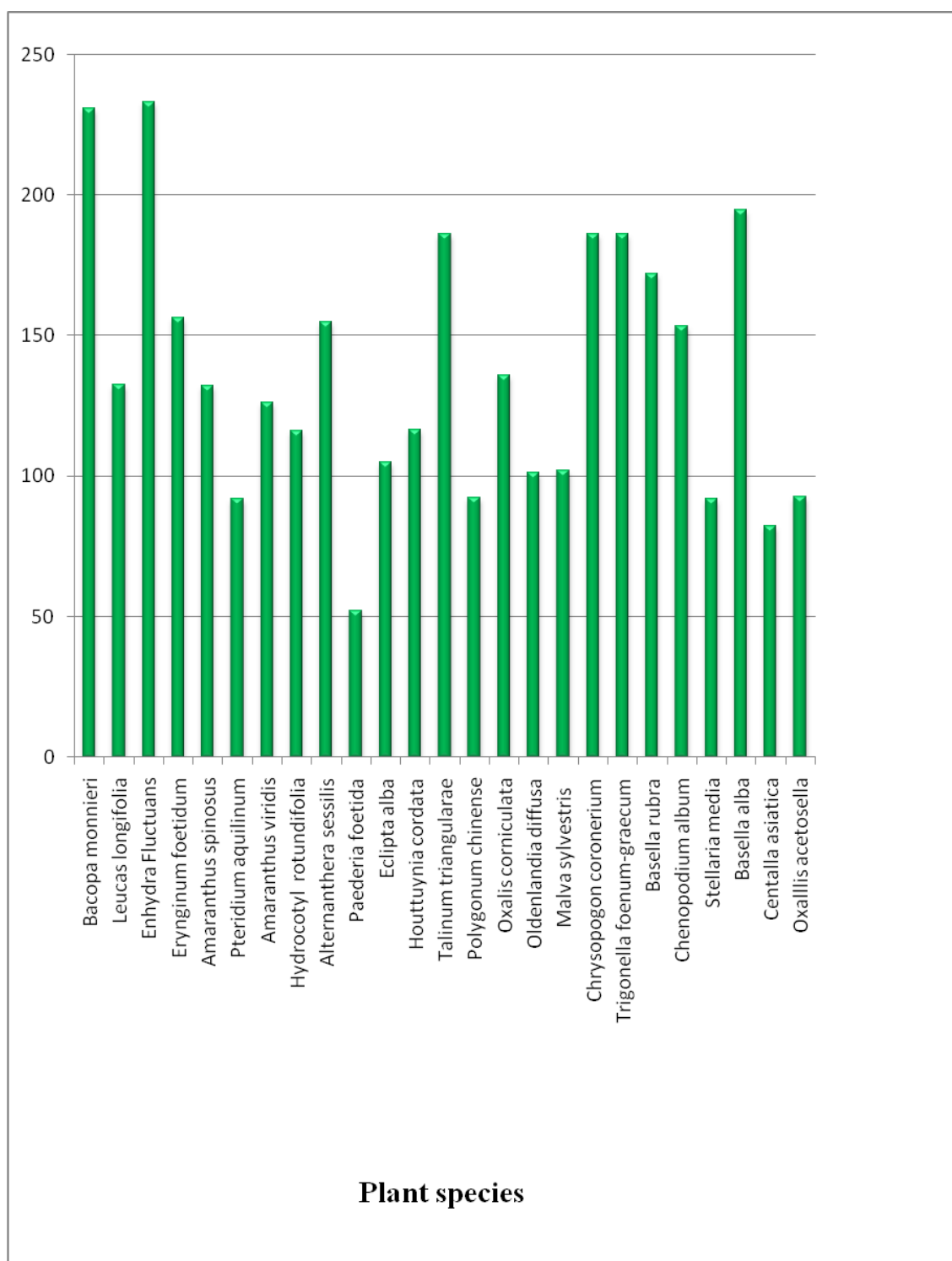


Fig. 11. Sodium content (milli gram percentage) of indigenous leafy vegetables

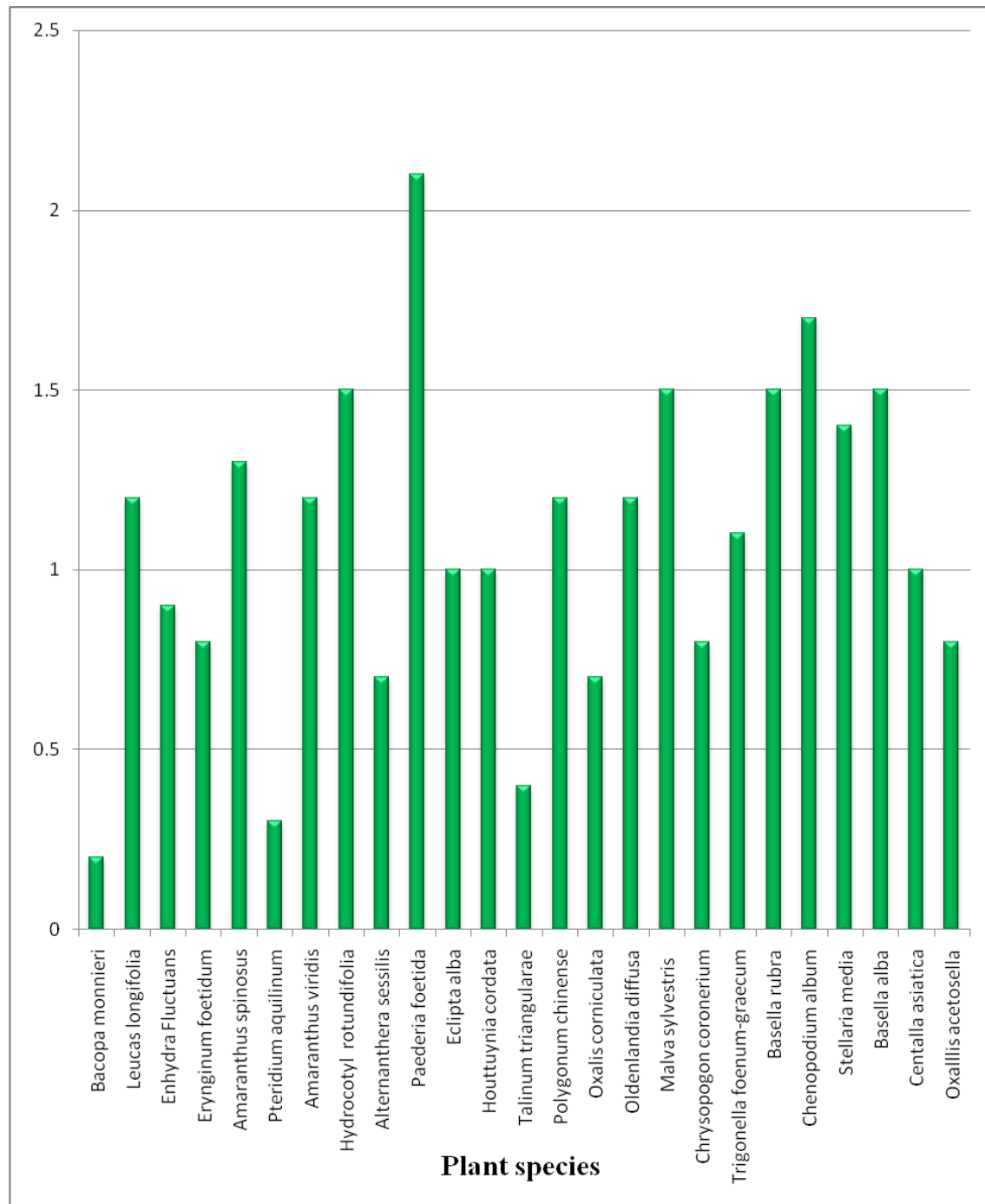


Fig. 12. Calcium content (gram percentage) of indigenous leafy vegetables

4.6 Micro nutrient content of indigenous leafy vegetables (mg %)

The data of iron content, copper content, zinc content, manganese content and arsenic content of different leafy vegetables are presented in the Table 7.

4.6.1 Iron content

Iron functions as haemoglobin in the transport of oxygen. In cellular respiration, it functions as essential component of enzymes involved in biological oxidation such as cytochromes C, C1, and A1 (Malhotra, 1998). Iron is an important constituent of succinate dehydrogenase as well as a part of the haeme of haemoglobin (Hb), myoglobin and the cytochromes (Chandra, 1990). Iron is required for proper myelination of spinal cord and white matter of cerebellar folds in brain and is a cofactor for a number of enzymes involved in neurotransmitter synthesis (Soetan *et al.*, 2010).

Iron is involved in synthesis and packaging of neurotransmitters, their uptake and degradation into other iron-containing proteins which may directly or indirectly alter brain function. Iron exists in the blood mainly as haemoglobin in the erythrocytes and as transferrin in the plasma. It is transported as transferrin; stored as ferritin or haemosiderin and it is lost in sloughed cells and by bleeding. Iron is required for making Hb and it is a pro-oxidant which is also needed by microorganisms for proliferation. Biologically important compounds of iron are haemoglobin, myoglobin, cytochromes, catalases and peroxidase. Factors affecting the absorption of iron are: low phosphate diet which increases iron absorption, whereas high phosphate diet decreases iron absorption by forming insoluble iron phosphates.

Excessive accumulation of iron in the liver, pancreas, heart, lungs and other tissues cause haemosiderosis and when this is accompanied by bronze pigmentation of the skin, the condition is called haemochromatosis (Malhotra, 1998; Murray *et al.*, 2000). Sources include red meat, spleen, heart, liver, kidney, fish, egg yolk, nuts, legumes, molasses, iron cooking ware, dark green leafy vegetables (Soetan *et al.*, 2010). A study by Mepba *et al.* (2007) indicated that blanching and cooking significantly ($P \leq 0.05$) decreased levels of iron in the test vegetables.

Iron has a critical role in the transport of oxygen. Iron coordinates the oxygen molecule into heme of haemoglobin so that it can be transported from lungs to the tissues. Therefore, over 65% of body iron is found in haemoglobin. One-third of body iron is a storage

form that can be mobilised during times of need. Non-heme sources of iron are found in plant and vegetable products and the absorption from these sources is generally lower and influenced to a greater extent by total diet composition. Vitamin C is the most significant enhancer of non-heme iron absorption, while plant phenolics such as tannins and phytates are some of the most potent inhibitors (Grace *et al.*, 2013).

Iron is an important trace element in the human body, it plays crucial roles in haemopoiesis, control of infection and cell mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Dioxin *et al.*, 2004).

The data of the present investigation revealed that Khotora (401.00 mg %) had the highest iron content followed by Matikandori (139.00 mg %) and Helosi sak, Hatikhutora, Dhekia and Puroi (red) (31.00 mg %) had the lowest iron content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Grace *et al.* (2013) in *Chenopodium album* and *Solanum nigrum* contains 120.4 mg/100 g and 177.1/100 g of iron on dry weight basis respectively; Singh *et al.* (2001) found total iron content of selected green leafy vegetables ranged from 22.3 to 84.4 mg/100 g on a dry matter basis and Kamga *et al.* (2013) reported that iron content varies from 3.34 mg to 38.79 mg/100 g on dry weight basis in three leafy vegetables.

4.6.2 Copper content

Copper is involved in the formation of [red blood cells](#), the absorption and utilization of iron, the metabolism of [cholesterol](#) and [glucose](#), and the synthesis and release of life-sustaining [proteins](#) and [enzymes](#). These enzymes in turn produce cellular energy and regulate nerve transmission, blood clotting, and oxygen transport. Copper stimulates the [immune system](#) to fight [infections](#), to repair injured tissues, and to promote healing. Copper also helps to neutralize "[free-radicals](#)", which can cause severe damage to cells.

Copper is involved in the formation of red blood cells, the synthesis of hemoglobin, and formation of bone. Copper has a role in energy production, wound healing, taste sensation, skin and hair color. Copper and zinc are both involved in catalytic or structural roles of many different enzymes (Grace *et al.*, 2013).

The data of the present investigation revealed that Halosi sak, Madhoholeng (0.020%) had the highest copper content followed by Khotora (0.01%) and Doron (0.003%) had the lowest copper content. This might be due to genetical, soil fertility, growth condition and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Grace *et al.* (2013) in *Chenopodium album* and *Solanum nigrum* contains 9.1 mg/100 g and 11.2/100 g of copper on dry weight basis respectively; Singh *et al.* (2001) reported that copper content of green leafy vegetables ranged from 0.8 to 1.0 mg/100 g and Zeghichi *et al.* (2003) reported that *S. pecten-veneris* contain copper 54 and 1.71 mg/100 g in Pakistan and Greece respectively.

4.6.3 Zinc content

Zinc is found as a co-factor in over 300 different enzymes including antioxidant enzymes. Zinc has a role in the regulation blood glucose levels via insulin function. Zinc also promotes wound healing, regulates immune function, and is necessary for protein synthesis. Regular consumption of these green leafy vegetables may assist in preventing the adverse effects of zinc deficiency such as growth retardation. Recommended Daily Allowance (RDA) for zinc is 11 mg/day (Grace *et al.*, 2013). Zinc is an essential micronutrient for human growth and immune functions (Black, 2003).

The data of the present investigation revealed that Puroi (green) (7.55 mg %) had the highest zinc content followed by Keheraj (6.29 mg %) and Bhedailata (0.013 mg %) had the lowest zinc content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Grace *et al.* (2013) in *Chenopodium album* and *Solanum nigrum* contains 23.0 mg/100 g and 26.1 mg/100 g of zinc on dry weight basis respectively; Kamga *et al.* (2013) reported that zinc content varies from 0.021 mg to 0.102 mg/100 g on dry weight basis in three leafy vegetables and Afolayan *et al.* (2009) reported that zinc content of four green leafy vegetables ranged from 4.2 to 5.5 mg/100 g.

Table 7. Micro nutrient content on dry weight basis of indigenous leafy vegetables (mg %)

Sl. No.	Local name	Botanical name	Fe	Cu	Zn	Mn	As
1	Brahmi	<i>Bacopa monnieri</i>	51	0.016	0.02	35.67	1.20
2	Doron	<i>Leucas longifolia</i>	151	0.003	0.046	44.00	0.27
3	Helosi sak	<i>Enhydra fluctuans</i>	31	0.020	0.055	39.67	0.30
4	Mandhania	<i>Erynginum foetidum</i>	51	0.009	0.015	24.00	0.60
5	Hatikhotora	<i>Amaranthus spinosus</i>	31	0.019	0.019	59.00	0.00
6	Dhekia	<i>Pteridium aquilinum</i>	31	0.043	0.016	27.67	0.00
7	Khotora	<i>Amaranthus viridis</i>	401	0.010	0.009	76.33	0.80
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	71	0.015	0.077	55.00	0.6
9	Matikandori	<i>Alternanthera sessilis</i>	139	0.016	3.73	12.00	0.00
10	Bhedailata	<i>Paederia foetida</i>	41	0.007	0.013	25.00	1.20
11	Keheraj	<i>Eclipta alba</i>	51	0.015	6.29	72.33	0.00
12	Masundori	<i>Houttuynia cordata</i>	91	0.013	0.088	82.00	0.00
13	Pirali paleng	<i>Talinum triangularae</i>	51	0.016	0.062	84.67	0.60
14	Madhuholang	<i>Polygonum chinense</i>	41	0.020	0.016	48.33	0.30
15	SaruTengesi	<i>Oxalis corniculata</i>	61	0.005	0.015	48.00	0.00
16	Bonjaluk	<i>Oldenlandia diffusa</i>	91	0.007	0.074	50.33	0.70
17	Laffa	<i>Malva sylvestris</i>	51	0.013	0.009	32.00	0.30
18	Baburi	<i>Chrysopogon coronarium</i>	221	0.013	0.019	132.00	0.77
19	Methi	<i>Trigonella foenum-graecum</i>	51	0.004	0.060	70.00	0.80
20	Puroi (red)	<i>Basella rubra</i>	31	0.006	0.018	13.33	0.00
21	Jilmil	<i>Chenopodium album</i>	91	0.015	0.031	75.67	0.70
22	Morolia	<i>Stellaria media</i>	121	0.006	6.08	41.00	0.00
23	Puroi (green)	<i>Basella alba</i>	106	0.013	7.55	70.00	1.70
24	Bor Manimoni	<i>Centalla asiatica</i>	101	0.005	0.025	68.67	0.00
25	Bor Tangeshi	<i>Oxalllis acetosella</i>	151	0.011	0.022	69.00	0.00
SED(±)			0.84	0.003	0.02	2.28	0.06

4.6.3 Manganese content

Very low intakes of manganese are associated with poor bone formation. This phenomenon is thought to be due to altered formation of the protein matrix that keeps minerals like calcium in place. Manganese is a required co-factor for an enzyme called *prolidase*, which is in turn necessary to make collagen as a structural component of skin. It is also a co-factor for an enzyme called manganese superoxide dismutase (MnSOD), which is a potent antioxidant associated with protection against ultra violet damage. Manganese is needed to help multiple enzymes in a process called gluconeogenesis. This is the process by which we build non-carbohydrate food products, for example, digested fats into sugars to burn as fuel (Gunter *et al.*, 2013).

Manganese is a co-factor for an enzyme called manganese superoxide dismutase (MnSOD), which is a potent antioxidant associated with protection against free radical damage. Diets low in manganese have been linked to conditions marked by increased oxidative stress, including skin problems and asthma (Treiber *et al.*, 2012).

The data of the present investigation revealed that Baburi (132.00 mg %) had the highest manganese content followed by Pirali paleng (84.67 mg %) and Matikandori (12.00 mg %) had lowest manganese content. This might be due to genetical, soil fertility and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Afolayan *et al.* (2009) in four green leafy vegetables ranged from 8.3 to 11.8 mg/100 g; Singh *et al.* (2001) in green leafy vegetables ranged from 1.8 to 10.2 mg/100 g and Asaolu *et al.* (2012) in in some green leafy vegetables ranges between 2.54 mg/100 g and 10.06 mg/100 g.

4.6.4 Arsenic content

Elements such as lead, chromium, cadmium and arsenic are likely to have cumulative side effects when large quantity of the leaves has been consumed. These elements could also affect the young nervous system of the child thereby adversely affecting the intellectual development and subsequent behaviour of the child (Grace *et al.*, 2013). The major arsenic species found in environment were arsenite, arsenate, arsenious acid, arsenic acid, monomethylarsenic acid (MMA), dimethylarsenic acid (DMA), arsenobetaine (AB) and arsenocholine (AC). Inorganic arsenic is the predominant form in the soil and is toxic to the plant. Methylated form of MMA and DMA is also present due to photooxidation and microbial biotransformation in the soil.

High arsenic irrigated water and soil appears to result in higher concentration of arsenic in root, stem and leaf of rice plants (Abedin *et al.*, 2002). Meharg *et al.* (2003) observed that both As (III) and MMA are phytotoxic to rice plants grown on nutrient solutions and the degree of arsenic uptake by rice followed as As (III)>MMA>As(V)>DMA. Arsenic is a non-essential element but phosphate is an essential element for the plant. Arsenic exists in environment in the form of organic and inorganic (WHO, 2001) of which inorganic is more acutely toxic.

Inorganic arsenic further exists in environment in the forms e.g As (III), (AsV). Phosphate is the 3rd most nutrient element for the plant; it is also necessary for energy transfer, photosynthesis, root development and flowering. Inorganic phosphate resembles pentavalent arsenic. Uptake of pentavalent arsenic is inhibited by the phosphate because they compete for the same sorption site and share the same physio-chemical properties.

The vegetables are important food crops of Assam. But vegetables that are grown in arsenic contaminated soil, people unknowingly consume such arsenic contaminated crops and become exposed to arsenic toxicity. To prevent the toxicity several measures or techniques are suggested to minimize arsenic mobility in the soil and uptake by the plants. There are many competing ions, which may alleviate or reduce the arsenic accumulation by the plants. Phosphate is one of such competing ions, which is used as a fertilizer in irrigated field, which may compete with arsenic for sorption site and accumulate in vegetable for nutrition purpose. The unknowing

ingestion of a toxic substance like arsenic may be hazardous to human health. Long time exposure of low doses of arsenic causes chronic toxicity.

In January 2006, the U.S. Environmental Protection Agency (EPA) set a maximum contaminant level (MCL) for arsenic in drinking water at 10 ppb. The EPA Reference Dose (RFD) for inorganic arsenic is 0.0003 milligrams per kilogram body weight per day (mg/kg/day). The Agency for Toxic Substances and Disease Registry's (ATSDR) Minimal Risk Level (MRL) for acute oral ingestion is 0.005 mg/kg/day and chronic oral ingestion is 0.0003 mg/kg/day.

The data of the present investigation revealed that Puroi (green) (1.70 mg %) had the highest arsenic content followed by Brahmi, Bhedailata (1.20 mg %) and Hatikhotura, Dhekia, Matikandori, Keheraj, Masundari, Saru tengeshi, Puroi (red), Morolia, Bor Manimoni, Bor tengeshi (0.0 mg %) had the lowest arsenic content. This might be due to genetical, soil fertility and environmental conditions of the plant species. There is a significant difference among the plant species. The results are little higher as compared to the results obtained by Jahanara (2007) in few green leafy vegetables ranges between 0.047 to 0.324 mg/100 g and are in agreement with the results obtained by Grace *et al.* (2013) in *Chenopodium album* and *Solanum nigrum* contains 1.8 mg/100 g and 1.9 mg/100 g of arsenic on dry weight basis respectively.

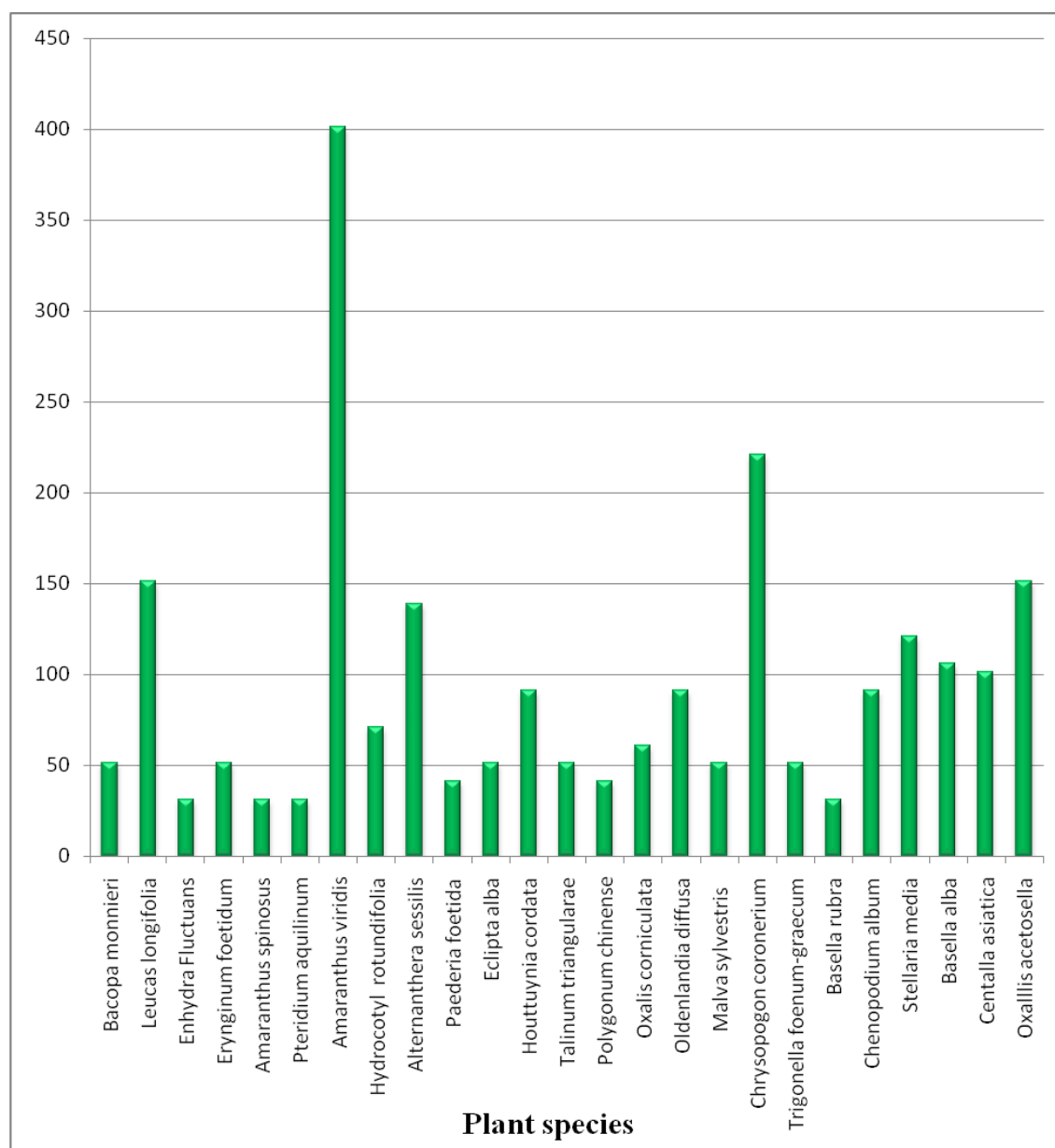


Fig. 13. Iron content (milli gram percentage) of indigenous leafy vegetables

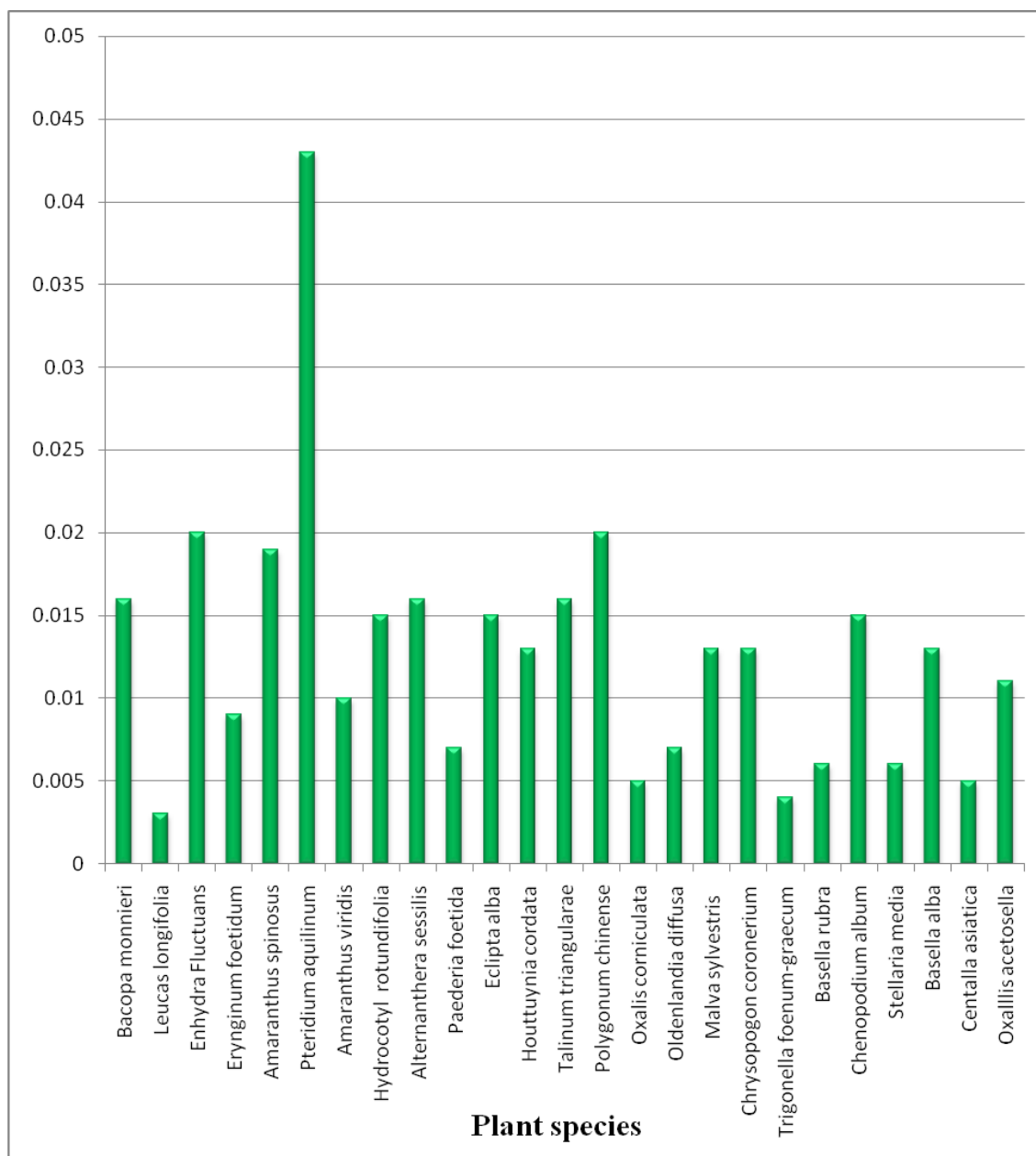


Fig. 14. Copper content (milli gram percentage) of indigenous leafy vegetables

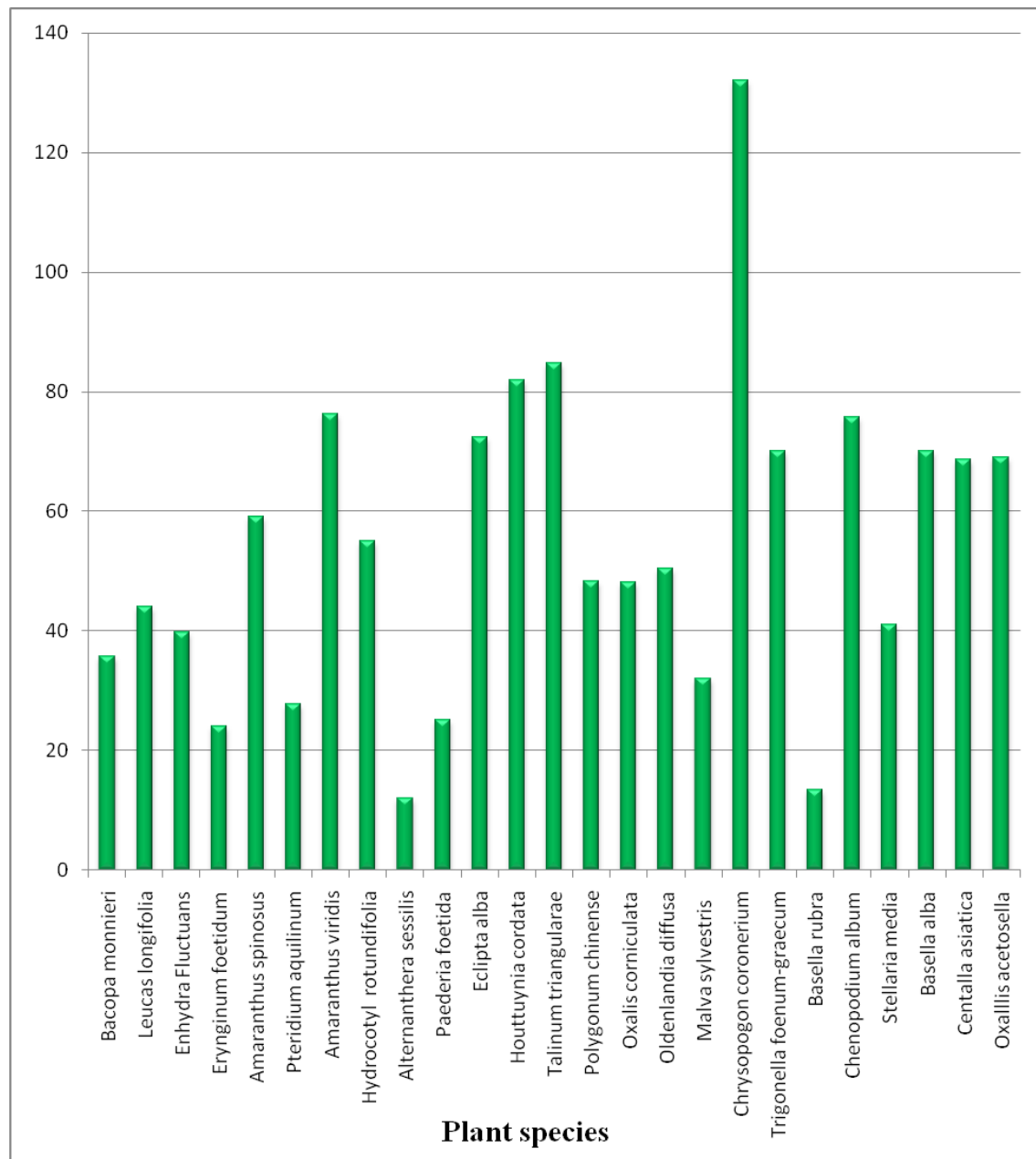


Fig. 15. Manganese content (milli gram percentage) of indigenous leafy vegetables

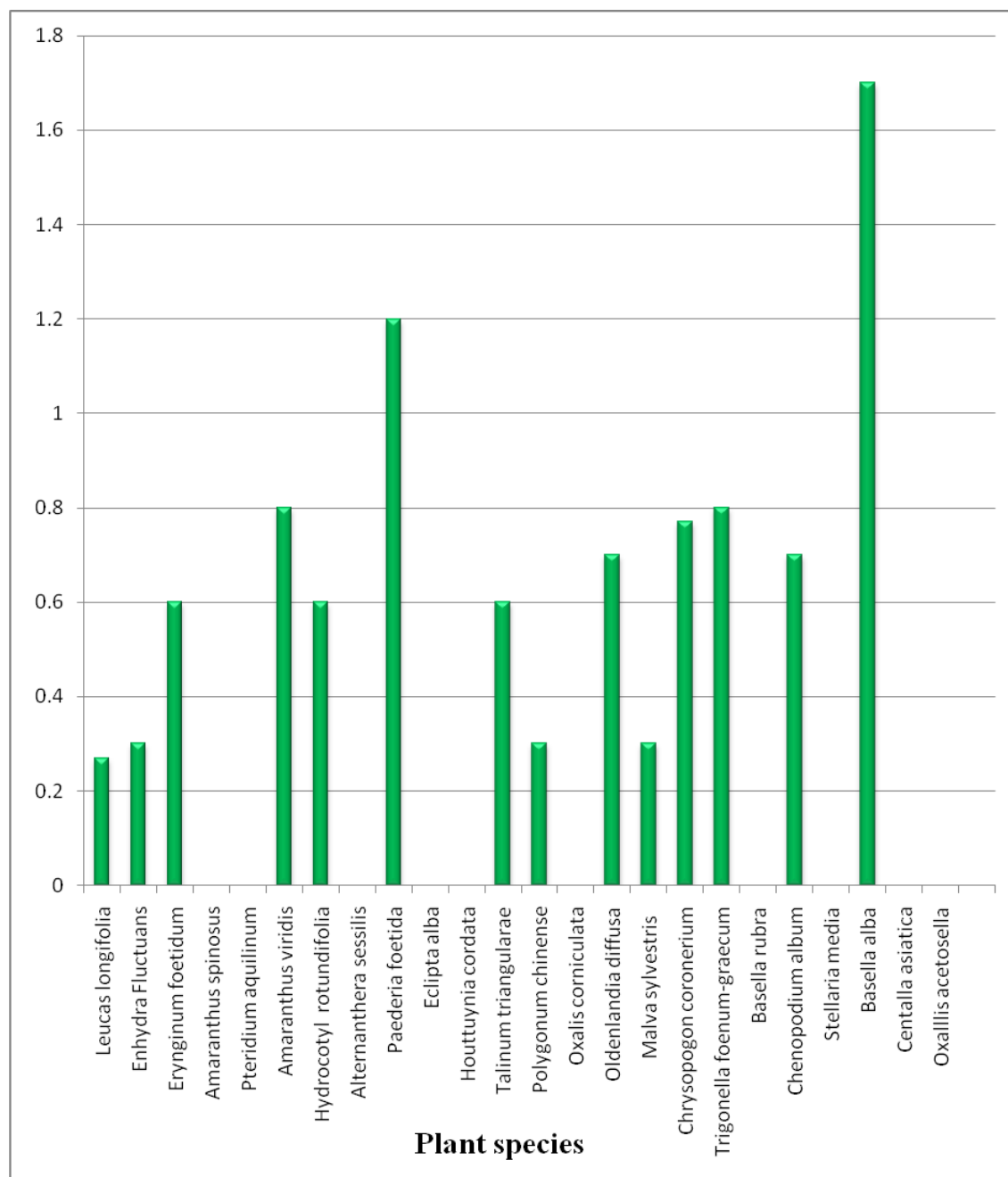


Fig. 16. Arsenic content (milli gram percentage) of indigenous leafy vegetables

4.7 Carbohydrates content (on dry weight basis) of different indigenous leafy vegetables (g %)

The data of starch content, total soluble sugar content and reducing sugar content of different leafy vegetables are presented in the Table 8.

4.7.1 Starch content

Starch constitutes a big source of calories in the diet. Starch belongs to the family of nutrients called carbohydrates, which contribute to over 50 percent of the energy consumed by most people. Starch used to be called a complex carbohydrate because it is made of a long chain of glucose, but this classification system is no longer used because the terms “complex” and “simple” carbs do not properly distinguish the impact of these nutrients in your body. Starch can be good for your body, but not all starches are created equal.

Starch is made of a long chain of glucose, and depending on how the glucose molecules are linked to one another, influence the speed at which they will be digested and turned into single molecules of glucose, or sugar. By turning into sugar, starches can elevate blood sugar levels and provide energy to all of cells and organs in body. If body doesn't require an immediate source of energy, the surplus of energy from the starches consumed will be stored, first as glycogen in liver and muscles, and the left overs will be converted to fat and stored in fat cells around waist, hips and thighs.

The data of the present investigation revealed that Hatikhutora (56.42%) had the highest starch content followed by Laffa (41.69%) and Madhuholang (18.78%) had the lowest starch content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Aberoumand (2011) in eight edible plants varies from 5.86 to 60.41% and Saunders and Beciker (1984) found starch content of pale-seeded leaves in the range from 48% for *A. cruentus* to 62% for *A. hypochondriacus*.

4.7.2 Total soluble sugar content

Total soluble sugar (TSS) content is not only the main photosynthate in higher plants, but also the main form of carbohydrate metabolism and temporary storage. Wilcox (2001) indicated that the soluble sugar content plays a very important role in carbohydrate metabolism and has a close relationship with photosynthesis and production. The level of soluble

sugar content was a sign of the supply ability of leaves and reflected transformation and ability of grains to use assimilates (Saratha *et al.*, 2001). Wang *et al.* (2007) reported that the photosynthate exists as a form of water soluble carbohydrate and its main components are soluble sugar, starch and cellulose.

The data of the present investigation revealed that Saru Manimoni (21.69%) had the highest total soluble sugar content followed by Masundori (18.37%) and Bor Tangeshi (7.37%) had lowest total soluble sugar content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Misra and Misra (2014) reported that among the 27 leafy vegetable studied, *Murraya koenigii* (18.76 g/100 g) registered the highest total sugar content and the lowest value was shown by *Tridax procumbens* (18.00 g/100 g). As per the report of Ejoh *et al.* (2007) total soluble sugar values for these *Vernonia calvoana* var. bitter, *V. amygdalina* var. bitter, *V. colorata* var. non bitter and *V. calvoana* var. non bitter ranged from 2.41% in *V. calvoana* var. bitter to 4.83% in *V. calvoana* var. non bitter on dry weight basis.

Table 8. Carbohydrates content (on dry weight basis) of different indigenous leafy vegetables (g %)

Sl. No.	Local name	Botanical name	Starch	TSS	Reducing Sugar
1	Brahmi	<i>Bacopa monnieri</i>	34.71	12.95	9.68
2	Doron	<i>Leucas longifolia</i>	28.86	15.08	10.40
3	Helosi sak	<i>Enhydra fluctuans</i>	37.52	16.69	12.72
4	Mandhania	<i>Eryngium foetidum</i>	27.68	13.27	5.86
5	Hatikhora	<i>Amaranthus spinosus</i>	56.42	9.50	5.52
6	Dhekia	<i>Pteridium aquilinum</i>	24.33	11.30	9.53
7	Khotor	<i>Amaranthus viridis</i>	24.57	10.72	6.37
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	34.44	21.69	18.58
9	Matikandori	<i>Alternanthera sessilis</i>	35.51	9.98	7.50
10	Bhedailata	<i>Paederia foetida</i>	26.35	16.54	14.13
11	Keheraj	<i>Eclipta alba</i>	26.86	14.68	12.66
12	Masundori	<i>Houttuynia cordata</i>	18.89	18.37	15.11
13	Pirali paleng	<i>Talinum triangulare</i>	30.40	10.31	7.48
14	Madhuholang	<i>Polygonum chinense</i>	18.78	14.73	13.26
15	SaruTengesi	<i>Oxalis corniculata</i>	33.75	15.17	14.10

Sl. No.	Local name	Botanical name	Starch	TSS	Reducing Sugar
16	Bonjaluk	<i>Oldenlandia diffusa</i>	20.40	11.19	10.46
17	Laffa	<i>Malva sylvestris</i>	41.69	16.49	15.34
18	Baburi	<i>Chrysopogon coronarium</i>	32.38	11.79	6.31
19	Methi	<i>Trigonella foenum-graecum</i>	36.40	8.17	5.97
20	Puroi (red)	<i>Basella rubra</i>	36.58	8.87	4.19
21	Jilmil	<i>Chenopodium album</i>	29.61	10.34	7.47
22	Morolia	<i>Stellaria media</i>	32.46	11.36	7.47
23	Puroi (green)	<i>Basella alba</i>	20.47	10.66	6.16
24	Bor Manimoni	<i>Centalla asiatica</i>	25.58	17.22	15.15
25	Bor Tangeshi	<i>Oxalllis acetosella</i>	23.87	7.37	5.10
SED(±)			0.17	0.04	0.10
CD at 5 %			0.28*	0.08*	0.16*

4.7.3 Reducing sugar content

A reducing sugar is any sugar that either has an aldehyde group or is capable of forming one in solution through [isomerism](#). The aldehyde functional group allows the sugar to act as a [reducing agent](#), for example in the [Tollens' test](#) or [Benedict's reagent](#), or the [Maillard reaction](#), important in the browning of many foods. Reducing sugar in diet can result in weight loss, increased energy and healthier.

Oxidation-reduction reactions are chemical processes that create energy, defined by the loss or gain of electrons in molecules. Oxidation occurs when a molecule loses one or more electrons, and reduction is when the molecule gains one or more electrons. This process is important in maintaining human life by creating an energy source for the body. This process requires a catalyst, called a reducing agent or oxidizing agent. Some types of sugars, or carbohydrates, are reducing agents. A reducing sugar contains aldehyde or ketone in its molecular structure. High sugar-sweetened beverage (SSB) intake is associated with risk of type 2 diabetes, obesity, hypertension and coronary heart disease (CHD).

The data of the present investigation revealed that Saru Manimoni (18.58%) had the highest reducing sugar content followed by Laffa (15.34%) and Puroi (red) (4.19%) had the lowest reducing sugar content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Verma *et al.* (2012) in *Bauhinia*

variegata buds and flowers contain reducing sugar 1.45% and 2.35% on dry weight basis respectively; Ejoh *et al.* (2007) found reducing sugar values in *Vernonia calvoana* var. bitter, *V. amygdalina* var. bitter, *V. colorata* var. non bitter and *V. calvoana* var. non bitter ranged from 13.08% in *V. calvoana* var.non bitter to 15.79% in *V. calvoana* var.non bitter on dry weight basis and Imran *et al.* (2007) found *Scandix pecten-veneris* contains reducing sugar 0.64%.

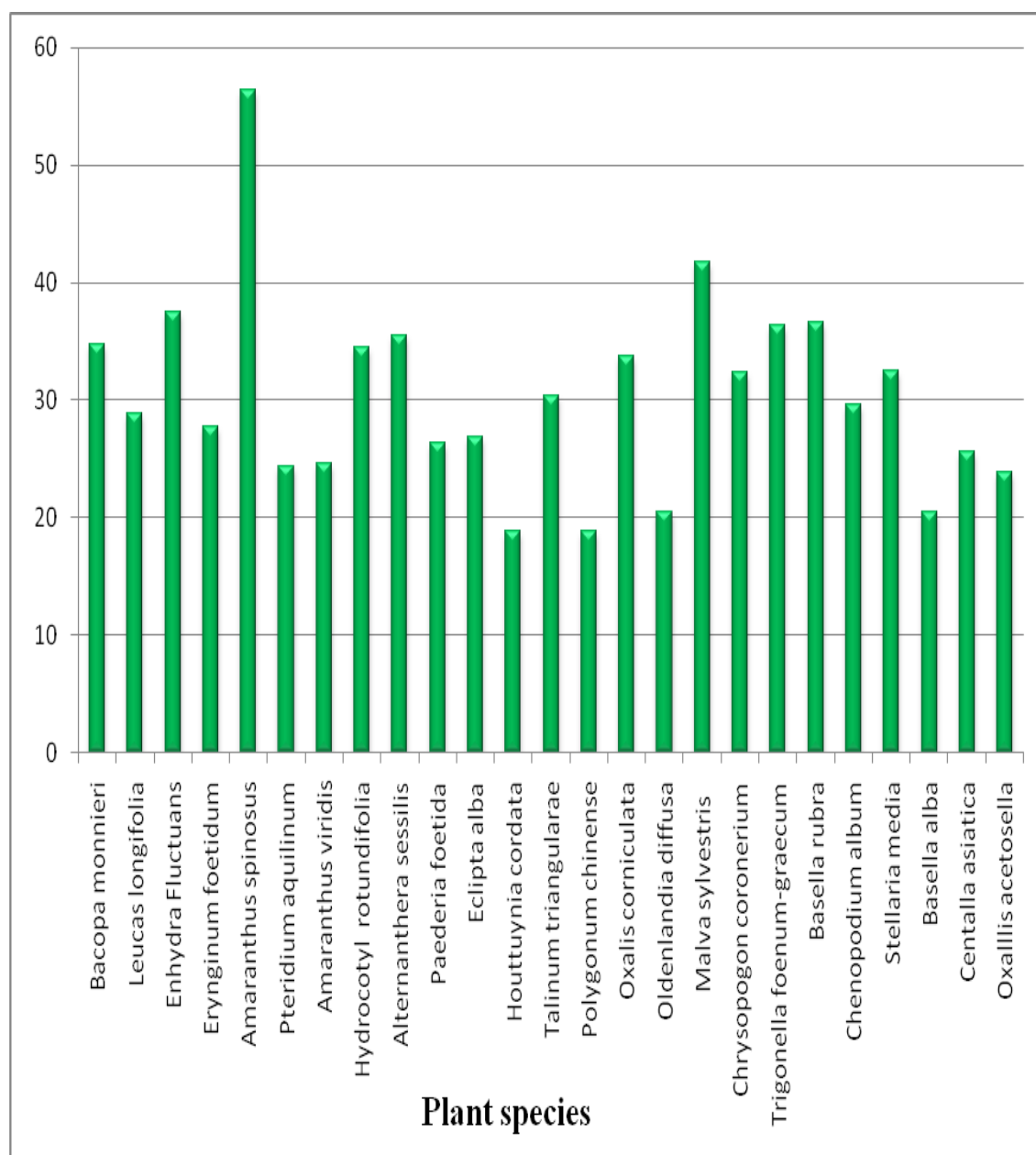


Fig. 17. Starch content (gram percentage) of indigenous leafy vegetables

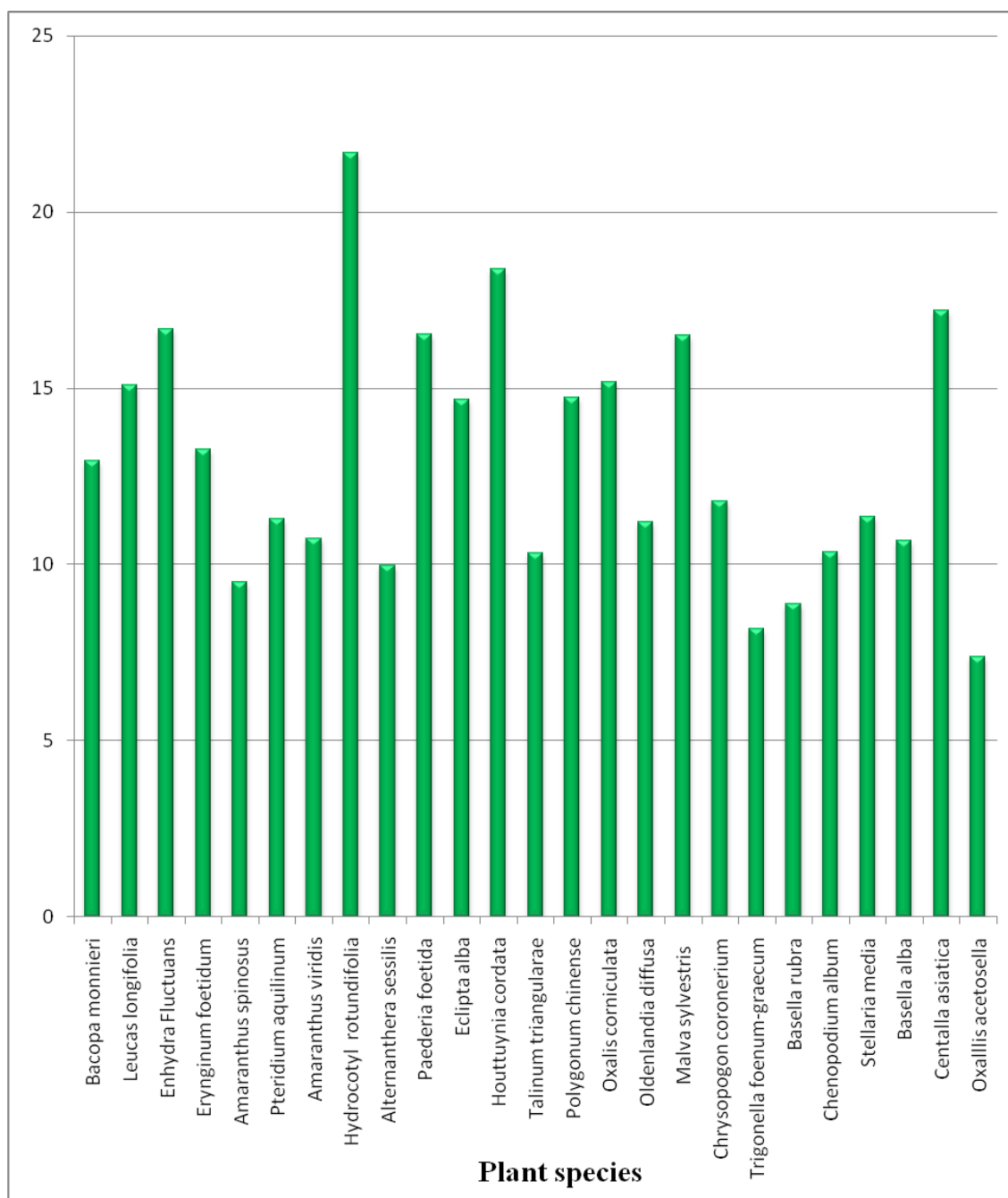


Fig. 18. Total soluble sugar content (gram percentage) of indigenous leafy vegetables

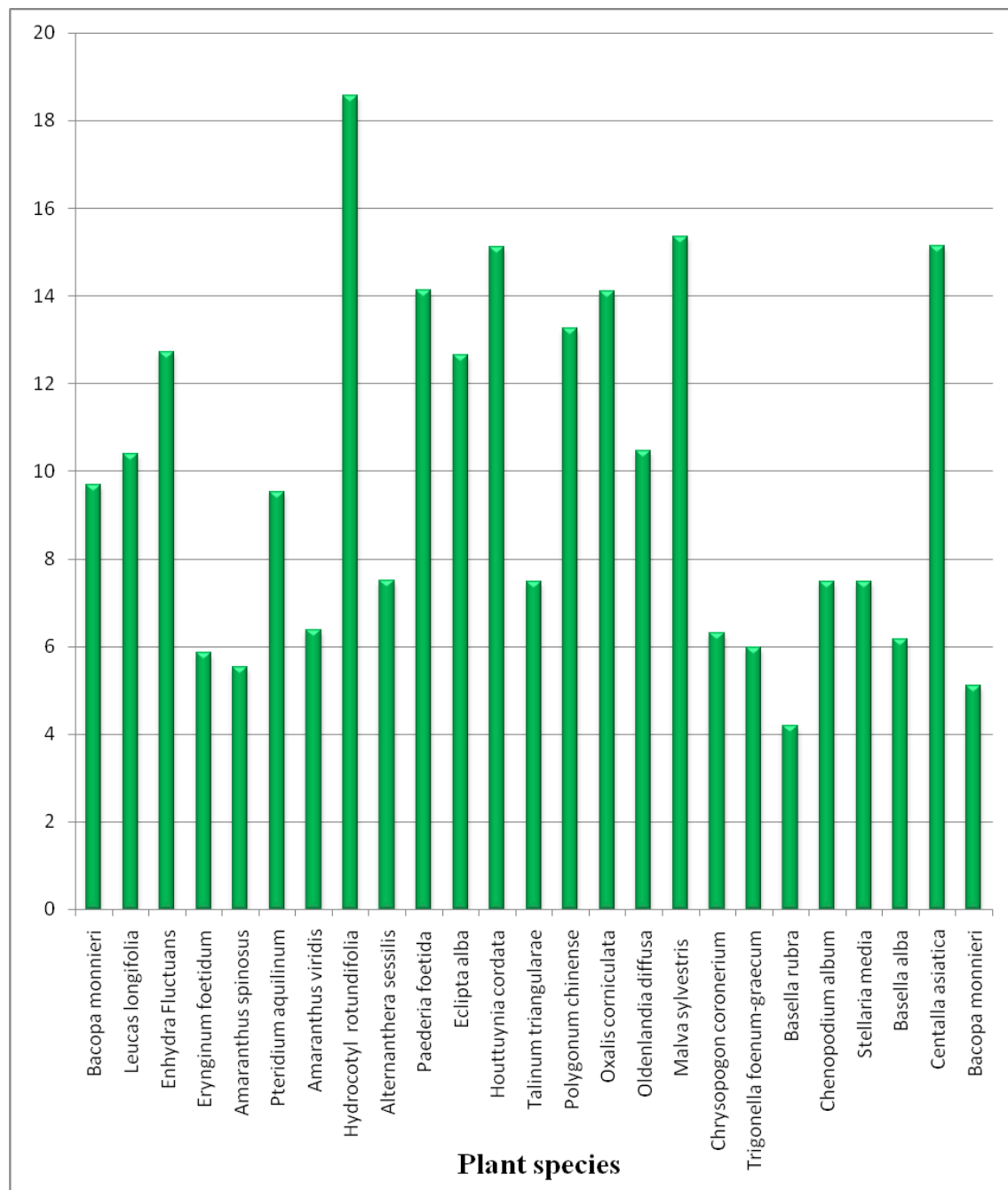


Fig. 19. Reducing sugar content (gram percentage) of indigenous leafy vegetables

4.8 Vitamin content (on dry weight basis) of different indigenous leafy vegetables (mg %)

The data of vitamin C content, riboflavin content, thiamine content and niacin content of different leafy vegetables are presented in the Table 9.

4.8.1 Vitamin C content

High concentration of ascorbic acid in plant samples might associated with attractive free radical scavenging capacity and health benefit like anti-carcinogenic and anti-atherogenic (Lui *et al.*, 2008). Ascorbic acid contributes to the antioxidant properties of vegetables by protecting the membrane erythrocyte, maintaining the blood vessels flexibility and improving blood circulation in the arteries of smokers as well as facilitating the absorption of iron in the body (Adegunwa *et al.*, 2011).

The data of the present investigation revealed that Helosi sak (119.67 mg %) had the highest vitamin C content followed by Saru Manimoni (119.33 mg %) and Masundori (39.00 mg %) had the lowest vitamin C content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Adegunwa *et al.* (2011) in *V. amygdalina* and *T. accidentalis* from 49.3 mg/100 g to 104.1 mg/100 g; Singh *et al.* (2001) found Ascorbic acid content in leafy vegetables varied from 4.9 to 110.4 mg/100 g and Adeniyi *et al.* (2012) found Ascorbic acid (vitamin C) ranges from 356.11 mg/100 g to 215.63 mg/100g in green leafy vegetables.

4.8.2 Riboflavin content

Riboflavin is a B vitamin. It can be found in certain foods such as milk, meat, eggs, nuts, enriched flour, and green vegetables. Riboflavin is frequently used in combination with other B vitamins in vitamin B complex products. Vitamin B complex generally includes vitamin B1 (thiamine), vitamin B2 (riboflavin), vitamin B3 ([niacin/niacinamide](#)), vitamin B5 (pantothenic acid), vitamin B6 ([pyridoxine](#)), [vitamin B12](#) ([cyanocobalamin](#)) and [folic acid](#).

Riboflavin is used for preventing low levels of riboflavin (riboflavin deficiency), [cervical cancer](#), and [migraine headaches](#). It is also used for treating riboflavin deficiency, [acne](#), [muscle cramps](#), [burning feet](#) syndrome, [carpal tunnel syndrome](#), and [blood disorders](#) such as congenital methemoglobinemia and red [blood](#) cell aplasia. Some people use riboflavin for [eye](#) conditions including eye [fatigue](#), [cataracts](#) and [glaucoma](#) (Saidu and Jideobi, 2009).

Other uses include increasing energy levels; boosting immune system function; maintaining healthy [hair](#), [skin](#), mucous membranes, and nails; slowing aging; boosting athletic performance; promoting healthy reproductive function; [canker sores](#); [memory loss](#), including [Alzheimer's](#) disease; ulcers; burns; [alcoholism](#); [liver](#) disease; [sickle cell anemia](#).

Riboflavin's primary role in the body is supporting metabolism, helping access energy from the nutrients that make up your diet. Vitamin B-2 helps body to break down the three macronutrients -- protein, carbohydrate and fat. Riboflavin, in the form of FAD, helps body break down fats and carbohydrates into fuel by contributing to the electron transport chain, a series of chemical reactions that give off usable energy for cells (Okwu, 2004).

Riboflavin also serves an antioxidant function due to its ability to activate antioxidant enzymes. Antioxidants protect cells from damage caused by harmful chemicals called reactive oxygen species, or ROS. ROS prove highly reactive in cells, interacting with proteins, cell membranes and even DNA, ultimately causing cellular damage. As an antioxidant, riboflavin helps neutralize ROS to prevent this damage, helping to preserve the health of cells.

The data of the present investigation revealed that Khotora (8.80 mg %) had the highest riboflavin content followed by Bhadailata (8.10 mg %) and Brahmi, Helosi sak (0.17 mg %) had the lowest riboflavin content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable to the results obtained by Ng *et al.* (2012) in *Limnophila aromaticoides* (1.37 mg/100 g, fresh weight) followed by *Crassocephalum crepidioides* (1.24 mg/100 g, fresh weight)

Table 9. Vitamin content (dry weight basis) of different indigenous leafy vegetables (mg %)

Sl. No.	Local name	Botanical name	Vitamin C (FWB)	Riboflavin	Thiamine	Niacin
1	Brahmi	<i>Bacopa monnieri</i>	100.33	0.17	0.004	0.86
2	Doron	<i>Leucas longifolia</i>	119.00	2.90	0.071	0.54
3	Helosi sak	<i>Enhydra fluctuans</i>	119.67	0.17	0.032	1.22
4	Mandhania	<i>Eryngium foetidum</i>	99.00	1.60	0.005	1.08
5	Hatikhotora	<i>Amaranthus spinosus</i>	118.00	3.30	0.018	0.56
6	Dhekia	<i>Pteridium aquilinum</i>	78.33	1.60	0.007	1.29
7	Khotora	<i>Amaranthus viridis</i>	100.00	8.80	0.007	0.15
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	119.33	0.14	0.032	1.41
9	Matikandori	<i>Alternanthera sessilis</i>	98.33	4.20	0.030	0.52
10	Bhedailata	<i>Paederia foetida</i>	78.33	8.10	0.062	1.13
11	Keheraj	<i>Eclipta alba</i>	79.67	1.27	0.028	2.60
12	Masundori	<i>Houttuynia cordata</i>	39.00	6.60	0.013	2.63
13	Pirali paleng	<i>Talinum triangularae</i>	81.00	1.57	0.032	1.93
14	Madhuholang	<i>Polygonum chinense</i>	79.00	6.60	0.017	1.78
15	Saru Tengesi	<i>Oxalis corniculata</i>	100.67	4.80	0.011	2.32
16	Bonjaluk	<i>Oldenlandia diffusa</i>	99.00	0.46	0.075	2.43
17	Laffa	<i>Malva sylvestris</i>	58.00	8.60	0.045	2.60
18	Baburi	<i>Chrysopogon coronarium</i>	78.33	3.60	0.080	1.89
19	Methi	<i>Trigonella foenum-graecum</i>	100.00	2.10	0.054	2.02
20	Puroi (red)	<i>Basella rubra</i>	98.67	2.77	0.067	1.61
21	Jilmil	<i>Chenopodium album</i>	78.33	3.70	0.017	1.08
22	Morolia	<i>Stellaria media</i>	59.33	5.60	0.032	1.43
23	Puroi (green)	<i>Basella alba</i>	118.00	5.35	0.025	0.91
24	Bor Manimoni	<i>Centalla asiatica</i>	78.00	5.97	0.022	1.47
25	Bor Tangeshi	<i>Oxalllis acetosella</i>	78.33	2.17	0.00	1.50
SED(±)			1.26	0.08	0.03	0.03
CD at 5 %			2.15*	0.14*	0.05*	0.05*

and Adeniyi *et al.* (2012) found riboflavin ranges from 0.18 mg/100 g to 0.06 mg/100 g in green leafy vegetables on dry weight basis.

4.8.3 Thiamine content

Thiamine derivatives and thiamine-dependent enzymes are present in all cells of the body, thus a thiamine deficiency would seem to adversely affect all of the organ systems. However, the nervous system is particularly sensitive to thiamine deficiency, because of its dependence on oxidative metabolism (Okwu, 2004).

Thiamine deficiency commonly presents sub acutely and can lead to metabolic coma and [death](#). A lack of thiamine can be caused by [malnutrition](#), a diet high in [thiaminase](#)-rich foods and/or foods high in anti-thiamine factors and by grossly impaired nutritional status associated with chronic diseases, such as alcoholism, gastrointestinal diseases, HIV-AIDS, and persistent vomiting (Butterworth, 2006). Well-known syndromes caused by thiamine deficiency include [beriberi](#), [Wernicke-Korsakoff syndrome](#), and optic neuropathy.

The data of the present investigation revealed that Baburi (0.08 mg %) had the highest thiamine content followed by Doron (0.071 mg %) and Bor tangeshi (0.00 mg %) had the lowest thiamine content. There is a significant difference among the plant species. The results are comparable to the results obtained by Ng *et al.* (2012) in *Limnophila aromaticoides* (1.66 mg/100 g, fresh weight basis), *Ceratopetris thalictroides* (0.090 mg/100 g, fresh weight basis) and *Etlingera elatior* (0.324 mg/100 g, fresh weight basis) and Adeniyi *et al.* (2012) found thiamin content ranges from 0.08 mg/100 g to 0.03 mg/100 g in green leafy vegetables.

4.8.4 Niacin content

Niacin is an important B vitamin found in animal foods like red meat, liver and fish. It's also found in some plant foods like beans, carrots and leafy green vegetables. Niacin helps protect against cardiovascular disease and it also supports cognitive function, the nervous system and the digestive tract, so it's important to get enough niacin into daily diet.

Niacin is one of the most important nutrients in diet, and it is water soluble. That means that body excretes excess niacin in urine, rather than storing it. Therefore, we need to eat a steady supply of niacin each day to ensure that we don't suffer from niacin deficiency (Lyimo *et al.*, 2003).

Niacin protects our heart by lowering blood levels of “bad” LDL cholesterol and preventing the accumulation of arterial plaque. Our digestive system, nervous system and brain need niacin to function. Niacin also helps our body make new DNA, and it helps our body use insulin efficiently to control blood sugar levels. Our body also needs niacin to process fats.

Niacin has a number of therapeutic uses. It's often used to treat atherosclerosis (coronary heart disease, or hardening of the arteries). Niacin can help keep blood cholesterol levels low to protect our heart. Niacin therapy has also been used to treat arthritis, diabetes and acne, as well as mental disorders like dementia, schizophrenia and psychosis.

The data of the present investigation revealed that Masundori (2.63 mg %) had the highest niacin content followed by Laffa (2.60 mg %) and Khotora (0.15 mg %) had the lowest niacin content. There is a significant difference among the plant species. The results are higher as compared to the results obtained by Adeniyi *et al.* (2012) that the niacin content ranges from 0.74 mg/100 g to 0.29 mg/100 g in green leafy vegetables.

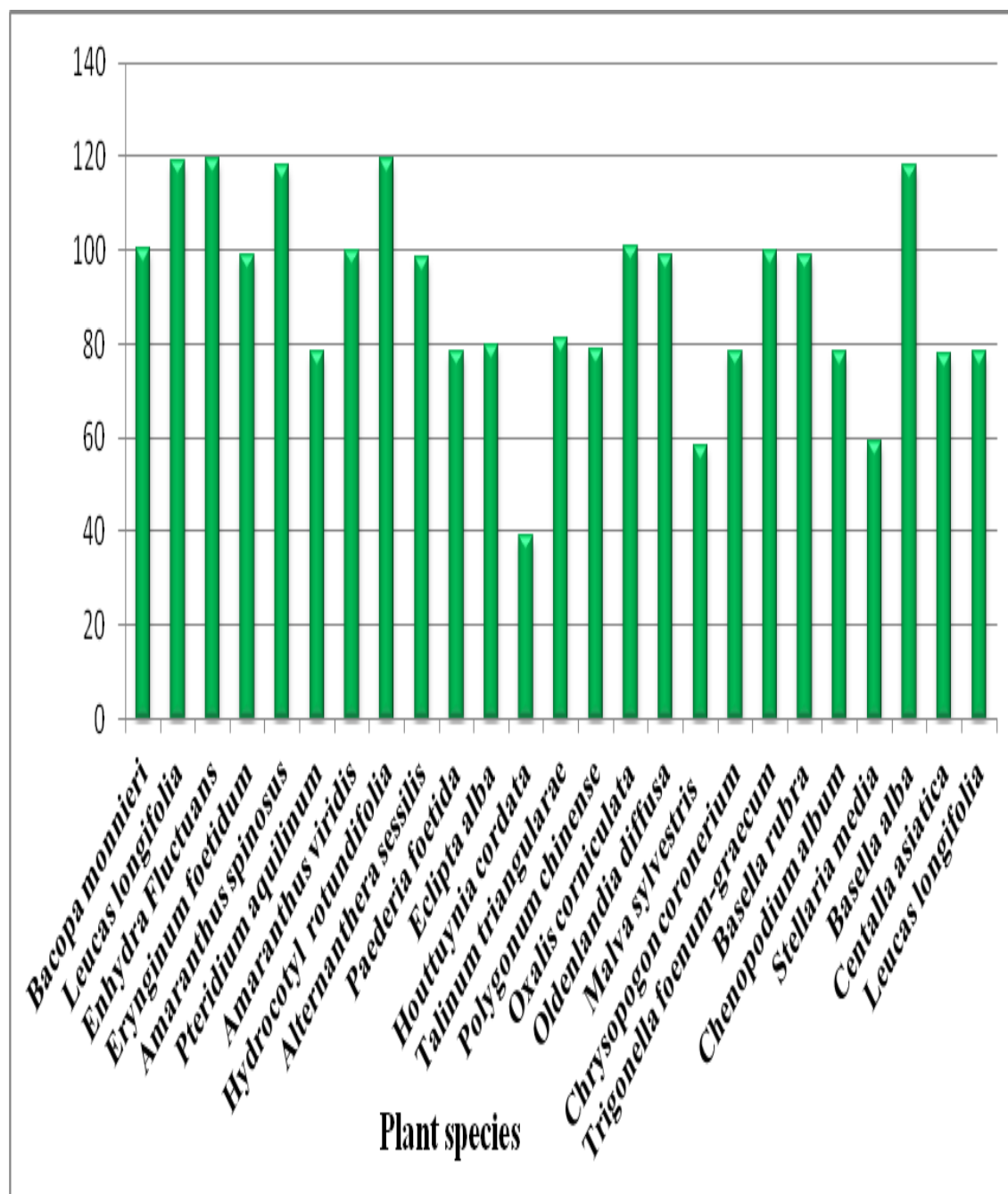


Fig. 20. Vitamin C content (milli gram percentage) of indigenous leafy vegetables

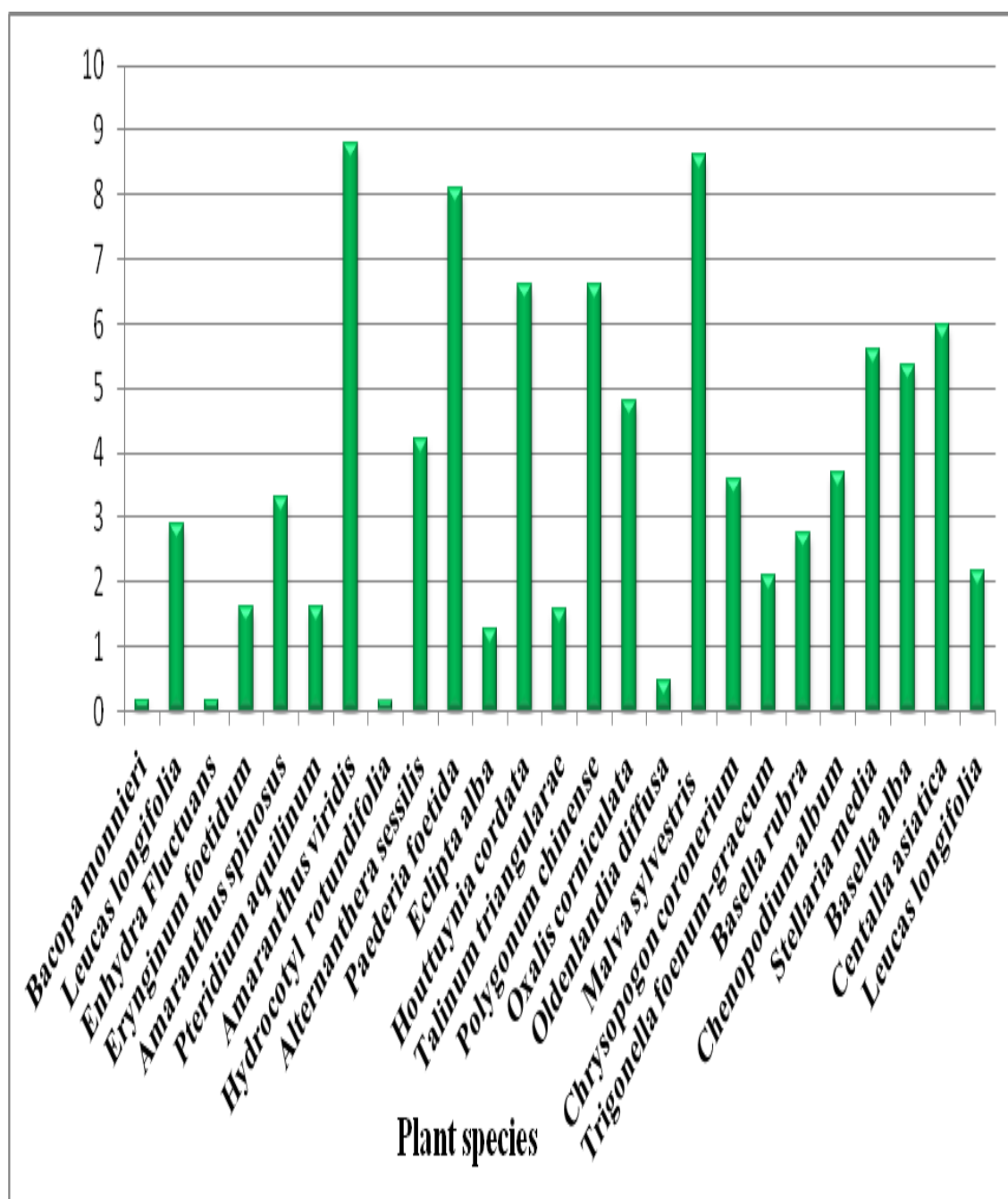


Fig. 21. Riboflavin content (milli gram percentage) of indigenous leafy vegetables

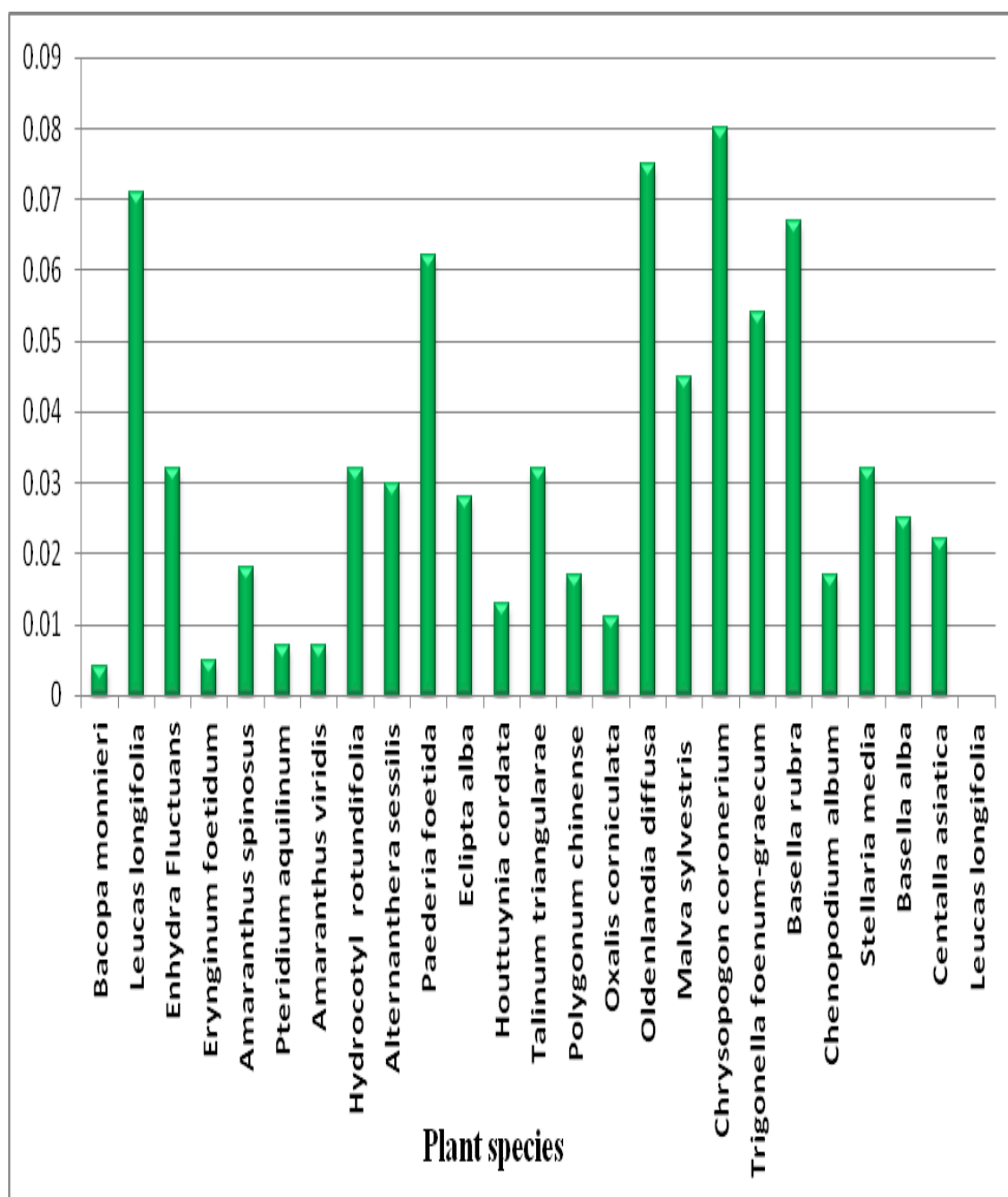


Fig. 22. Thiamine content (milli gram percentage) of indigenous leafy vegetables

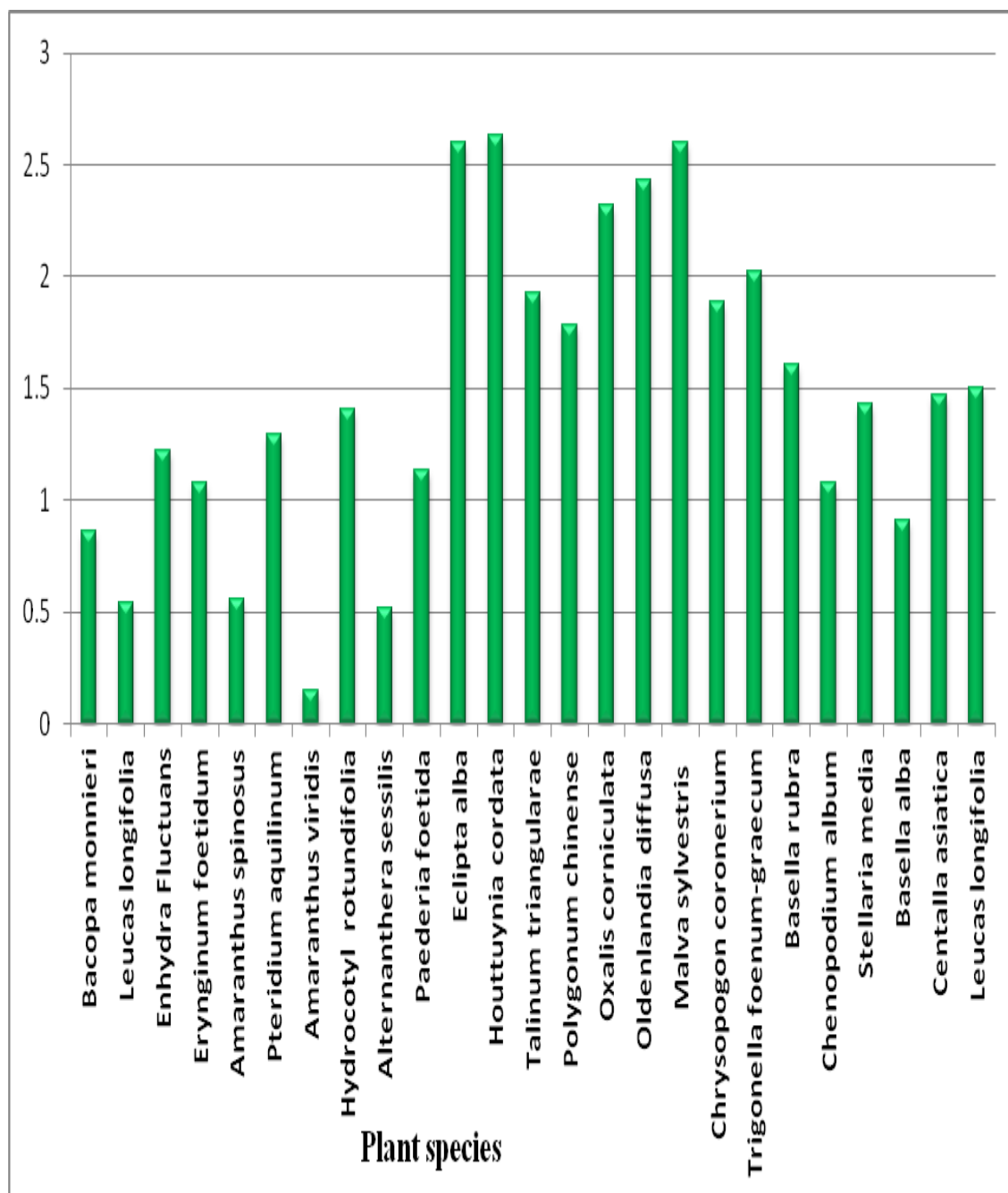


Fig. 23. Niacin content (milli gram percentage) of indigenous leafy vegetables

4.9 Anti-nutritional factors (on dry weight basis) of different indigenous leafy vegetables (g %)

Anti nutritional factors can be divided into several groups based on their chemical and physical properties such as non protein amino acids, quinolizidine alkaloids, cynogenic glycosides, pyrimidine glycosides, isoflavones, tannis, oligosaccharides, saponin, phytates, lectins or protease inhibitor. Plant which produces seeds, rich in energy supplies (Carbohydrates, lipids, proteins) usually produces potent chemical defense compounds. This occurs in many grain legumes with comparable large and protein rich seeds. They often contains substantial amount of anti nutritive factors (ANF) such as non protein amino acids, quinolizidine alkaloids, cynogenic glycosides, pyrimidine glycosides, isoflavones, tannis, oligosaccharides, saponin, phytates, lectins or protease inhibitor (Bell and Charlewood, 1980). Many feedstuffs that are commonly fed animals contain factors that interfere with the utilization of dietary nutrients. These factors, which cause depressions in growth and feed efficiency and/or affect animal health, can be defined as antinutritional factors (ANFs).

Green leafy vegetables are important component of the dietary regime of humans because they provide the necessary vitamins, and minerals (Fasuyi, 2006). They however, also contain antinutrients which reduce the bioavailability of these nutrients (Akindahunsi and Salawu, 2005; Binita and Khetarpaul, 1997). Aletor and Adeogun (1995) however, reported that some antinutrients exhibit protective effects thus making them serve dual purpose. Oxalate binds to calcium to form calcium oxalate crystals; these prevent the absorption and utilization of calcium by the body thereby causing diseases such as ricket and osteomalacia (Ladeji *et al.*, 2004). The calcium crystals may also precipitate around renal tubules causing renal stones. Phytic acid combines with some essential elements such as Fe, Ca, Zn and P to form insoluble salts called the phytates which are not absorbed by the body therefore making these minerals biounavailable. Saponins are naturally oily glycosides occurring in wide variety of plants. When eaten, they are non-poisonous to warm blooded animals but are poisonous when injected into the blood stream. Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur almost in all vascular plants. They combine with digestive enzymes thereby making them unavailable for digestion (Binita and Khetarpaul, 1997).

The data of saponin content, tannin content, phytic acid content and oxalate content of different leafy vegetables are presented in the Table 10.

4.9.1 Saponin content

Saponins are nitrogen free glycosides each consisting of a sapogenin and a sugar. The sapogenin may be a steroid or a triterpene and the sugar is generally glucose, galactose, pentose or methyl pentose. They impart bitter taste and tend to foam in aqueous solution. Saponins are poorly absorbed and most of their effects are attributable to their hydrophilic/hydrophobic asymmetry and consequently their capacity to reduce interfacial tension (Champ, 2002).

Stuardo and San Martin (2008) reported antifungal activity of *Chenopodium* saponins due to its capacity to associate with steroids of fungal membranes causing damage to its integrity and pore formation. Saponins in plants have been known to protect the plant from fungal and insect attacks (Ridout *et al.*, 1991). Other studies have proven that saponins have the ability to reduce the cholesterol levels in man and animals (Gee *et al.*, 1989).

The data of the present investigation revealed that Pirali paleng (0.74%) had the highest saponin followed by Methi (0.61%) and Mandhania (0.23%) had lowest saponin content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Sood *et al.* (2012) in different cultivars of *Chenopodium* ranged from 0.027 to 0.867% and Ejoh *et al.* (2007) in *Vernonia calvoana* var. bitter, *V. amygdalina*, *V. colorata* and *V. calvoana* var. non bitter ranged from 0.83 to 1.91 mg/100 g on dry weight basis.

4.9.2 Tannin content

Tannins (commonly referred to as tannic acid) are water-soluble polyphenols that are present in many plant foods. They have been reported to be responsible for decreases in feed intake, growth rate, feed efficiency, net metabolizable energy, and protein digestibility in experimental animals. Therefore, foods rich in tannins are considered to be of low nutritional value. However, recent findings indicate that the major effect of tannins was not due to their inhibition on food consumption or digestion but rather the decreased efficiency in converting the absorbed nutrients to new body substances. Incidences of certain cancers, such as esophageal

cancer, have been reported to be related to consumption of tannins-rich foods such as betel nuts and herbal teas, suggesting that tannins might be carcinogenic.

However, other reports indicated that the carcinogenic activity of tannins might be related to components associated with tannins rather than tannins themselves. Interestingly, many reports indicated negative association between tea consumption and incidences of cancers. Tea polyphenols and many tannin components were suggested to be anti-carcinogenic. Many tannin molecules have also been shown to reduce the mutagenic activity of a number of mutagens. Many carcinogens and/or mutagens produce oxygen-free radicals for interaction with cellular macromolecules. The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their anti-oxidative property, which is important in protecting cellular oxidative damage including lipid peroxidation. The generation of superoxide radicals was reported to be inhibited by tannins and related compounds.

The antimicrobial activities of tannins are well documented. The growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins. We have also found that tannic acid and propyl gallate, but not gallic acid, were inhibitory to food borne bacteria, aquatic bacteria, and off-flavor-producing microorganisms. Their antimicrobial properties seemed to be associated with the hydrolysis of ester linkage between gallic acid and polyols hydrolyzed after ripening of many edible fruits. Tannins in these fruits thus serve as a natural defense mechanism against microbial infections. The antimicrobial property of tannic acid can also be used in food processing to increase the shelf-life of certain foods, such as catfish fillets. Tannins have also been reported to exert other physiological effects, such as to accelerate blood clotting, reduce blood pressure, decrease the serum lipid level, produce liver necrosis, and modulate immune responses. The dosage and kind of tannins are critical to these effects (Chung *et al.*, 1998).

The data of the present investigation revealed that Bhedailata (2.82%) had the highest tannin content followed by Dhekia (2.38%) and Morolia (0.37%) had the lowest tannin content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Gupta *et al.* (2005) reported that tannin values ranged between 0.061 and 0.205 g/100g with the exception of *Coleus aromaticus* (0.015 g/100 g) and *Delonix elata* (1.330 g/100 g) on fresh weight basis and Adegunwa *et al.* (2011) found in sun-

dried samples the tannin content ranged from 0.18 g/100 g in *B. alba* to 0.34 g/100 g in *V. amygdalina*.

Table 10. Anti-nutritional factors (on dry weight basis) of different indigenous leafy vegetables (g %)

Sl. No.	Local name	Botanical name	Saponin	Tannin	Phytic acid	Oxalate
1	Brahmi	<i>Bacopa monnieri</i>	0.54	1.63	0.23	0.08
2	Doron	<i>Leucas longifolia</i>	0.29	1.91	0.32	0.11
3	Helosi sak	<i>Enhydra fluctuans</i>	0.41	0.66	0.27	0.07
4	Mandhanian	<i>Eryngium foetidum</i>	0.23	1.45	0.18	0.06
5	Hatikhotora	<i>Amaranthus spinosus</i>	0.50	0.76	0.17	0.09
6	Dhekia	<i>Pteridium aquilinum</i>	0.32	2.38	0.15	0.03
7	Khotora	<i>Amaranthus viridis</i>	0.29	1.10	0.10	0.04
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	0.40	0.70	0.2	0.04
9	Matikandori	<i>Alternanthera sessilis</i>	0.38	0.57	0.10	0.03
10	Bhedailata	<i>Paederia foetida</i>	0.31	2.82	0.07	0.04
11	Keheraj	<i>Eclipta alba</i>	0.34	2.14	0.27	0.07
12	Masundori	<i>Houttuynia cordata</i>	0.56	1.36	0.26	0.08
13	Pirali paleng	<i>Talinum triangularae</i>	0.74	1.35	0.08	0.02
14	Madhuholang	<i>Polygonum chinense</i>	0.33	1.33	0.06	0.03
15	Saru Tengesi	<i>Oxalis corniculata</i>	0.43	0.82	0.08	0.04
16	Bonjaluk	<i>Oldenlandia diffusa</i>	0.40	1.85	0.19	0.02
17	Laffa	<i>Malva sylvestris</i>	0.30	2.43	0.22	0.13
18	Baburi	<i>Chrysopogon coronarium</i>	0.33	1.39	0.15	0.05
19	Methi	<i>Trigonella foenum-graecum</i>	0.61	1.92	0.13	0.09
20	Puroi (red)	<i>Basella rubra</i>	0.50	2.7	0.21	0.12
21	Jilmil	<i>Chenopodium album</i>	0.41	1.15	0.06	0.02
22	Morolia	<i>Stellaria media</i>	0.38	0.37	0.03	0.03

Sl. No.	Local name	Botanical name	Saponin	Tannin	Phytic acid	Oxalate
23	Puroi (green)	<i>Basella alba</i>	0.47	0.89	0.22	0.13
24	Bor Manimoni	<i>Centalla asiatica</i>	0.26	1.16	0.21	0.03
25	Bor Tangeshi	<i>Oxalllis acetosella</i>	0.61	1.12	0.09	0.07
SED(±)			0.01	0.08	0.01	0.008
CD at 5 %			0.02*	0.14*	0.14*	0.01*

4.9.3 Phytic acid content

Phytic acid (hexa phosphate of inositol) is a complex class of naturally occurring anti-nutritional factor present in plant foods and is a major phosphorus storage compound in green leafy vegetables. It affects the digestion and absorption of minerals. Phytic acid though considered as an anti - nutritional factor in a common storage of phosphorus in seeds and in few tubers and fruits. The complexities of phytic acid with nutritionally essentials minerals are suggested to be responsible for the anti- nutritional activity (Oboh *et al.*, 2005).

The data of the present investigation revealed that Doron (0.32%) has the highest phytic acid followed by Helosi sak, Keheraj (0.27%) and Morolia (0.03%) has the lowest phytic acid content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Sood *et al.* (2012) in different *Chenopodium* cultivars ranged from 0.24-0.27 g/100 g; Yadav and Sehgal (2003) found 0.24 g/100 g phytic acid content in *bathua* (*C. album*) leaves and Khan *et al.* (2012) reported that *Scandix pecten-veneris* contains 0.025% phytate.

4.9.4 Oxalate content

Oxalates is a dicarboxylic acid and is found in the form of soluble salts of potassium and sodium and as insoluble salts of calcium, magnesium and iron in algae, fungi, lichens, ferns and higher plants. Insoluble oxalate is excreted in the feces while the soluble oxalate is absorbed by the body. Soluble oxalate forms strong chelates with dietary calcium,

rendering it unavailable for absorption and assimilation. However, high dietary intake of soluble oxalate can lead to the formation of kidney stones. A diet high in oxalates may require supplementation of divalent minerals to prevent deficiencies. Addition of a source of calcium to vegetables containing high levels of soluble oxalate has been shown to reduce the intestinal available oxalate content in such food (Radek and Savage, 2008). Oxalic acid is one of the anti-nutritional factors, which are widely distributed in plant foods. Oxalic acid is known to interfere with calcium absorption by forming insoluble salts of calcium.

The data of the present investigation revealed that Puroi (green), Laffa (0.13%) had the highest oxalate content followed by Puroi (red) (0.12%) and Pirali paleng, Bonjaluk (0.02%) had the lowest oxalate content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Sood *et al.* (2012) in different cultivars of *Chenopodium* ranged from 0.52 g/100 g to 0.39 g/100 g and Khan *et al.* (2012) reported that *Scandix pecten-veneris* contains 0.225% oxalate.

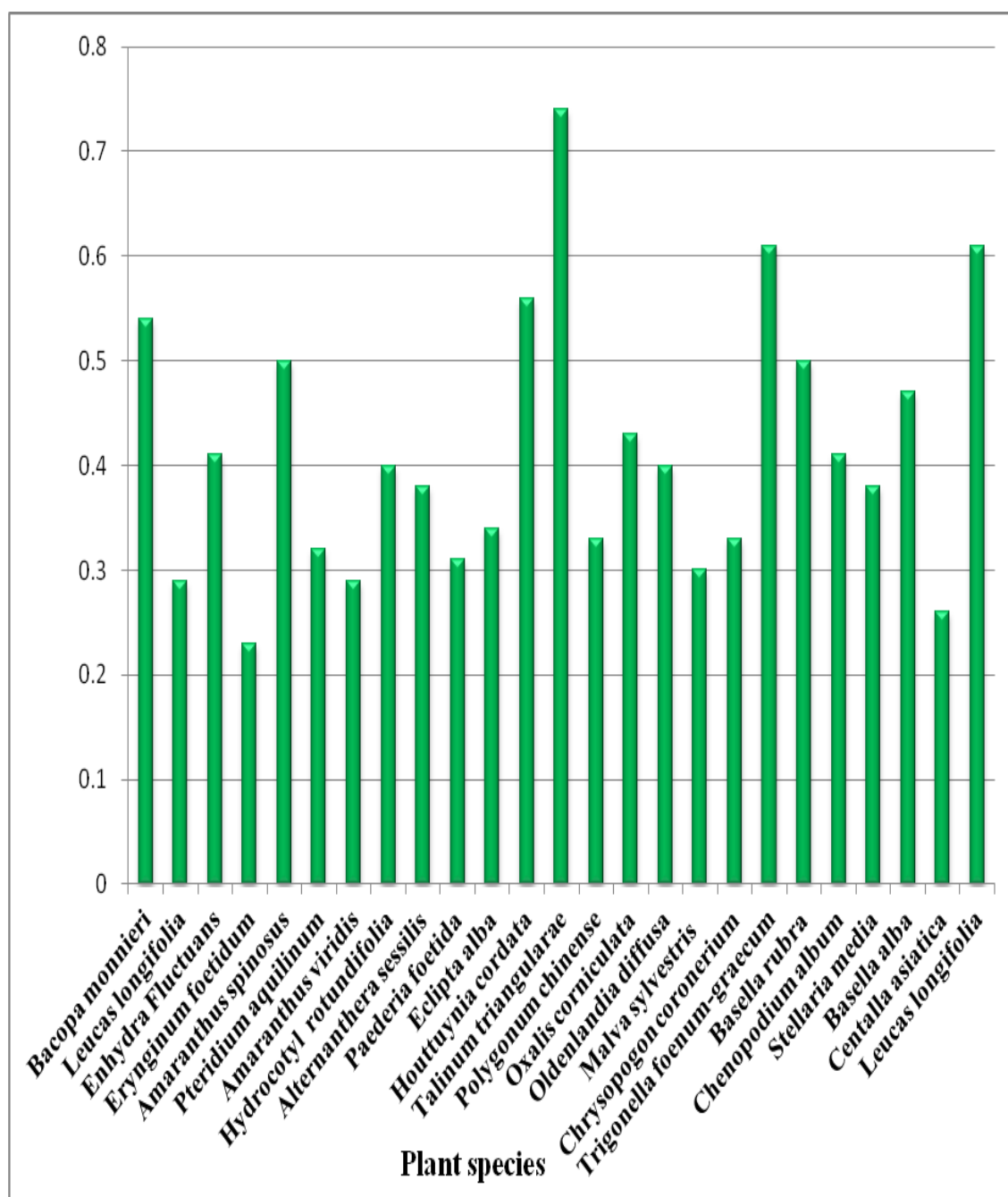


Fig. 24. Saponin content (gram percentage) of indigenous leafy vegetables

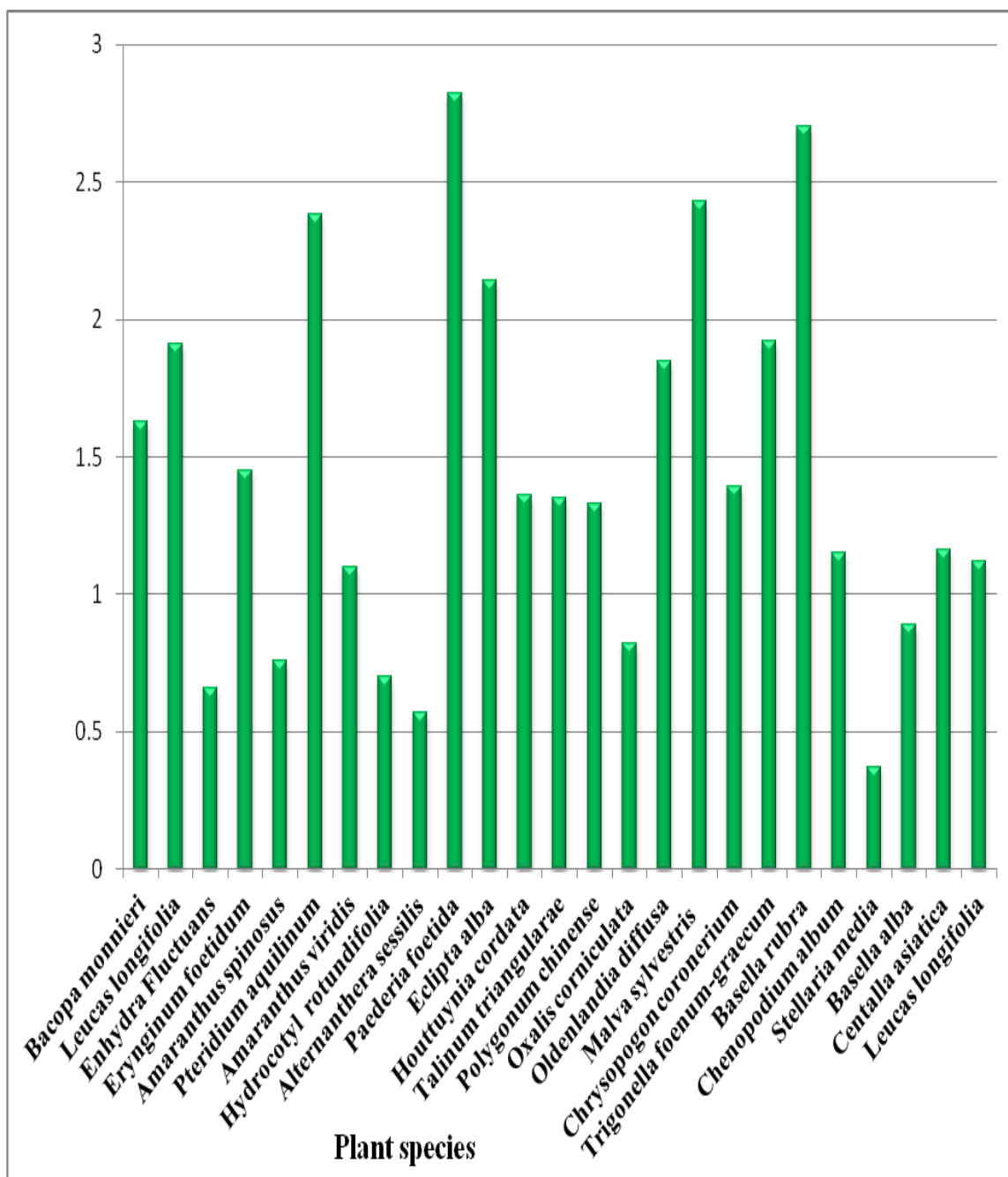


Fig. 25. Tannin content (gram percentage) of indigenous leafy vegetables

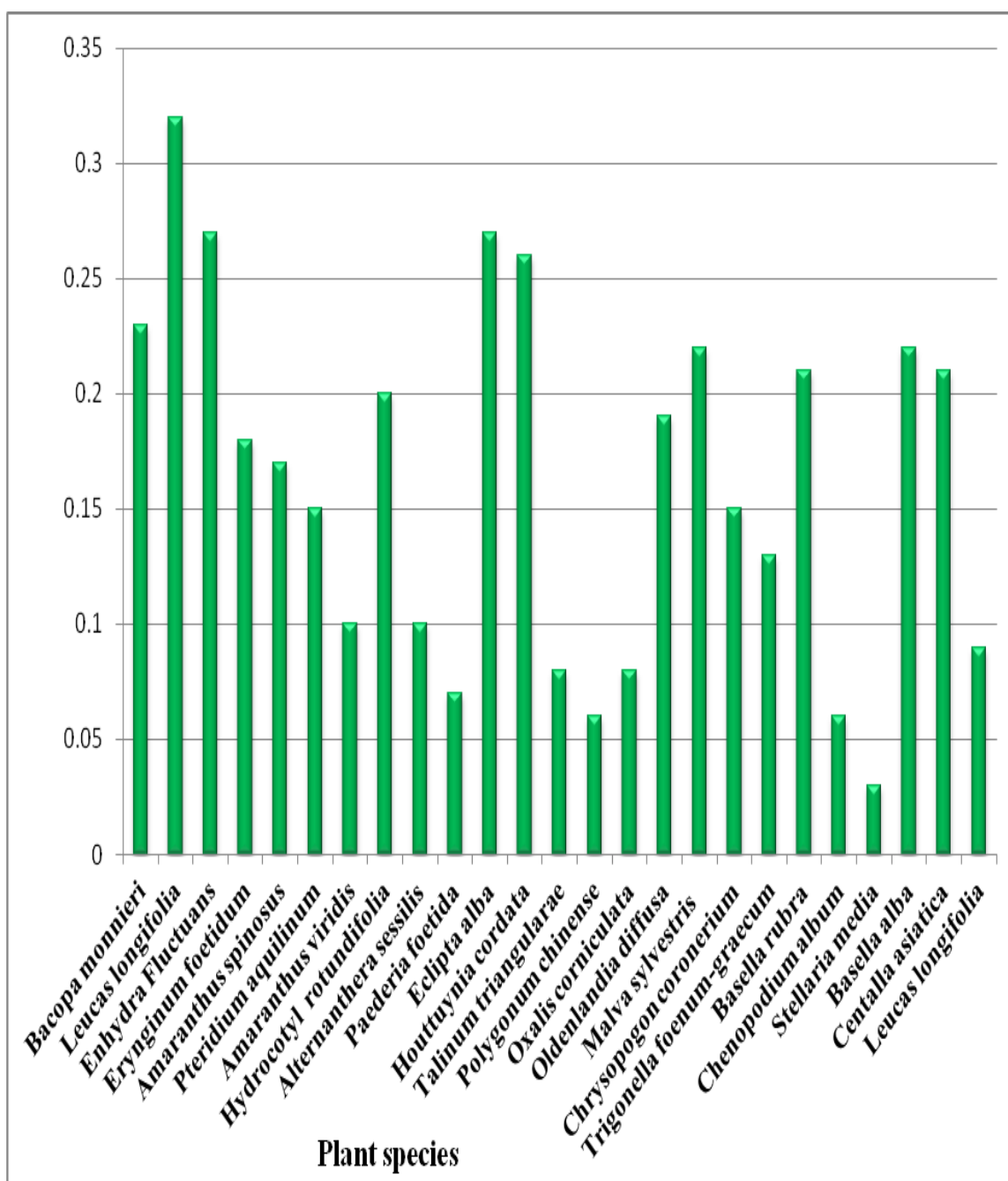


Fig. 26. Phytic acid content (gram percentage) of indigenous leafy vegetables

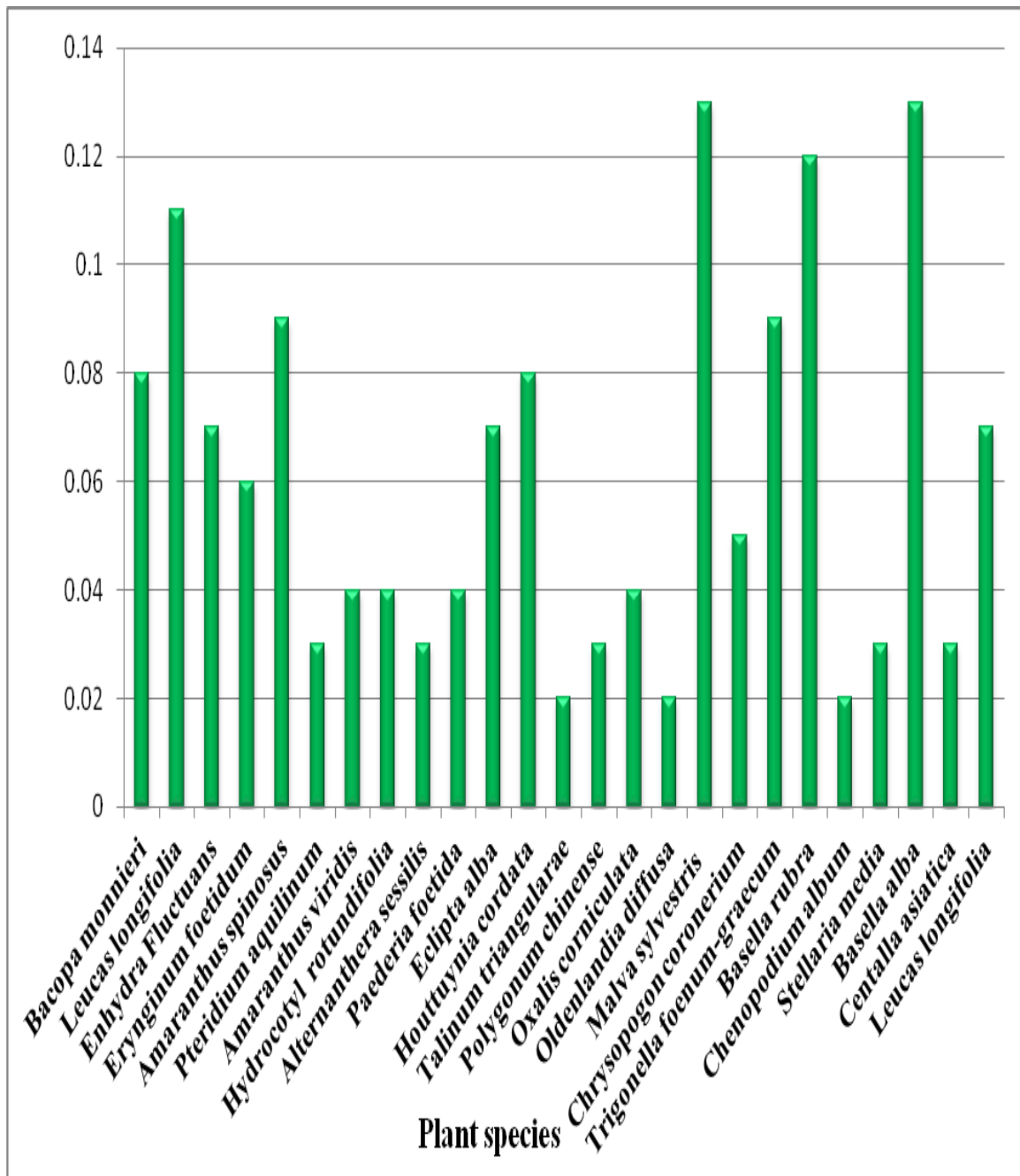


Fig. 27. Oxalate content (gram percentage) of indigenous leafy vegetables

4.10 Other significant phytochemicals of Indigenous leafy vegetables (g %)

The data of total alkaloid content, total phenol content, total flavanoid content and total flavanol of different leafy vegetables are presented in the Table 11.

4.10.1 Total alkaloid content

Alkaloids are a group of naturally occurring [chemical compounds](#) that contain mostly [basic nitrogen](#) atoms, this group also includes some related compounds with neutral and even weakly [acidic](#) properties. Some synthetic compounds of similar structure are also attributed to alkaloids, in addition to [carbon](#), [hydrogen](#) and [nitrogen](#) alkaloids may also contain [oxygen](#), [sulfur](#) and more rarely other elements such as [chlorine](#), [bromine](#), and [phosphorus](#) (Manske, 1965).

Alkaloids are a class of naturally occurring organic nitrogen containing bases. Most common alkaloids include morphine, strychnine, quinine, ephedrine and nicotine. The bitterness of some plants might be due to the presence of alkaloids which affect the consumer acceptability of product. These compounds also possess medicinal properties.

Alkaloids are a special group of secondary compounds and are part of an organism's adoption mechanism to its living environment. They are not toxic when stored, but become toxic as a result of pH change. The defensive function of alkaloids is only secondary and connected to internal immune and regulation process. Animal response to alkaloids are very diverse. Some animals can tolerate alkaloids relatively well, while others are harmed or even poisoned by them.

The data of the present investigation revealed that Hatikhutora (3.23%) had the highest total alkaloid followed by Saru Manimoni (3.10%) and Madhuholang (0.13%) had the lowest total alkaloid content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are higher as compared to the results obtained by Adedapo *et al.* (2011) in leaves of *C. album* as 1.8 mg/100 g; Jimoh *et al.* (2010) found in *Amaranthus asper* leaves 0.3

mg/100 g (on dry weight basis) alkaloid and Sood *et al.* (2012) found in different cultivars of *Chenopodium* ranged from 0.69 to 1.67 mg/100 g.

4.10.2 Total phenol content

Phenolics are nonnutritive secondary metabolites found in plants that promote significant health benefit and prevent various diseases. Phenols exhibit antioxidant potential (Awika *et al.*, 2003) due to their redox properties which allow them to act as reducing agents, hydrogen donors and single oxygen quenchers (Chang *et al.*, 2001).

Plant phenolics are biosynthesized following different routes, the shikimic acid pathway being the most biosynthetic route involved. This pathway as reported by Rivero *et al.* (2001) is thought to be an acclimatization mechanism of plant external stress (Temperature, injury, infection etc.), the cold having the highest phenylalanine ammonia-lyase activating effect and the highest peroxidase and polyphenol oxidase inhibiting effect.

The data of the present investigation revealed that Brahmi (2.2%) had the highest total phenol content followed by Doron (2.1%) and Bor Manimoni (0.25%) had the lowest total phenol content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Silva *et al.* (2007) in some of the plant species found in Amazonian region such as *Bauhinia forficata* (1.66 g, gallic acid equivalents (GAE)/100 g, on fresh weight basis), *Davilla kunthii* (3.64 g GAE/100 g, on fresh weight basis) and *Byrsonima crassifolia* (4.55 g GAE/100 g, on fresh weight basis) have 10 fold higher phenolic contents; Sood *et al.* (2012) found in different cultivars of *Chenopodium* ranged from 0.304 g GAE/100 g to 0.225 g GAE/100 g and Kaur and Kapoor (2002) reported 0.254 g GAE/100 g total phenol content in the leaves of *Chenopodium album*.

Table 11. Other significant phytochemicals (on dry weight basis) of Indigenous leafy vegetables (g %)

Sl. No.	Local name	Botanical name	Total alkaloid	Total phenol	Total Flavanoid	Total flavanol (mg%)
1	Brahmi	<i>Bacopa monnieri</i>	2.30	2.2	1.96	0.44
2	Doron	<i>Leucas longifolia</i>	1.80	2.1	2.36	0.82
3	Helosi sak	<i>Enhydra fluctuans</i>	2.70	0.47	1.56	0.42
4	Mandhania	<i>Erynginum foetidum</i>	3.07	0.31	1.72	0.44
5	Hatikhotora	<i>Amaranthus spinosus</i>	3.23	0.32	1.15	0.50
6	Dhekia	<i>Pteridium aquilinum</i>	2.97	1.13	2.02	0.41
7	Khotora	<i>Amaranthus viridis</i>	2.80	0.44	1.41	0.62
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	3.10	0.44	2.25	0.45
9	Matikandori	<i>Alternanthera sessilis</i>	1.50	0.26	2.32	0.71
10	Bhedailata	<i>Paederia foetida</i>	2.10	1.08	2.94	0.65
11	Keheraj	<i>Eclipta alba</i>	2.93	0.53	1.82	0.93
12	Masundori	<i>Houttuynia cordata</i>	0.43	1.13	1.53	0.51
13	Pirali paleng	<i>Talinum triangularae</i>	0.33	0.37	1.38	0.41
14	Madhuholang	<i>Polygonum chinense</i>	0.13	0.49	1.89	0.47
15	Saru Tengesi	<i>Oxalis corniculata</i>	0.37	0.39	1.36	0.50
16	Bonjaluk	<i>Oldenlandia diffusa</i>	1.97	1.15	2.76	0.40
17	Laffa	<i>Malva sylvestris</i>	0.64	1.22	2.23	0.37
18	Baburi	<i>Chrysopogon coronarium</i>	1.20	1.05	1.78	0.80
19	Methi	<i>Trigonella foenum-graecum</i>	1.20	1.33	2.90	0.52
20	Puroi (red)	<i>Basella rubra</i>	0.60	1.18	2.13	1.39
21	Jilmil	<i>Chenopodium album</i>	0.80	0.96	3.40	0.60
22	Morolia	<i>Stellaria media</i>	0.79	0.44	1.92	1.63
23	Puroi (green)	<i>Basella alba</i>	0.82	0.44	1.84	0.51
24	Bor Manimoni	<i>Centalla asiatica</i>	0.83	0.25	0.86	0.50
25	Bor Tangesi	<i>Oxalllis acetosella</i>	1.2	1.10	2.42	0.33
SED(±)			0.11	0.02	0.05	0.03
CD at 5 %			0.18*	0.03*	0.04*	0.06*

4.10.3 Total flavanoid content

Flavonoids are a group of polyphenolic compounds naturally present in most edible fruits and vegetable plants. They are secondary plant metabolite and are a natural antioxidant which helps to maintain the body's health and to protect against diseases. They also exhibit anti-nutritional properties due to their metal chelating properties. However, epidemiological and clinical studies have provided evidence of a potential role for flavonoids in lowering the risk of coronary heart diseases prevention, cardiovascular diseases, alzheimers disease, neurodegenerative diseases, diabetes, osteoporosis and lung cancer through antioxidative action and / or the modulation of several protein functions. However, nutritionally flavonoid is considered as an anti-nutrient as its polyphenol structure is a metal chelator therefore capable of binding iron and facilitating its removal from the body.

Flavonols and flavonones are flavonoids of particular importance because they have been found to possess antioxidant and free radical scavenging activity in vegetable (Amic *et al.*, 2003). Even though flavonoids are important phenolic compounds contributing to the antioxidant activity of the vegetables, apparently it is also possible that the antioxidant activities of these vegetables are due to other phenolic compounds.

Furthermore, the type of flavonoids present in the plant samples could be another factor that determines their antioxidant capability. As Tsimogiannis and Oreopoulou (2006) found that antioxidant properties of flavonoids depend on their C-ring structure, flavonoids with full substitution C-ring like rutin and luteolin, which their ether bonded with three oxygen demonstrate better free radical scavenging capacity and higher reaction rate compare to flavonoids that lack of one or more C-ring structural elements.

The data of the present investigation revealed that Jilmil (3.40%) had the highest total flavanoids followed by Bhedailata (2.94%) and Bor Manimoni (0.86%) had the lowest total flavanoids content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Sood *et al.* (2012) in different cultivars of

Chenopodium ranged from 0.22 g/100 g to 0.41 g/100 g and Adedapo *et al.* (2011) found 0.18 g/100 g flavonoid content in *C. album* leaves.

4.10.4 Total flavanol content

Phenolic compounds are secondary metabolites in plants. These compounds are one of the most widely occurring groups of phytochemicals that exhibit a wide range of physiological properties, such as antiallergenic, anti artherogenic, anti-inflammatory, anti-microbial, antioxidant, antithrombotic, cardioprotective and vasodilatory effects (Manach *et al.*, 2005; Middleton *et al.*, 2000).

Many phenolics, such as flavonoid, have antioxidant properties that are much stronger than those of vitamins C and E. Flavonol and flavonoid are of particular importance because they have been found to possess antioxidant and free radical scavenging activity in vegetables (Amic *et al.*, 2003). Some evidences have also shown that flavonoids could protect membrane lipids from oxidation (Amic *et al.*, 2003).

The data of the present investigation revealed that Morolia (1.63%) had the highest total flavanol content followed by Puroi (red) (1.39%) and Bor Tangeshi (0.33%) had the lowest total flavanol content. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Jimoh *et al.* (2010) in *Amaranthus asper* leaves contained 0.96 g/100 g (dry weight basis) total flavanols and Afolayan *et al.* (2009) reported that total flavonols (g/100 g) content in *C. album*, *S. asper*, *S. nigrum* and *U. urens* are 0.98 ± 0.41 , 0.91 ± 0.26 , 0.71 ± 0.00 , 0.78 ± 0.08 , respectively.

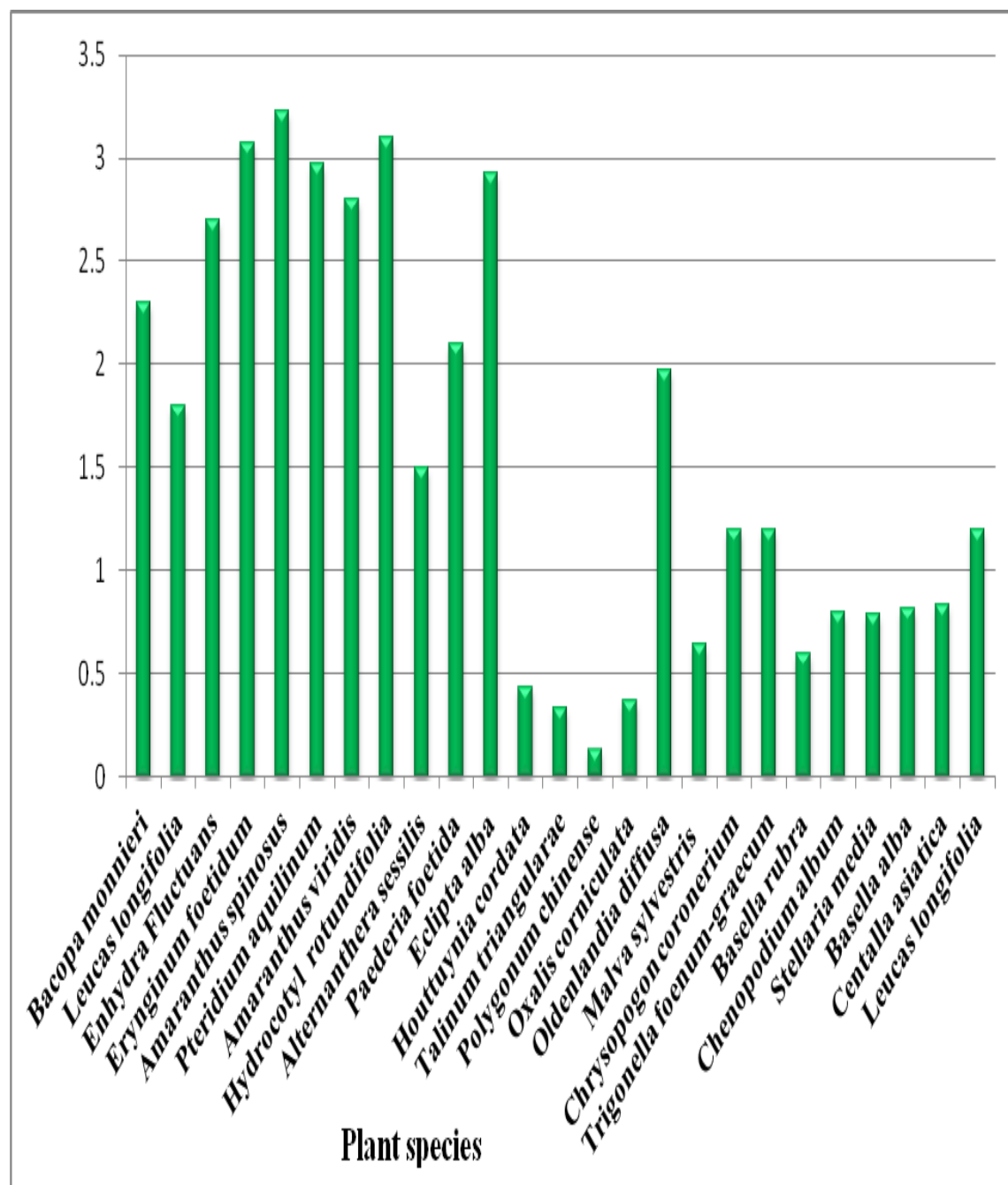


Fig. 28. Total alkaloid content (gram percentage) of indigenous leafy vegetables

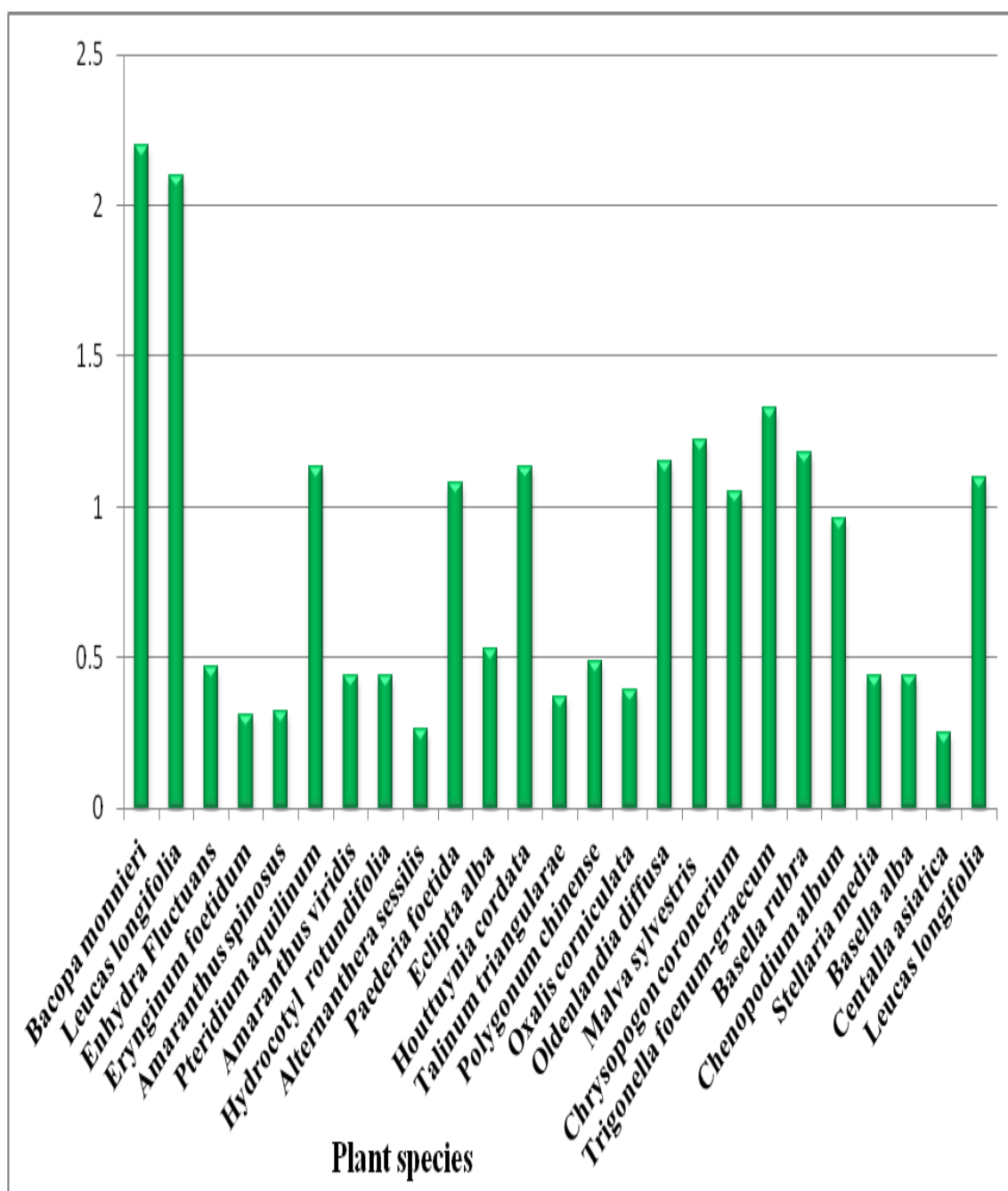


Fig. 29. Total phenol content (gram percentage) of indigenous leafy vegetables

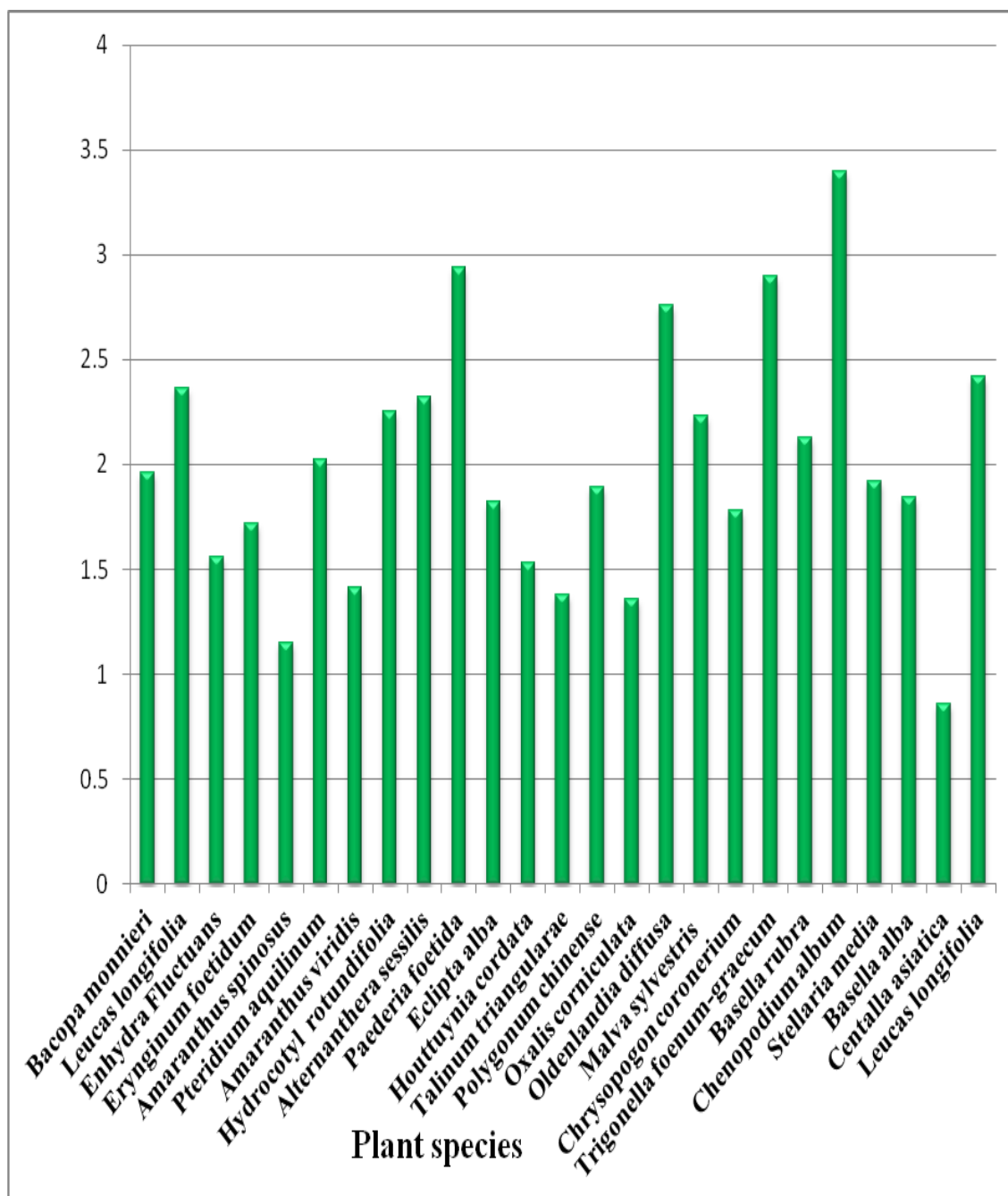


Fig. 30. Total flavanoid content (gram percentage) of indigenous leafy vegetables

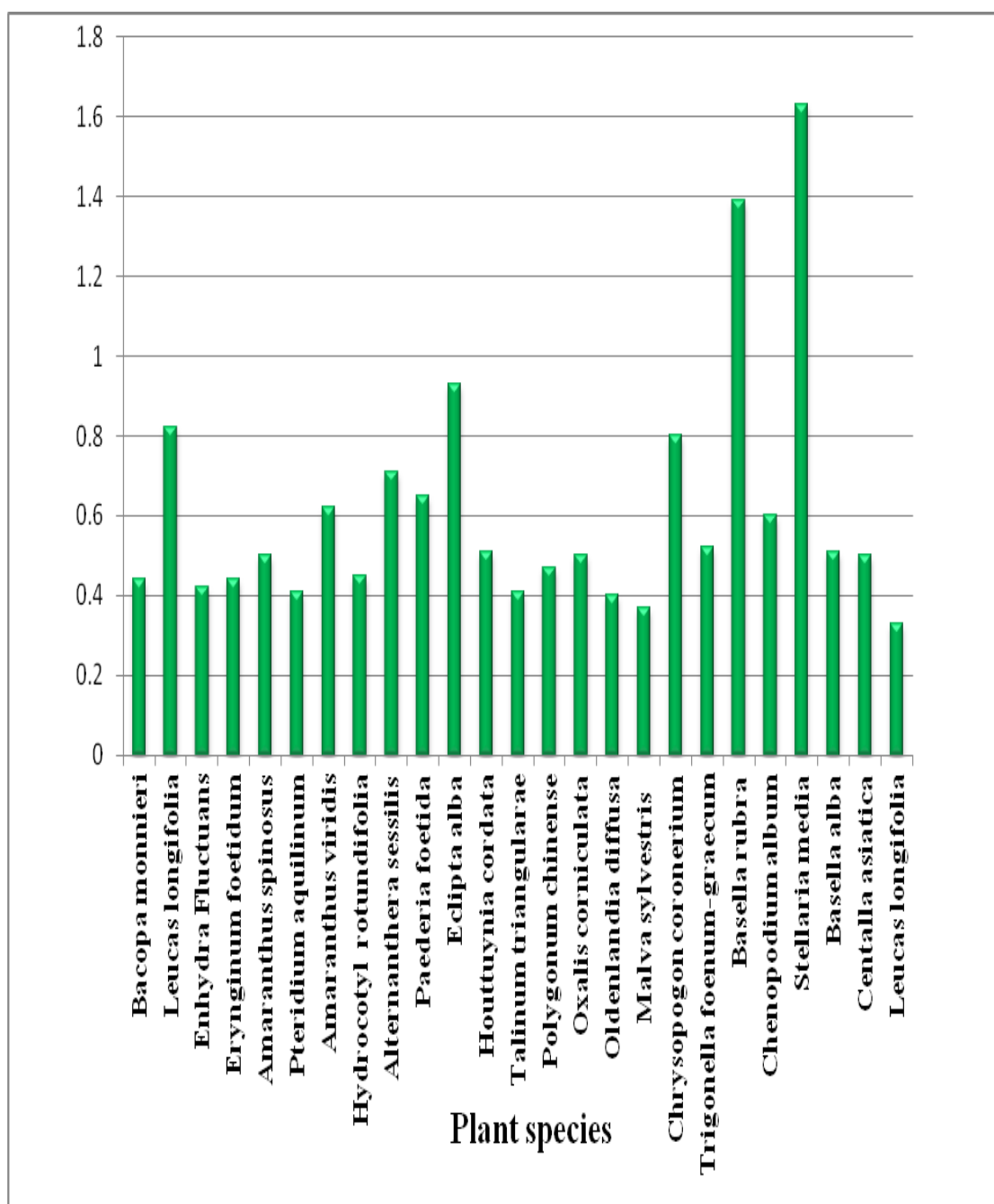


Fig. 31. Total flavanol content (milli gram percentage) of indigenous leafy vegetables

4.11 Anti oxidants of Indigenous leafy vegetables (unit min⁻¹ mg⁻¹protein)

Native plant species are used for daily diet as vegetables, spices and condiments. Some of them are also employed for traditional medicine. There are several varieties of these leafy vegetables either in the wild or under cultivation in the rural areas. Migration to urban centres has a great influence on the choice of vegetables used as food. High consumption of vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are considered to be related to the various antioxidants contained in them. The oxidative stress experienced by a tissue, organelle or organ results from the balance between the production and removal of potentially damaging reactive oxygen species (ROS). Since the ROS removal rate is mostly controlled by a variety of low molecular weight antioxidants, there is a great interest in determining their levels and the way they are related to pathological states and whether they can be controlled by an antioxidant-rich diet and/or by the ingestion of an antioxidant supplementation (Urquiaga and Leighton, 2000; Crozier *et al.*, 2000).

Antioxidants have become synonymous with good health. They are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by a variety of sources including pesticides, smoking and exhaust fumes. Destroying free radicals may help fight cancer, heart disease, stroke and other immune compromising diseases (Aruoma, 2003). Many of the natural antioxidants, especially flavonoids, seem to be very important in the prevention of these diseases. Fruits and vegetables have long been viewed as a rich source of antioxidant compounds. Health officials have been urging consumers for years to eat more fruits and vegetables in order to gain the health benefits of antioxidants. Epidemiological studies show that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames *et al.*, 1993). Spices and herbs are recognized as sources of natural antioxidants that can protect from oxidative stress and thus play an important role in the chemoprevention of diseases that have their etiology and pathophysiology in reactive oxygen species (Velioglu *et al.*, 1988; Lee and Shibamoto, 2002; Dragland *et al.*, 2003; Odukoya *et al.*, 2005a-b; Atawodi, 2005).

The data of free radical scavenging activity, super oxide dismutase activity, catalase activity and peroxidase activity of different leafy vegetables are presented in the Table 12.

4.11.1 Free radical scavenging activity

Reactive oxygen and nitrogen free radicals are produced during immune activity, and are triggered by several environmental factors such as pollution, smoke, and sunlight. Harmful effects of these reactive species include cellular damage to RNA, DNA, proteins and lipids. In humans several diseases including those connected with the heart, lung, and the eye are associated with the accumulation of reactive oxygen and nitrogen species (ROS/RNS). Antioxidants in blood, cells, and tissue fluids play an important role in neutralizing the normal level of oxidative damage caused by these free radicals. In an effort to minimize the impact of environmental pollution on humans, identification of natural product antioxidants has become a realistic and powerful tool in the dietary and natural products industry.

Free radicals are produced in normal and or pathological cell metabolism. Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, the uncontrolled production of oxygen derived free radicals is involved in triggering many diseases such as cancer, rheumatoid arthritis, cirrhosis and arteriosclerosis as well as in degenerative processes associated with aging. Exogenous chemical and endogenous metabolic processes in the human body or in the digestive system might produce highly reactive free radicals, especially oxygen derived radicals, which are capable of oxidizing biomolecules, resulting in cell death and tissue damage (Mau *et al.*, 2001). Almost all organisms are well protected against free radical damage by oxidative enzymes such as superoxide dismutase (SOD) and catalase (CAT), or by chemicals such as α -tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione (Gulcin *et al.*, 2002). When the process of antioxidant protection becomes unbalanced, deterioration of physiological functions may occur, resulting in diseases and accelerated aging. Antioxidant food supplements may be used to help the human body to reduce oxidative damage. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells from damage brought on by oxidative stress (Cazzi *et al.*, 1997).

The data of the present investigation revealed that Bonjaluk (91.72%) had the highest free radical scavenging activity followed by Helosi sak (91.35%) and Puroi (green)

(74.81%, 10 mg/ml) had the lowest free radical scavenging activity. This might be due to genetical, soil fertility, growth condition and environmental conditions of the plant species. There is a significant difference among the plant species. The results are comparable with the results obtained by Kiruthiga *et al.* (2014) in the methanolic leaves and callus extracts of *C. pallida* exhibited highest antioxidant activity of 94.47 ± 0.54 and 96.5 ± 0.51 at 50 mg/ml concentration respectively.

4.11.2 Super oxide dismutase activity

Super oxide dismutase (SOD) out-competes damaging reactions of superoxide, thus protecting the cell from superoxide toxicity. The reaction of superoxide with non-radicals is [spin-forbidden](#). In biological systems, this means that its main reactions are with itself (dismutation) or with another biological radical such as [nitric oxide](#) (NO) or with a transition-series metal. The superoxide anion radical (O_2^-) spontaneously dismutates to O_2 and hydrogen peroxide (H_2O_2) quite rapidly ($\sim 10^5 M^{-1}s^{-1}$ at pH 7). SOD is necessary because superoxide reacts with sensitive and critical cellular targets. Super oxide radicals are inactivated by the enzyme superoxide dismutase (SOD), the only enzyme known to use a free radical as a substrate. The radical scavenging activity of SOD is effective only when it is followed by increase in activity of catalase and other peroxidases (Ramasarma, 1990).

SOD is the most indispensable enzyme for protecting the cells from the toxicity of the reactive oxygen species that are generated during aerobic respiration for energy production; it converts more toxic superoxide anion radicals to less toxic hydrogen peroxide (Vangronsveld and Clijsters, 1994).

The data of the present investigation revealed that Keheraj, Dhekia ($0.24 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the highest super oxide dismutase activity followed by Bhedailata ($0.23 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) and Saru Tangeshi, Methi, Morolia ($0.11 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the lowest super oxide dismutase activity. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results obtained by Kumar *et al.* (2012) in *Cassia fistula* ($2.29 \pm 0.325 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) and Kiruthiga *et al.* (2014) found superoxide dismutase activity of *C. pallida* fresh leaves (*in vivo*) and callus (*in vitro*) exhibited 0.78 ± 0.036 and $0.826 \pm 0.050 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$ respectively.

Table 12. Anti oxidants (on fresh weight basis) of Indigenous leafy vegetables (unit min⁻¹ mg⁻¹ protein)

Sl. No.	Local name	Botanical name	Free radical scavenging activity (% inhibition)	SOD	Catalase	Peroxidase
1	Brahmi	<i>Bacopa monnieri</i>	85.71	0.17	0.13	0.03
2	Doron	<i>Leucas longifolia</i>	80.82	0.21	0.03	0.05
3	Helosi sak	<i>Enhydra fluctuans</i>	91.35	0.15	0.005	0.06
4	Mandhania	<i>Erynginum foetidum</i>	76.69	0.21	0.03	0.07
5	Hatikhotora	<i>Amaranthus spinosus</i>	75.56	0.22	0.02	0.03
6	Dhekia	<i>Pteridium aquilinum</i>	87.21	0.24	0.005	0.04
7	Khotora	<i>Amaranthus viridis</i>	80.07	0.17	0.008	0.05
8	Saru Manimoni	<i>Hydrocotyl rotundifolia</i>	86.46	0.27	0.008	0.08
9	Matikandori	<i>Alternanthera sessilis</i>	90.22	0.15	0.027	0.03
10	Bhedailata	<i>Paederia foetida</i>	85.71	0.23	0.026	0.03
11	Keheraj	<i>Eclipta alba</i>	86.09	0.24	0.037	0.02
12	Masundori	<i>Houttuynia cordata</i>	84.21	0.17	0.016	0.03
13	Pirali paleng	<i>Talinum triangularae</i>	81.57	0.14	0.031	0.05
14	Madhuholang	<i>Polygonum chinense</i>	81.95	0.12	0.09	0.02
15	SaruTengesi	<i>Oxalis corniculata</i>	80.45	0.11	0.06	0.08
16	Bonjaluk	<i>Oldenlandia diffusa</i>	91.72	0.14	0.053	0.02
17	Laffa	<i>Malva sylvestris</i>	88.34	0.13	0.037	0.03
18	Baburi	<i>Chrysopogon coronarium</i>	89.47	0.12	0.023	0.02
19	Methi	<i>Trigonella foenum-graecum</i>	90.22	0.11	0.037	0.04
20	Puroi (red)	<i>Basella rubra</i>	88.34	0.16	0.027	0.03
21	Jilmil	<i>Chenopodium album</i>	85.38	0.16	0.04	0.04
22	Morolia	<i>Stellaria media</i>	75.18	0.11	0.027	0.02
23	Puroi (green)	<i>Basella alba</i>	74.81	0.15	0.03	0.01
24	Bor Manimoni	<i>Centalla asiatica</i>	78.94	0.17	0.037	0.03
25	Bor Tangeshi	<i>Oxallis acetosella</i>	89.47	0.16	0.043	0.03
SED(±)			1.90	0.01	0.02	0.01

Sl. No.	Local name	Botanical name	Free radical scavenging activity (% inhibition)	SOD	Catalase	Peroxidase
		CD at 5 %	3.25*	0.02*	0.03*	0.02*

4.11.3 Catalase activity

Catalase is a tetrahedral protein, constituted by four heme groups which catalyze the dismutation of hydrogen peroxide in water and oxygen. An enzyme that splits hydrogen peroxide to yield oxygen and water; an important part of the body's antioxidant defences. Catalase is a common enzyme present in most all living organisms and a very potent catalyst. Catalase converts the harmful by product of metabolism- hydrogen peroxide into water and oxygen. It also oxidizes toxins in the body such as formaldehyde, alcohols, phenols and formic acid. Catalase is present in every known animal and is vital to life processes (Scandalios, 1987).

Catalase is a common antioxidant enzyme which is produced naturally in almost all living organisms. The reactions in catalyses are important to life, for example, it helps the body to break down hydrogen peroxide (a powerful and harmful oxidizing agent) into oxygen and water, thus it prevents the accumulation of carbon dioxide bubbles in the blood. Catalase is a very potent enzyme; one molecule of catalase can decompose millions of hydrogen peroxide molecules into oxygen and water. It also uses hydrogen peroxide to oxidize potentially harmful toxins in the body including formaldehyde, formic acid, alcohol, and phenol.

The data of the present investigation revealed that Bhrami ($0.13 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the highest catalase activity followed by Bonjaluk ($0.053 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) and Helosisak, Dhekia ($0.005 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the lowest catalase activity. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. The results obtained by Kiruthiga *et al.* (2014) in *Crotalaria pallid* is $0.434 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$ and Kumar *et al.* (2012) reported that catalase activity was in *Cassia fistula* $1.196 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$.

4.11.4 Peroxidase activity

A peroxidase is one of a number of [enzymes](#) that act as catalysts to allow a variety of biological processes to take place. Specifically, they promote the oxidation of various compounds using naturally occurring peroxides, especially hydrogen peroxide (H_2O_2), which are reduced, forming water. Peroxides are created as byproducts of various biochemical reactions within organisms, but can cause damage as they are oxidizing agents.

Peroxidases break these compounds down into harmless substances by adding hydrogen, obtained from another molecule known as a donor molecule in a reduction-oxidation (redox) reaction in which the peroxide is reduced to form water, and the other molecule is oxidized. There are a large number of these enzymes, and they are found in plants and animals, including humans.

A number of peroxidases are found in plants, where they may help minimize damage caused by stress factors or insect pests. When plants are subjected to stress such as drought or high temperatures or to attack by pests, this tends to result in the release of reactive [oxygen](#) species (ROS). These are forms of oxygen, or compounds of this element, including hydrogen peroxide, in which the oxygen is very reactive, and can damage or kill cells. It is thought that peroxidases remove ROS, helping prevent damage (Schenk *et al.*, 2000).

In humans, and other [mammals](#), a group of these enzymes called glutathionases, which contain the element selenium, are found both within and outside cells. Some of these catalyze reactions involving H_2O_2 , while others use peroxide compounds of [lipids](#) (fats and oils). Their main role seems to be to remove these potentially harmful oxidizing agents. Peroxidases in the saliva also enable redox reactions between H_2O_2 and chemicals called thiocyanates, producing compounds that can kill potentially harmful microorganisms. [Thyroid](#) peroxidase releases [iodine](#) from nutrients to form essential thyroid hormones.

The data of the present investigation revealed that Saru Manimoni, Madhohuleng ($0.08 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the highest peroxidase activity followed by Mandhania ($0.07 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) and Puroi (green) ($0.01 \text{ unit min}^{-1} \text{ mg}^{-1} \text{ protein}$) had the lowest peroxidase activity. This might be due to genetical, soil fertility, growth status and environmental conditions of the plant species. There is a significant difference among the plant species. These

results are less than the results obtained by Kiruthiga *et al.* (2014) in *Crotalaria pallid* is 0.229 unit min⁻¹ mg⁻¹ protein.

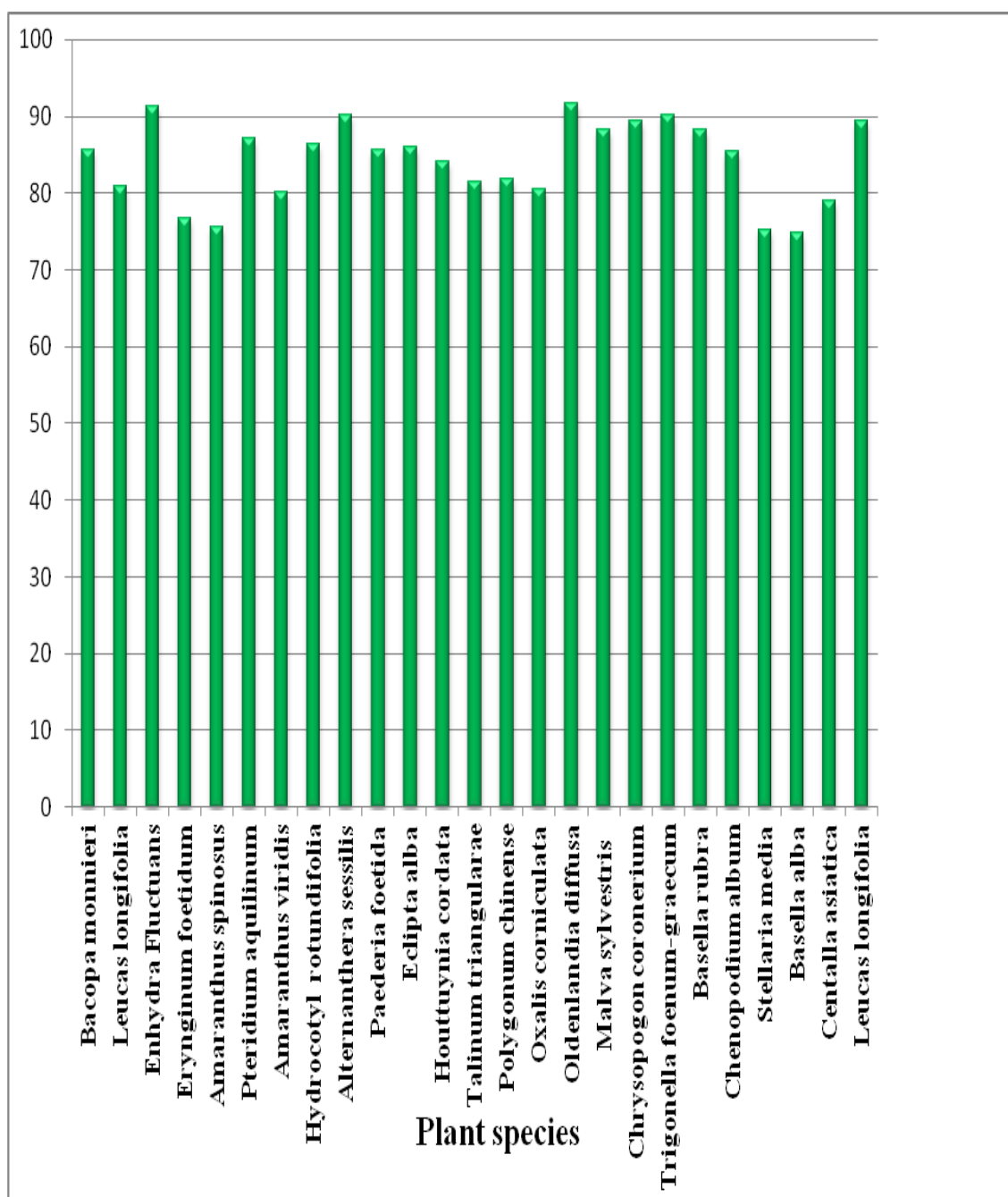


Fig. 32. Free Radical Scavenging Activity (percentage) of indigenous leafy vegetables

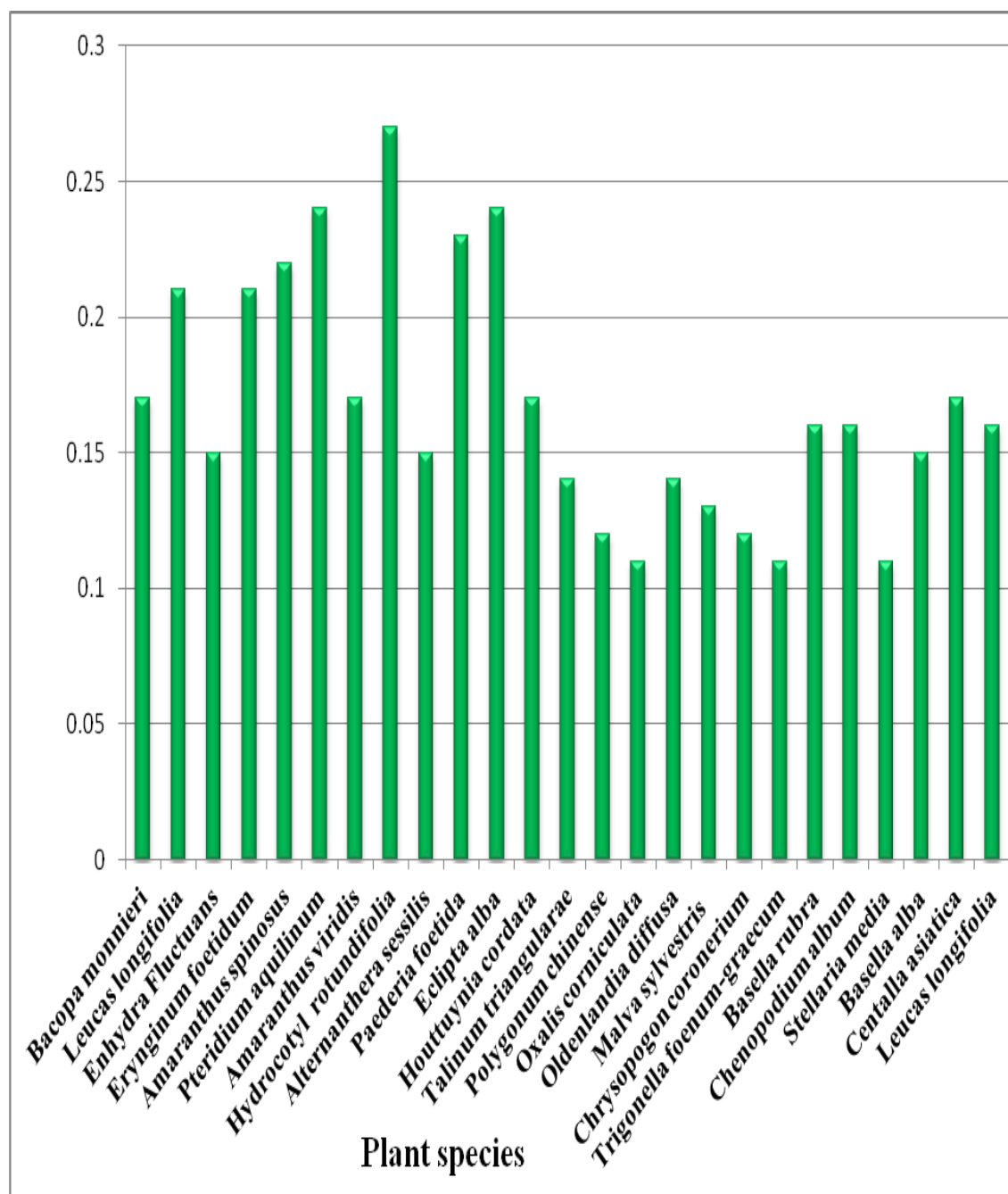


Fig. 33. Super oxide dismutase activity of indigenous leafy vegetables

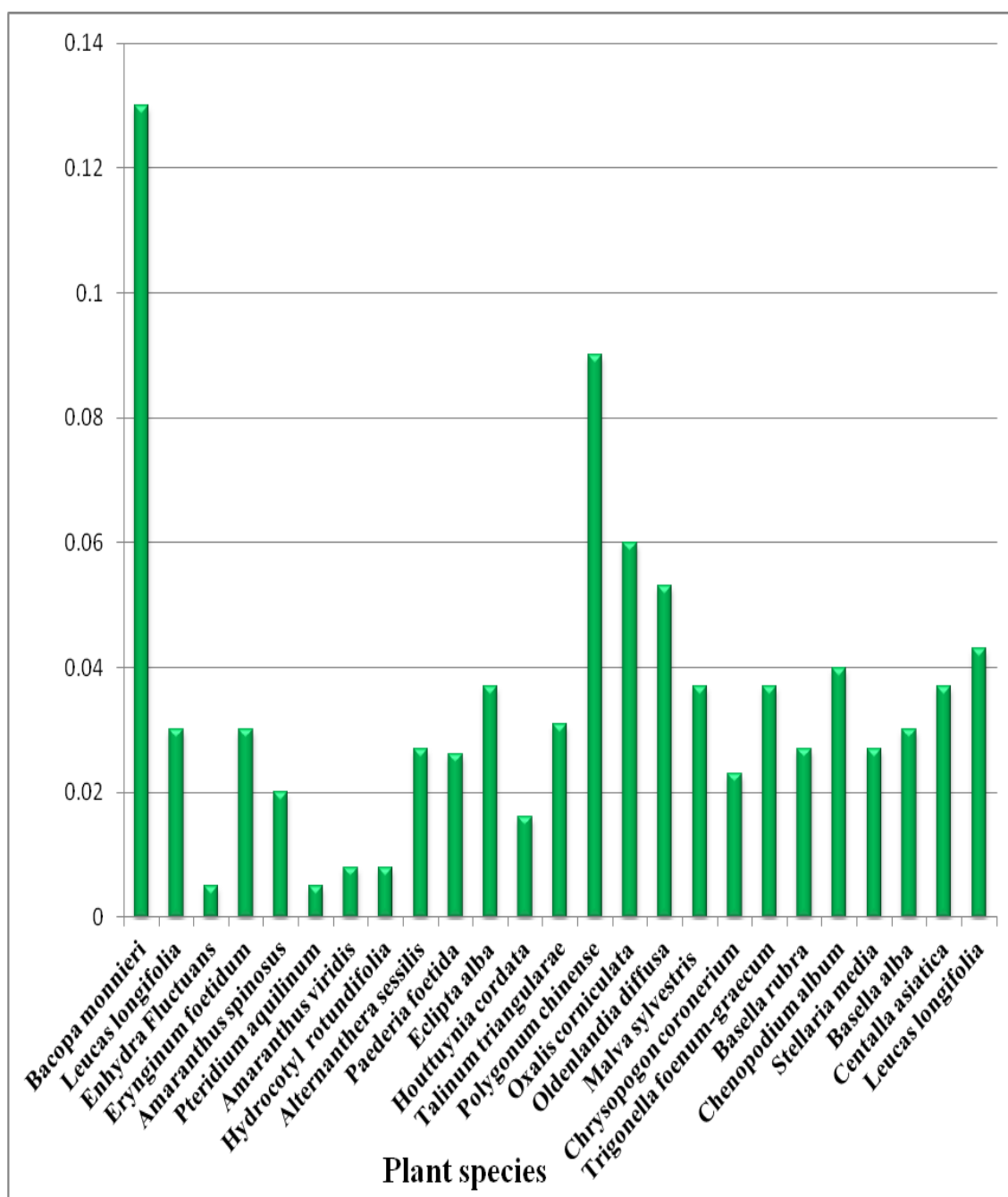


Fig. 34. Catalase activity of indigenous leafy vegetables

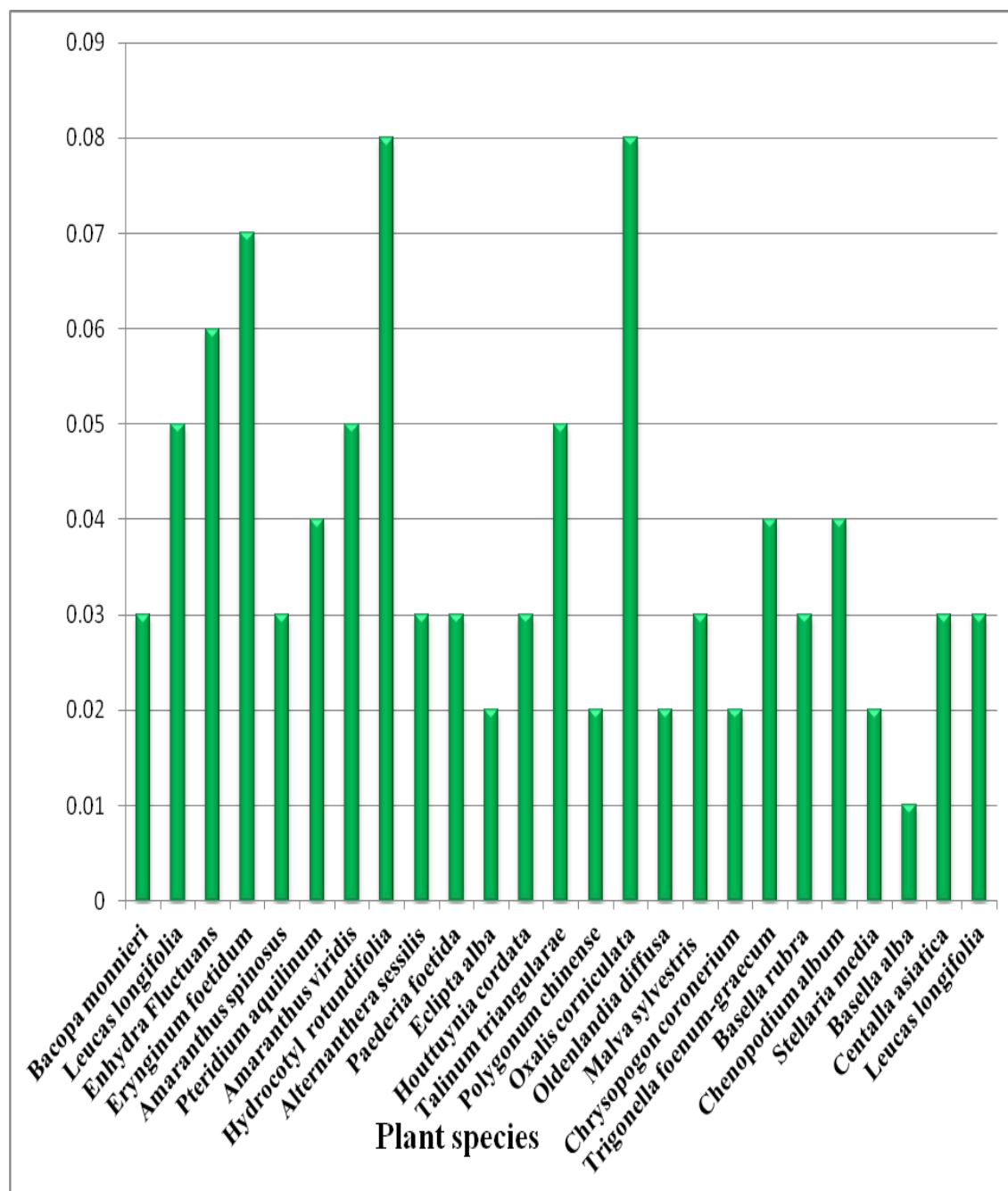


Fig. 35. Peroxidase activity of indigenous leafy vegetables

4.12 Randomly amplified polymorphic DNA (RAPD) analysis of few indigenous leafy vegetables

DNA fingerprinting is a test to identify and evaluate the genetic information called [DNA](#) in a person's cells. It is called a "fingerprint" because it is very unlikely that any two people would have exactly the same DNA information, in the same way that it is very unlikely that any two people would have exactly the same physical fingerprint. The test is used to determine whether a family relationship exists between two people, to identify organisms causing a disease, and to solve crimes.

DNA fingerprinting is a way of identifying a specific individual, rather than simply identifying a species or some particular trait. It is also known as genetic fingerprinting or [DNA profiling](#). DNA fingerprinting is currently used both for identifying paternity or maternity and for identifying criminals or victims. DNA fingerprinting uses a specific type of DNA sequence, known as a [microsatellite](#), to make identification much easier. Microsatellites are short pieces of DNA which repeat many times in a given person's DNA. In a given area, microsatellites tend to be highly variable, making them ideal for DNA fingerprinting. By comparing a number of microsatellites in a given area, one can identify a person relatively easily.

RAPD fingerprints are very useful and promising for genotype identification (Caetans Anolles *et al.*, 1991) population and pedigree analysis, phylogenetic studies and genetic mapping.

Isozyme analysis has gained wide applications in many fields of study, including systematics, population genetics, genomic organization, plant breeding, sociobiology and basic genetics. Apart from isozyme electrophoresis, many novel techniques of molecular analyses have been developed since the invention of the polymerase chain reaction (PCR) in 1987. Random amplified polymorphic DNA (RAPD) is a powerful PCR-based technique (William *et al.*, 1990). Genetic analysis using RAPD as markers can be applied to any organisms one wants to study, even if they have not been studied at the molecular level before. Extensive uses of this technique bring about a revolution in genetic analysis of plants and animals or even micro-organisms. RAPD can be used in fingerprinting, genome analysis, gene mapping, pedigree assessment, population genetics, phylogenetic analysis, and germplasm evaluation (Demeke *et al.*, 1992; Hendrick, 1992; Dweikat *et al.*, 1993; Williams *et al.*, 1990; Buren *et al.*, 1994; Caetan-Anollés

et al., 1991; Aboelwafa and Shimoda, 1995; Graham and McNicol, 1995; Rieseberg and Gerber, 1995).

Information on genetic diversity and relationships within and among crop species and their wild relatives is essential for the efficient utilization of plant genetic resource collections. Several molecular approaches have been employed to assess genetic diversity and relationships, but isozyme or random amplified polymorphic DNA (RAPD) data can be generated faster and with less labor than other methods, such as RFLP (restriction fragment length polymorphism) and the use of microsatellites. However, isozyme studies are limited by the numbers of enzymes and loci that can be resolved, and reveal only genetic changes in coding regions of the genome that have resulted in an altered amino-acid sequence. In many cases, lack of allozyme polymorphism may further restrict its application in plant germplasm classification. In contrast, RAPD analysis enables the detection of informative genetic markers at a large number of loci in both coding and non-coding regions of the genome (Williams *et al.*, 1990). However, the dominant nature of RAPD markers requires that more individuals and loci be sampled, compared to co-dominant markers such as RFLP and isozymes (Lynch and Milligan, 1994), and the low homology between co-migrating fragments from different species may limit its application in phylogenetic studies at the interspecific level (Thormann *et al.*, 1994; Rieseberg, 1996). Thus, for comparative studies of crops and their wild relatives in plant germplasm collections, a complementary approach using both isozymes and RAPDs may be more appropriate for generating accurate estimates of genetic diversity and relationships than either method used alone.

Twenty five number of plant species have been studied during the present investigation. Out of these only four was selected for molecular investigation, since these four plants were occupying position in the ranking of biochemical constituents based on consideration of one to fourth position. Matikandori occupying first position in the ranking having nine highest biochemical constituents viz. ash, crude fat, phosphorous, copper, tannin, starch, total flavanol, free radical scavenging activity, total phenol. Helosi sak occupying second position in the ranking having seven highest biochemical constituents viz. iron, potassium, sodium, starch, total soluble sugar, vitamin C, peroxidase activity. Jilmil occupying third position in the ranking having five highest biochemical constituents viz. potassium, calcium, total flavanoid, crude protein, oxalate. Hatikhotura occupying third position in the ranking having five highest

biochemical constituents' viz. potassium, starch, total alkaloid, tanin, crude protein. Remaining plants were occupying lower position in the ranking of biochemical constituents, that is why, only four plants were selected for molecular investigation occupying one to third position in the ranking.

4.12.1 Extraction of genomic DNA from *Alternanthera* spp. landraces (Matikandori)

DNA purity has been implicated as one of the most important factor in RAPD reproducibility. And, as such it is desirable to optimize a method of DNA isolation that allows fast extraction of DNA ensuring uniformity of yield and purity of extracted DNA and with minimum shearing. The protocol employed in the present investigation could yield appreciable amount of good quality DNA with minimum shearing (Plate 1).

Spectrophotometric Analysis

A common practice in molecular biology is to perform a quick assessment of the purity of nucleic acid samples by determining the ratio of spectrophotometric absorbance of the sample at 260nm to that of 280 nm. The assessment of the purity of a nucleic acid sample is often performed by a procedure commonly referred to as the A_{260}/A_{280} ratio. Pure DNA and RNA preparations expected to have A_{260}/A_{280} ratios of 1.8 and 2.0 respectively (Maniatis *et al.*, 1982). Quantification of nucleic acids is commonly performed to determine the average concentrations of [DNA](#) or [RNA](#) present in a mixture, as well as their purity.

The DNA extracted using protocol described by Murray and Thomson (1980) showed good yield of DNA. The yield varied from 11.69 μg to 511.84 μg per gram of fresh leaves. The A_{260} to A_{280} ratio was also found to be less than 2 in most of the samples or more than 2 in few samples. The least ratio was recorded as 1.27 and highest as 2.22. The A_{260} to A_{280} ratio was found 2.22 in M_9 which was highest and 1.27 in M_{17} which was lowest. The spectral analysis data is shown in (Table 13).

Table 13. Quantification and purity determination of genomic DNA by spectral analysis

Sample	A_{260/280}	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
M ₁	1.75	665.5	79.86
M ₂	1.82	770.70	92.48
M ₃	1.80	134.80	16.18
M ₄	1.79	436.60	52.39
M ₅	1.95	2328.00	279.36
M ₆	1.70	97.40	11.69
M ₇	1.68	389.50	46.74
M ₈	1.86	3280.00	393.60
M ₉	2.22	1072.10	128.65
M ₁₀	2.16	1682.30	201.88
M ₁₁	2.13	1802.60	216.31
M ₁₂	1.78	3753.00	450.36
M ₁₃	1.87	1323.90	158.87
M ₁₄	1.89	1957.50	234.90
M ₁₅	1.45	4246.90	509.63
M ₁₆	1.40	4151.30	498.16
M ₁₇	1.27	2478.60	297.43
M ₁₈	1.52	4265.30	511.84
M ₁₉	1.37	4263.20	511.58
M ₂₀	1.54	4139.20	496.70
M ₂₁	1.81	3416.60	409.99
M ₂₂	1.53	4218.90	506.27
M ₂₃	1.56	4184.40	502.13
M ₂₄	1.70	3926.40	471.17

M = Matikandori

4.12.2 Polymorphism of *Alternanthera* spp. (Matikandori) land races revealed by RAPD

Twenty decamer random primers were used to generate RAPD finger prints of the studied land races. The PCR products were resolved in 1.5% agarose gel to separate the amplified DNA fragments according to molecular size and visualized under ultra violet after staining with ethidium bromide. The maximum level of polymorphism was generated by primer OPA-04 and was recorded as 100% followed by primer OPB-05 (87.50%). Primer OPB-06 showed monomorphic banding pattern. The percentage of polymorphism generated is summarized in Table 14. Representative RAPD banding patterns of *Alternanthera* spp. land races are shown in (Plate 2, 3, 4, 5, 6). Well resolved reproducible bands were considered for the analysis and scored as present (1) or absent (0) for further bioinformatics analysis to obtain Jacard's Correlation Coefficient Similarity matrix (Table 21) and the dendrogram (Fig. 36). Similarity matrix value ranged from 0.14 (M₁, M₆) to 0.67 (M₁₉, M₂₀) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.14) were observed between M₁ and M₆ and lowest genetic distance were observed between M₁₉ and M₂₀ (0.67) among the land races. High genetic variability within land races and significant differentiation between land races indicate rich genetic resource of a species. In our RAPD analysis, significant genetic polymorphism was observed among the land races of *Alternanthera sessilis*. From the dendrogram (Fig. 36) it is also clearly seen that the land races has higher genetic diversity among themselves and in few cases two or more landraces could resolved into one group.

Group I (M₁ and M₂) has 26% similarity with the rest of population. Group II (M₃, M₄, M₁₂ and M₁₃) has 43% similarity with the rest of population. Group III (M₅, M₉, M₁₀ and M₁₁) has 41% similarity with the rest of population. Group IV (M₂₁ and M₂₂) has 33% similarity with the rest of population. Group V (M₁₆, M₁₇, M₁₈, M₁₉ and M₂₀) has 42% similarity with the rest of population. Group VI (M₁₄, M₁₅ and M₁₆) has 41 % similarity with the rest of population and group VII (M₁₇, M₁₈, M₂₃ and M₂₄) has 32% similarity with the rest of population.

The land race M₁ and M₆ contain the highest genetic variation, while land race M₁₉ and M₂₀ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race M₁ and M₆ could provide the opportunities for selecting as parental future breeding program to improve *Alternanthera sessilis*.

Table 14. Percent polymorphism revealed by RAPD among landraces of *Alternanthera* spp.

Sl. No.	Primer	% Polymorphism
1`	OPA-01	80.00
2	OPA-02	83.34
3	OPA-03	47.80
4	OPA-04	100.00
5	OPA-05	50.00
6	OPA-06	45.14
7	OPA-07	23.32
8	OPA-08	46.76
9	OPA-09	87.60
10	OPA-10	80.34
11	OPB-01	88.89
12	OPB-02	72.14
13	OPB-03	66.67
14	OPB-04	87.50
15	OPB-05	87.50
16	OPB-06	0.00
17	OPB-07	85.71
18	OPB-08	83.40
19	OPB-09	57.24
20	OPB-10	60.15

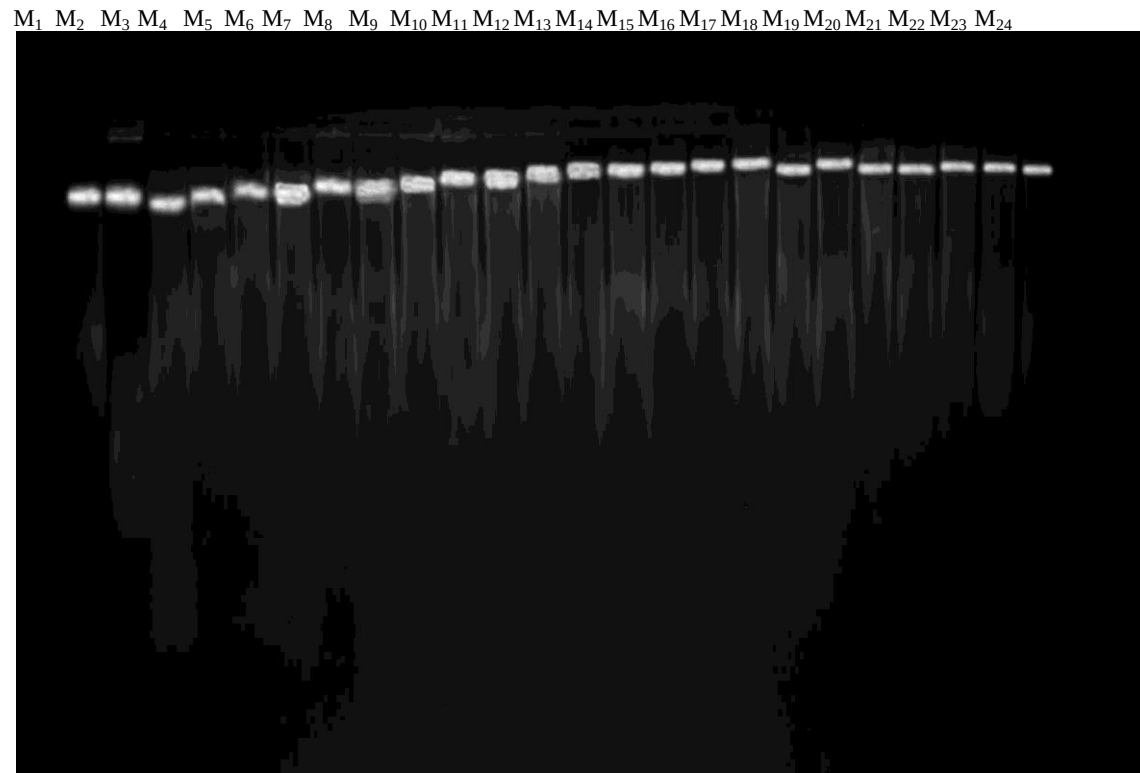


Plate 1. Genomic DNA of of *Alternanthera* spp. (Matikandori) land races

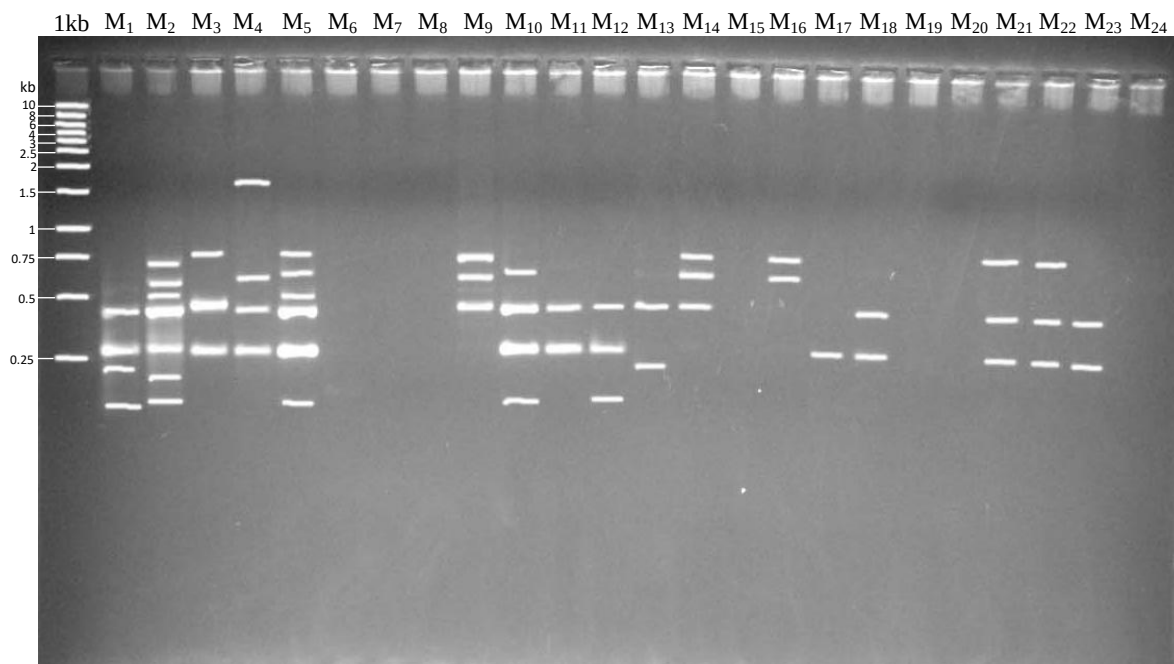


Plate 2. OPA-01(5' CAGGCCCTTC 3')

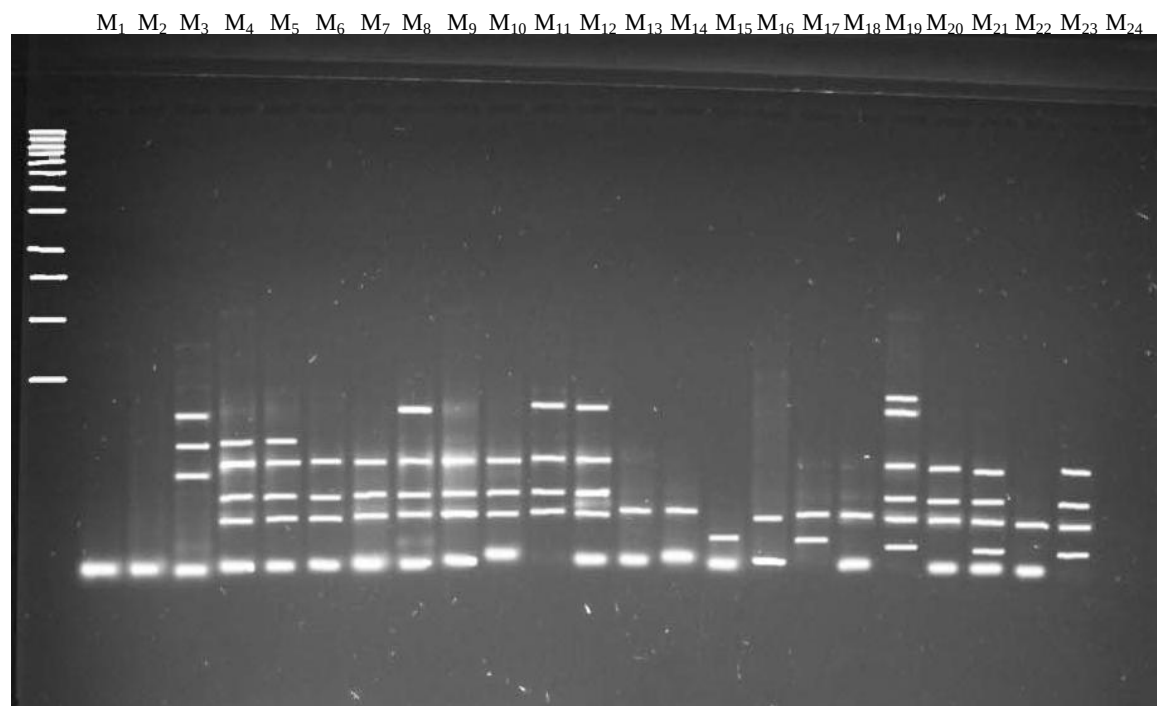


Plate 3. OPA-04 (5' AATCGGGCTG 3')

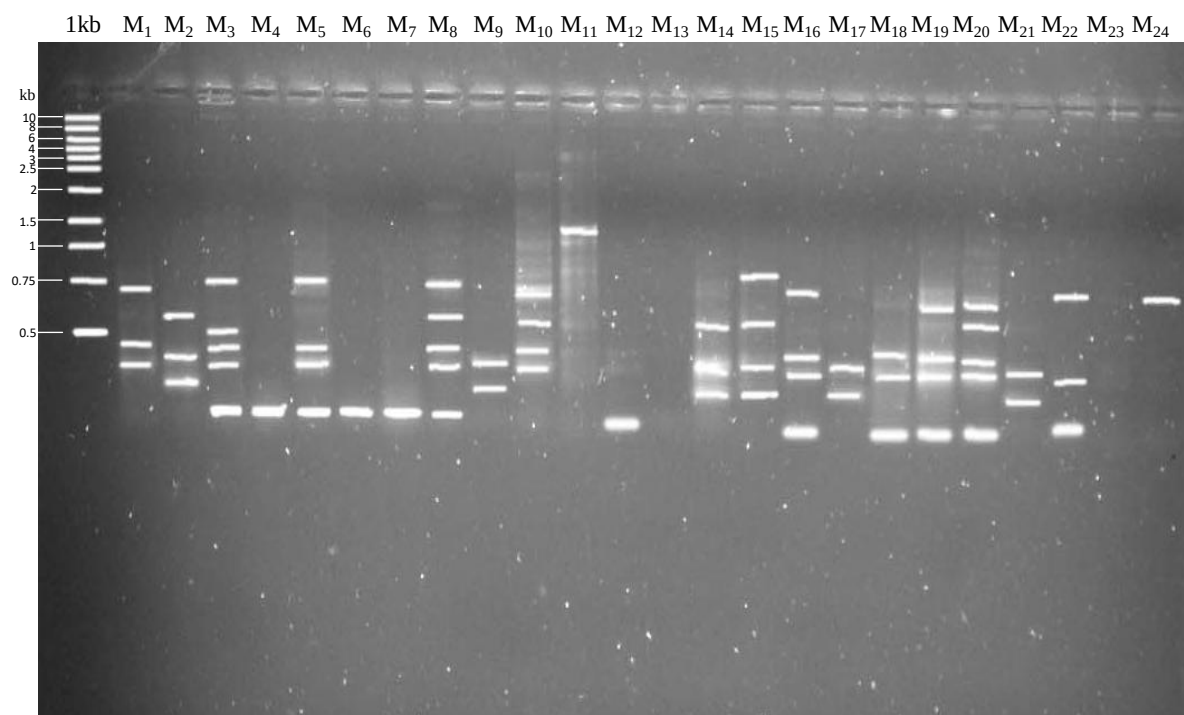


Plate 4. OPB-01 (5' GTTTCGCTCC 3')

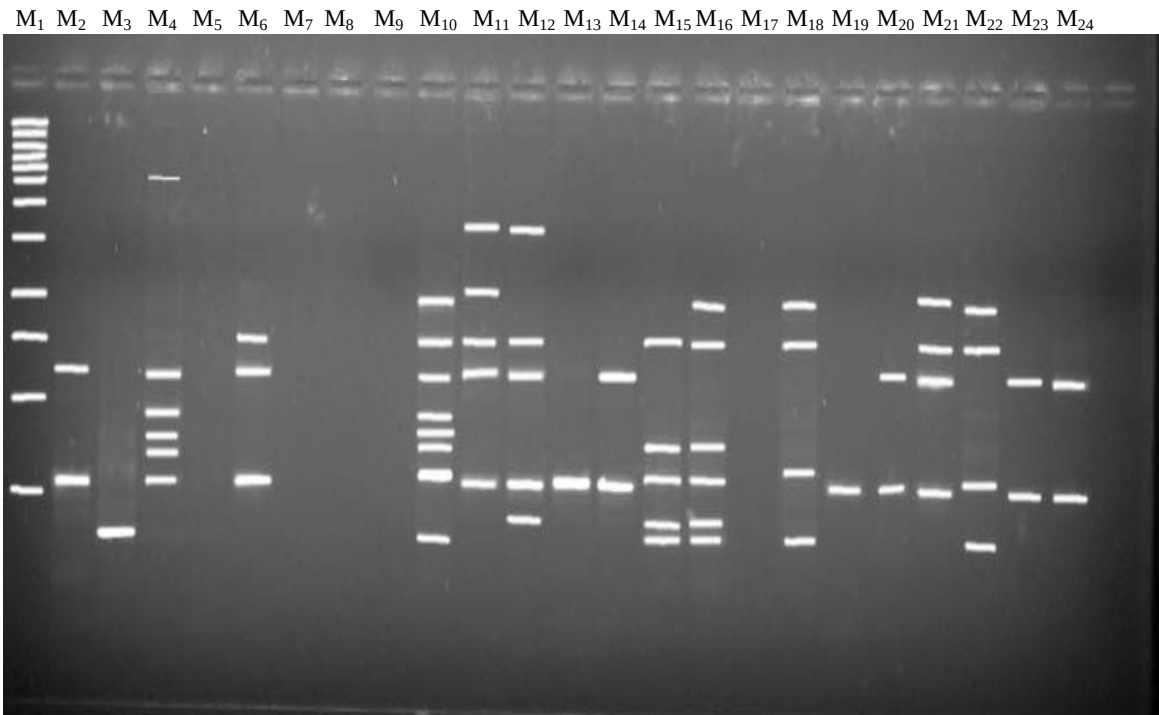


Plate 5. OPB-05 (5' TGCGCCCTTC 3')

1kb M₁ M₂ M₃ M₄ M₅ M₆ M₇ M₈ M₉ M₁₀ M₁₁ M₁₂ M₁₃ M₁₄ M₁₅ M₁₆ M₁₇ M₁₈ M₁₉ M₂₀ M₂₁ M₂₂ M₂₃ M₂₄

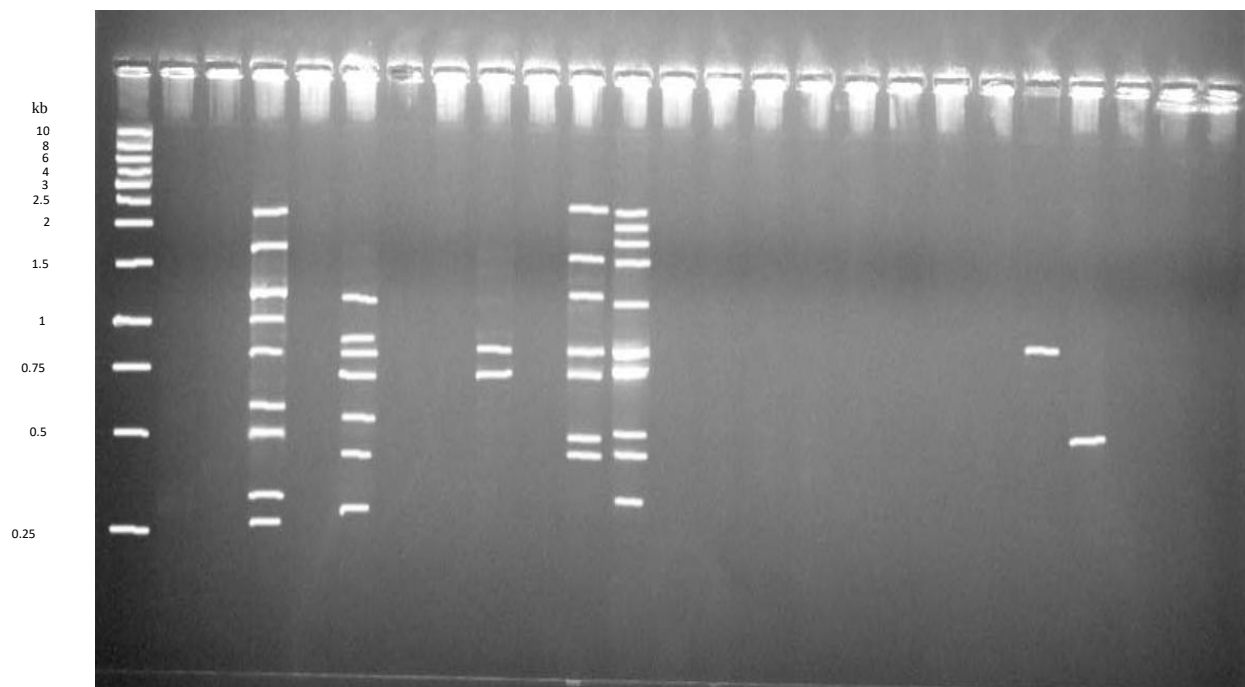


Plate 6. OPB-07 (5' GGTGACGCAC 3')

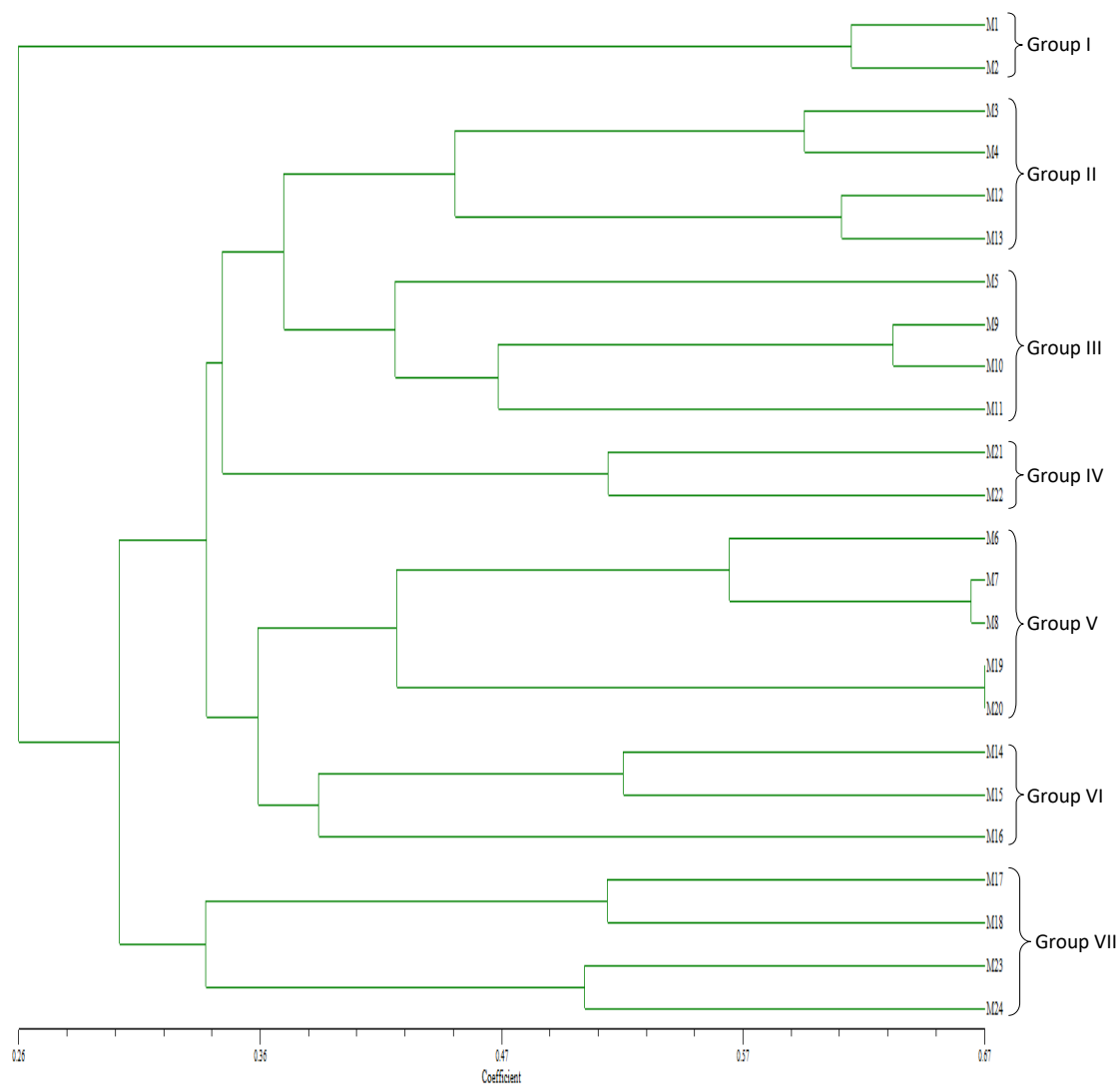


Fig. 36. RAPD dendrogram based on Jaccard's coefficient of similarity using NTSYS programme

4.12.3 Extraction of genomic DNA from *Amaranthus* spp. (Hatikhotura)

DNA purity has been implicated as one of the most important factor in RAPD reproducibility. And, as such it is desirable to optimize a method of DNA isolation that allows fast extraction of DNA ensuring uniformity of yield and purity of extracted DNA and with minimum shearing. The protocol employed in the present investigation could yield appreciable amount of good quality DNA with minimum shearing (Plate 7).

Spectrophotometric Analysis

The DNA extracted using protocol described by Murray and Thomson (1980) showed good yield of DNA. The yield varied from 291.44 µg to 546.77 µg per gram of fresh leaves. The A_{260} to A_{280} ratio was also found to be less than 2 in all the samples. The least ratio was recorded as 1.11 and highest as 1.89. The A_{260} to A_{280} ratio was found 1.89 in H₂ which was highest and 1.11 in H₁₃ which was lowest. The spectral analysis data is shown in (Table 15).

Table 15. Quantification and purity determination of genomic DNA by spectral analysis

Sample	$A_{260/280}$	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
H ₁	1.31	4511.92	541.43
H ₂	1.89	2963.47	355.62
H ₃	1.34	4474.92	536.99
H ₄	1.81	3127.62	375.31
H ₅	1.81	3231.63	387.80
H ₆	1.82	2850.91	342.11
H ₇	1.18	4521.89	542.63
H ₈	1.35	4376.69	525.20
H ₉	1.76	3405.89	408.71
H ₁₀	1.84	2428.67	291.44
H ₁₁	1.31	4406.54	520.88
H ₁₂	1.19	4457.34	534.89
H ₁₃	1.11	4556.45	546.77
H ₁₄	1.61	4121.00	494.52
H ₁₅	1.12	4409.91	529.19
H ₁₆	1.19	4514.89	541.79
H ₁₇	1.12	4485.86	538.30

Sample	A _{260/280}	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
H ₁₈	1.53	4281.57	513.79
H ₁₉	1.31	4412.35	529.48
H ₂₀	1.59	3967.84	476.14
H ₂₁	1.25	4371.85	524.62
H ₂₂	1.31	4466.08	535.93
H ₂₃	1.20	4480.50	537.66
H ₂₄	1.22	4519.20	542.30

H = Hatkhotura

4.12.4 Polymorphism of *Amaranthus* spp. (Hatikhotura) land races revealed by RAPD

Twenty decamer random primers were used to generate RAPD finger prints of the studied land races. The PCR products were resolved in 1.5% agarose gel to separate the amplified DNA fragments according to molecular size and visualized under UV after staining with ethidium bromide. The maximum level of polymorphism was generated by primer OPB-01 and was recorded as 71.42% followed by primer OPB-07 (66.67%). Primer OPA-09 and primer OPB-03 has monomorphic banding pattern. The percentage of polymorphism generated is summarized in Table 16. The few RAPD banding patterns of *Amaranthus* spp. land races generated by various primers are shown in (Plate 8, 9, 10, 11). Well resolved reproducible bands were considered for the analysis and scored as present (1) or absent (0) for further bioinformatics analysis to obtain Jacard's Correlation Coefficient Similarity matrix (Table 22) and the dendrogram (Fig. 37). Similarity matrix value ranged from 0.10 (H₁, H₁₀) to 0.74 (H₈, H₉) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.10) was observed between H₁ and H₁₀ and lowest genetic distance (0.74) were observed between H₈ and H₉ among the land races. High genetic variability within land races and significant differentiation between land races indicate rich genetic resource of a species. In our RAPD analysis, significant genetic polymorphism was observed among the land races of *Amaranthus spinosus*.

From the dendrogram (Fig. 37) it is also clearly seen that the land races has higher degree of genetic diversity among themselves and in few cases two or more landraces could resolved into one group.

Group I (H₁ and H₂) has 50% similarity with the rest of population. Group II (H₃, H₁₁, H₄, H₁₂, H₁₃, H₁₀, H₁₄ and H₂₁) has 40% similarity with the rest of population. Group III (H₅, H₆, H₇, H₈, H₉, H₁₉ and H₂₀) has 51% similarity with the rest of population. Group IV (H₁₅, H₁₆, H₁₇ and H₁₈) has 44% similarity with the rest of population. Group V (H₂₂, H₂₃ and H₂₄) has 36% similarity with the rest of population.

The land race H₁ and H₁₀ contain the highest genetic variation, while land race H₈ and H₉ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race H₁ and H₁₀ could provide the opportunities for selecting as parental source in future breeding program to improve *Amaranthus spinosus*.

Table 16. Percent polymorphism revealed by RAPD of *Amaranthus* spp. land races

Sl. No.	Primer	% Polymorphism
1`	OPA-01	57.45
2	OPA-02	56.62
3	OPA-03	34.12
4	OPA-04	46.70
5	OPA-05	23.42
6	OPA-06	31.24
7	OPA-07	40.17
8	OPA-08	43.23
9	OPA-09	0.00
10	OPA-10	14.17
11	OPB-01	71.42
12	OPB-02	43.12
13	OPB-03	0.00
14	OPB-04	66.66
15	OPB-05	40.00
16	OPB-06	66.12
17	OPB-07	66.67
18	OPB-08	66.14
19	OPB-09	66.66

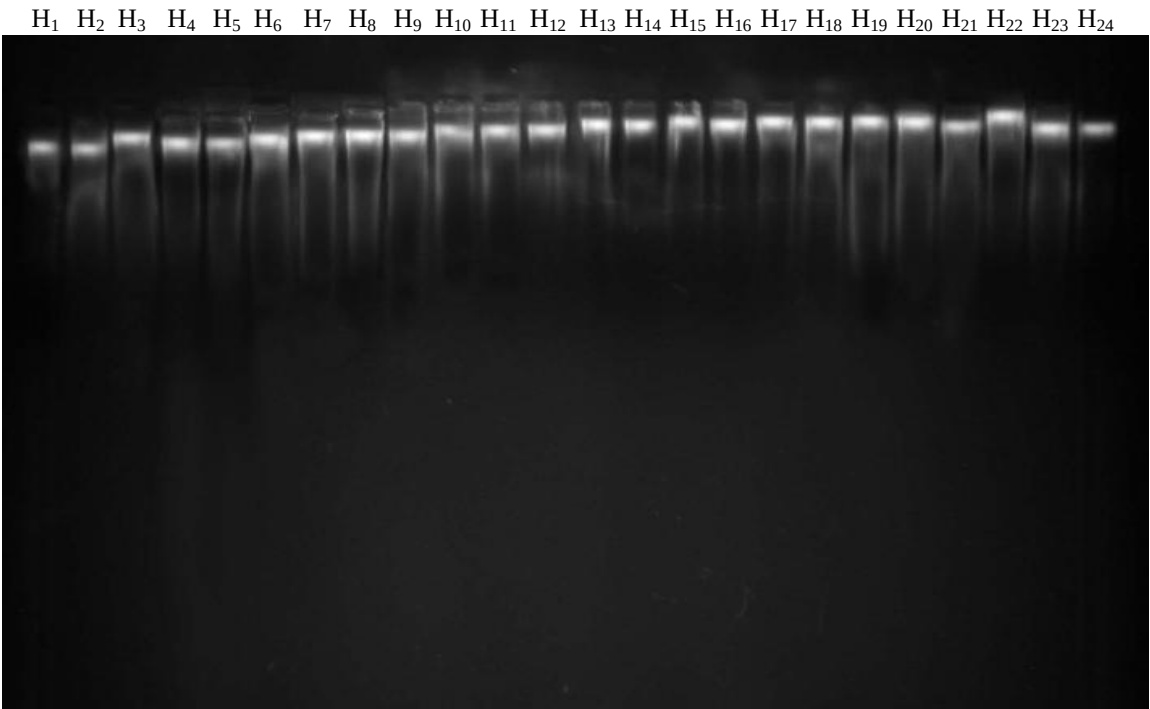


Plate 7. Genomic DNA of *Amaranthus* spp. land races

1kb H₁ H₂ H₃ H₄ H₅ H₆ H₇ H₈ H₉ H₁₀ H₁₁ H₁₂ H₁₃ H₁₄ H₁₅ H₁₆ H₁₇ H₁₈ H₁₉ H₂₀ H₂₁ H₂₂ H₂₃ H₂₄

kb
10
8
4
3
2.5
2
1.5
1
0.75
0.5
0.25
0.2
0.1

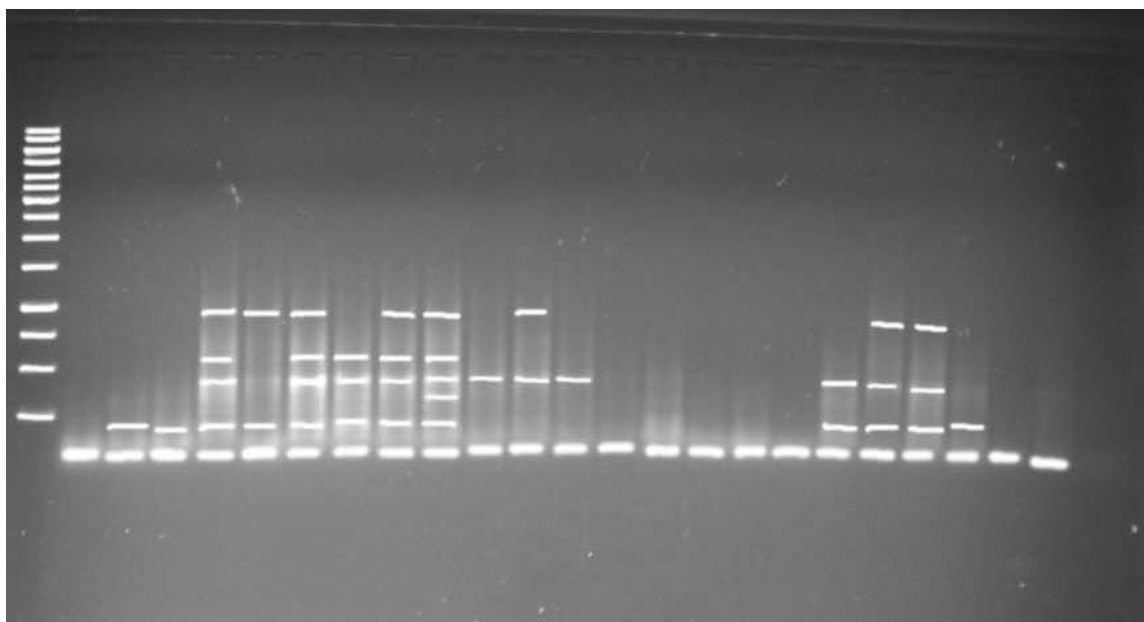


Plate 8. OPB-01 (5'GTTTCGCTCC3')

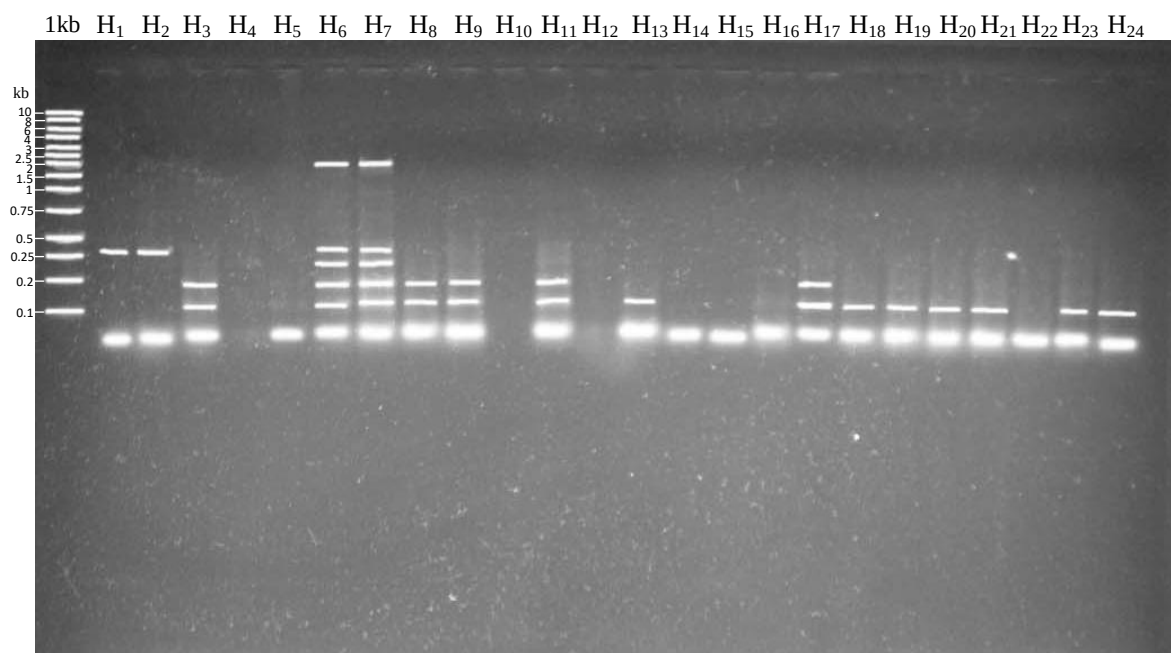


Plate 9. OPB-04 (5'GGACTGGAGT')

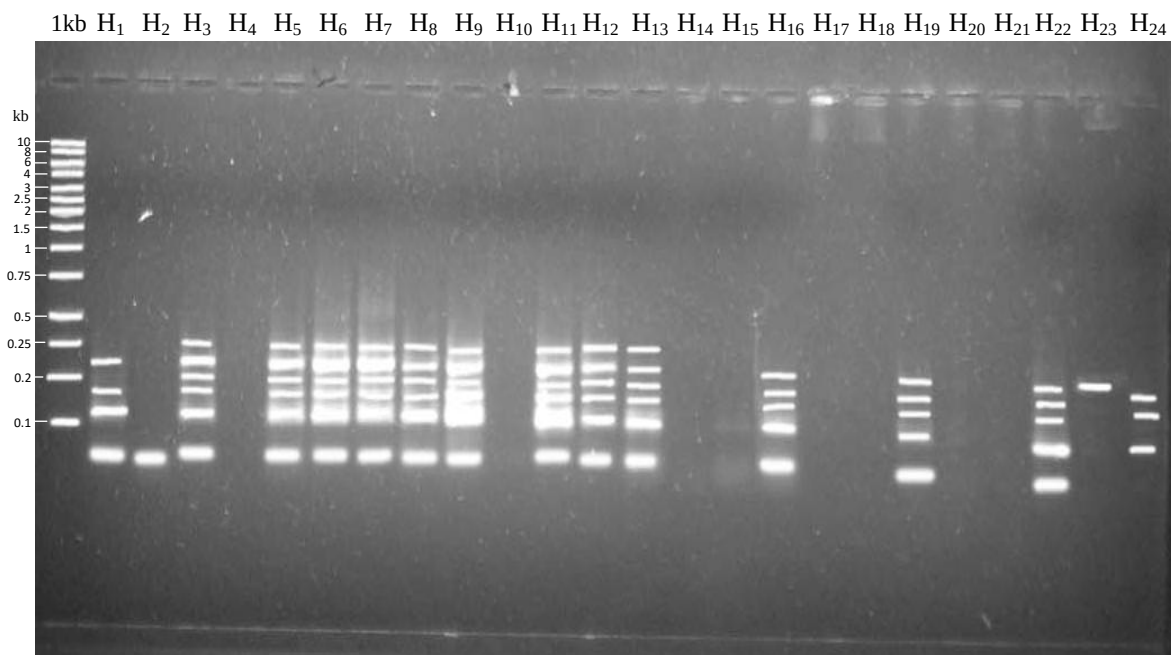


Plate 10. OPB-05 (5'TGCGCCCTTC3')

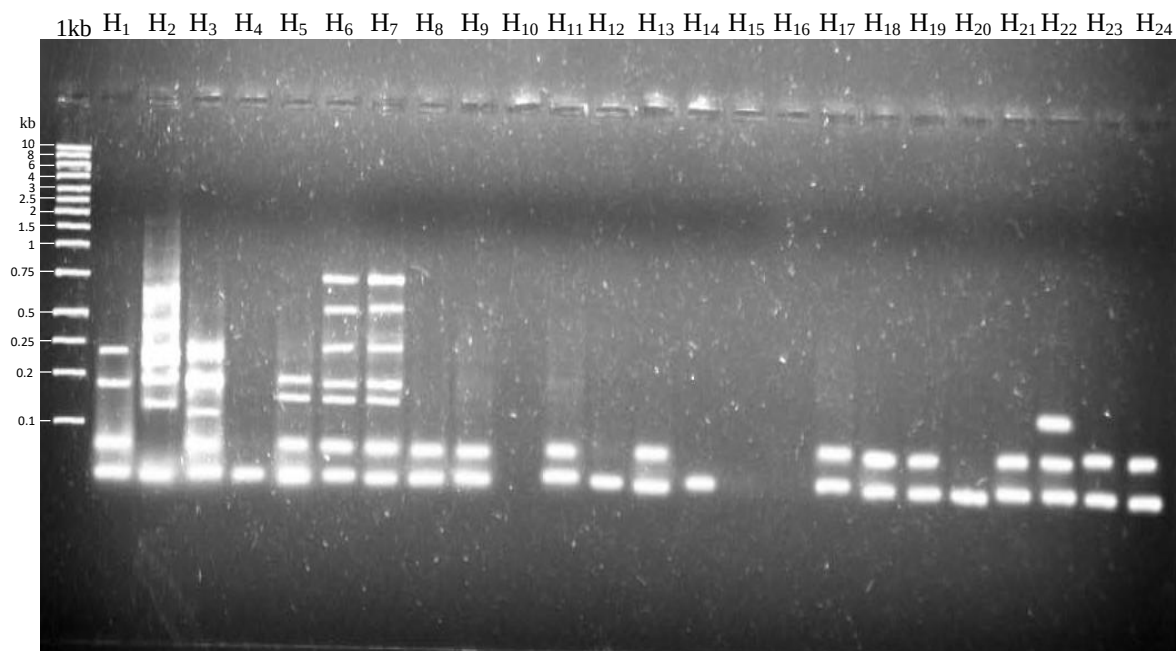


Plate 11. OPB-07 (5'GGTGACGCAG3')

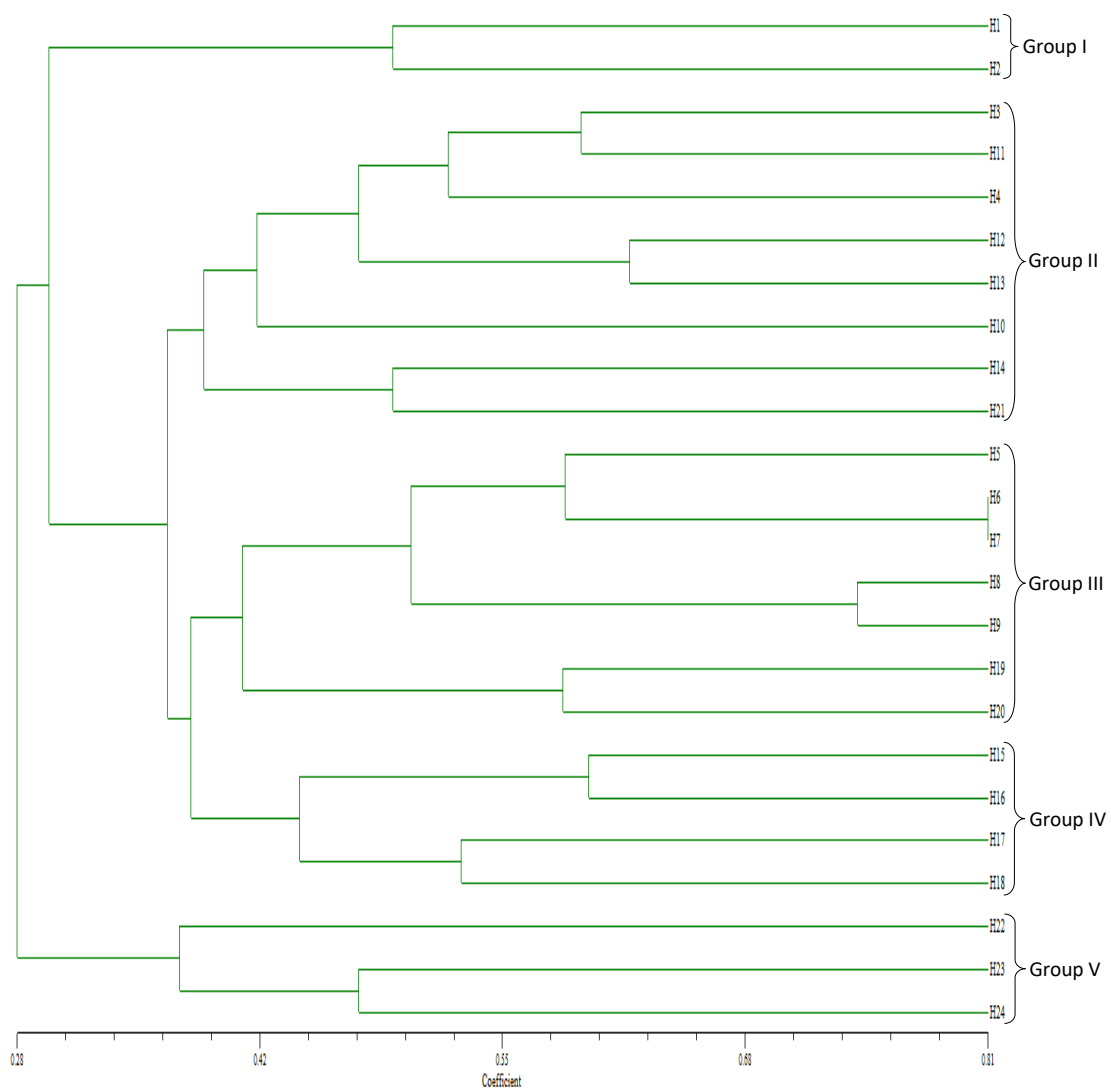


Fig. 37. RAPD dendrogram based on Jaccard's coefficient of similarity using NTSYS programme

4.12.5 Extraction of genomic DNA from *Chenopodium* spp. (Jilmil)

DNA purity has been implicated as one of the most important factor in RAPD reproducibility. And, as such it is desirable to optimize a method of DNA isolation that allows fast extraction of DNA ensuring uniformity of yield and purity of extracted DNA and with minimum shearing. The protocol employed in the present investigation could yield appreciable amount of good quality DNA with minimum shearing (Plate 12).

Spectrophotometric Analysis

The DNA extracted using the protocol described by Murray and Thomson (1980) showed good yield of DNA. The yield varied from 297.50 to 550.17 µg per gram of fresh leaves. The A_{260} to A_{280} ratio was also found to be less than 2 in most the samples and above 2 in one sample. The least ratio was recorded as 1.07 and highest as 2.03. The A_{260} to A_{280} ratio was found 2.03 in J₁₁ which was highest and 1.07 in J₁₈ which was lowest. The spectral analysis data is shown in (Table 17).

Table 17. Quantification and purity determination of genomic DNA by spectral analysis

Sample	$A_{260/280}$	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
J ₁	1.39	4515.36	541.84
J ₂	1.38	4489.36	538.72
J ₃	1.23	4584.75	550.17
J ₄	1.39	4409.42	529.13
J ₅	1.24	4257.72	510.93
J ₆	1.86	3596.00	431.52
J ₇	1.52	3049.46	365.92
J ₈	1.83	3691.23	442.95
J ₉	1.63	4243.19	509.18
J ₁₀	1.51	4384.37	526.12
J ₁₁	2.03	2748.48	329.82
J ₁₂	1.66	4136.00	496.32
J ₁₃	1.08	4278.80	513.46
J ₁₄	1.13	4203.67	504.44

Sample	A _{260/280}	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
J ₁₅	1.38	4351.90	522.23
J ₁₆	1.90	2479.17	297.50
J ₁₇	1.96	2709.84	325.18
J ₁₈	1.07	4551.20	546.14
J ₁₉	1.40	4481.14	537.74
J ₂₀	1.29	4438.15	532.58
J ₂₁	1.31	4507.77	540.93
J ₂₂	1.37	4530.89	543.71
J ₂₃	1.44	4353.05	522.37
J ₂₄	1.59	4207.67	504.92

4.12.6 Polymorphism of *Chenopodium* spp. (Jilmil) land races revealed by RAPD

Twenty decamer random primers were used to generate RAPD finger prints of the studied land races. The PCR products were resolved in 1.5% agarose gel to separate the amplified DNA fragments according to molecular size and visualized under UV after staining with ethidium bromide. The maximum level of polymorphism was generated by primer OPA-04 and was recorded as 100% followed by primer OPB-02 (77.21%). Primer OPA-02 and primer OPA-05 has showed monomorphic band. The percentage of polymorphism generated is summarized in Table 18. The few RAPD banding patterns of *Chenopodium* spp. land races generated by various primers are shown in (Plate 13, 14, 15, 16). Well resolved reproducible bands were considered for the analysis and scored as present (1) or absent (0) for further bioinformatics analysis to obtain Jacard's Correlation Coefficient Similarity matrix (Table 23) and the dendogram (Fig.38). Similarity matrix value ranged from 0.19 (J₃, J₁₇) to 0.75 (J₇, J₈) indicating high degree of genetic diversity among the landraces studied. The highest genetic distance(0.19) were observed between J₃ and J₁₇ and lowest genetic distance (0.75) were observed between J₇ and J₈ among the land races. High genetic variability within land races and significant differentiation between land races indicates rich genetic resource of a species. In our RAPD analysis, significant genetic polymorphism was observed among the land races of *Chenopodium album*.

From the dendrogram (Fig. 38) it is also clearly seen that the land races has high genetic diversity among themselves and in few cases two or more landraces could resolved into one group. Group I (J₁, J₂, J₃, J₁₅, J₁₆ and J₂₄) has 35% similarity with rest of the population. Group II (J₄, J₁₈ and J₁₉) has 50% similarity with rest of the population. Group III (J₅, J₆, J₁₄, J₁₂, J₁₃, J₂₀ and J₂₁) has 48% similarity with rest of the population. Group IV (J₇, J₈, J₉, J₁₀ and J₁₁) has 51% similarity with rest of the population. Group V (J₂₂, J₂₃ and J₁₇) has 35% similarity with rest of the population.

The land race J₃ and J₁₇ contain the highest genetic variation, while land race J₇ and J₈ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race J₃ and J₁₇ could provide the opportunities for selecting as parental source in future breeding program to improve *Chenopodium album*.

Table 18. Percent polymorphism revealed by RAPD of *Chenopodium* spp. land races

Sl. No.	Primer	% Polymorphism
1`	OPA-01	25.00
2	OPA-02	0.00
3	OPA-03	75.00
4	OPA-04	100.00
5	OPA-05	0.00
6	OPA-06	66.67
7	OPA-07	46.21
8	OPA-08	56.35
9	OPA-09	46.77
10	OPA-10	56.46
11	OPB-01	66.77
12	OPB-02	77.21
13	OPB-03	71.14
14	OPB-04	23.24
15	OPB-05	56.18
16	OPB-06	24.36

17	OPB-07	64.12
18	OPB-08	42.12
19	OPB-09	34.23
20	OPB-10	16.16

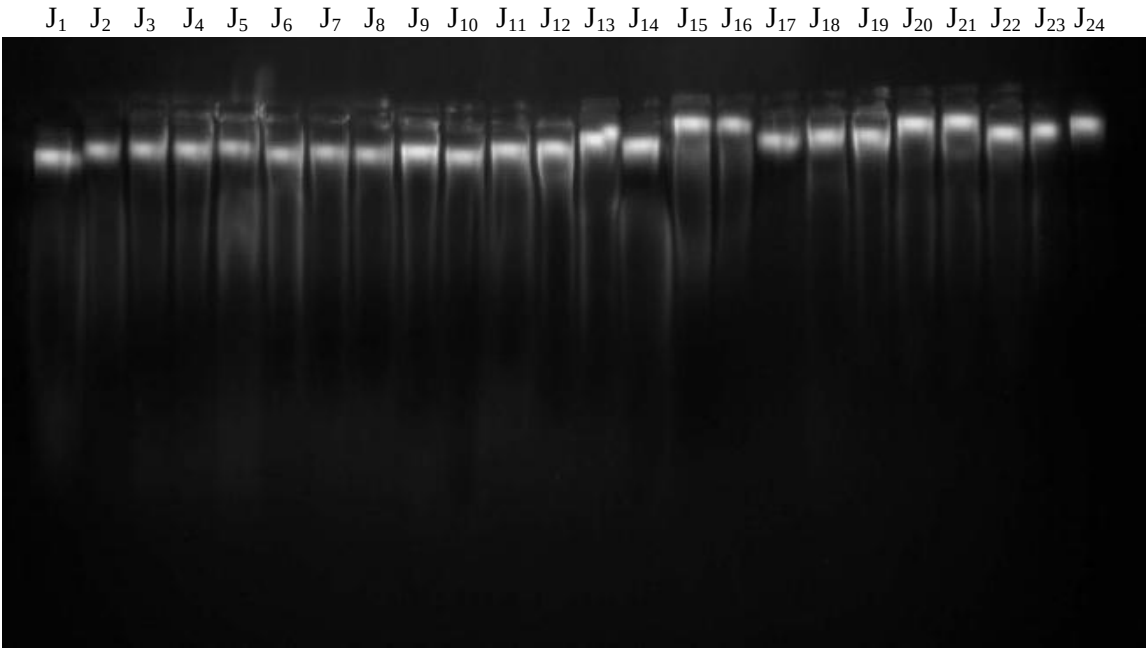


Plate 12. Genomic DNA of *Chenopodium* spp. land races

1kb J₁ J₂ J₃ J₄ J₅ J₆ J₇ J₈ J₉ J₁₀ J₁₁ J₁₂ J₁₃ J₁₄ J₁₅ J₁₆ J₁₇ J₁₈ J₁₉ J₂₀ J₂₁ J₂₂ J₂₃ J₂₄

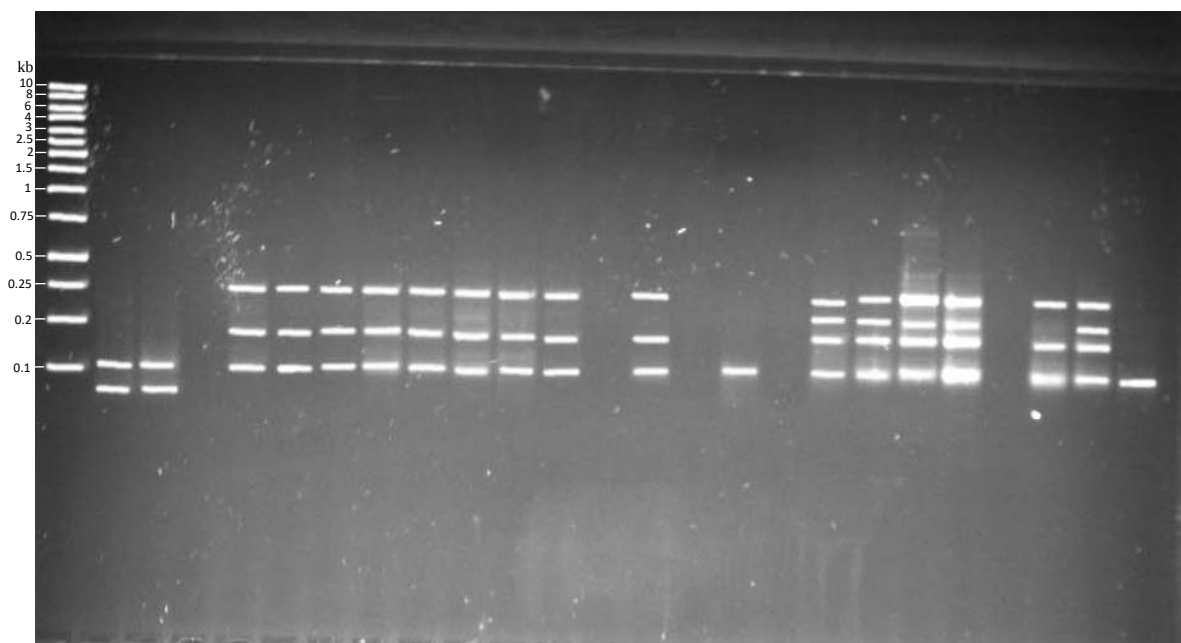


Plate 13. OPA-01 (5' CAGGCCCTTC 3')

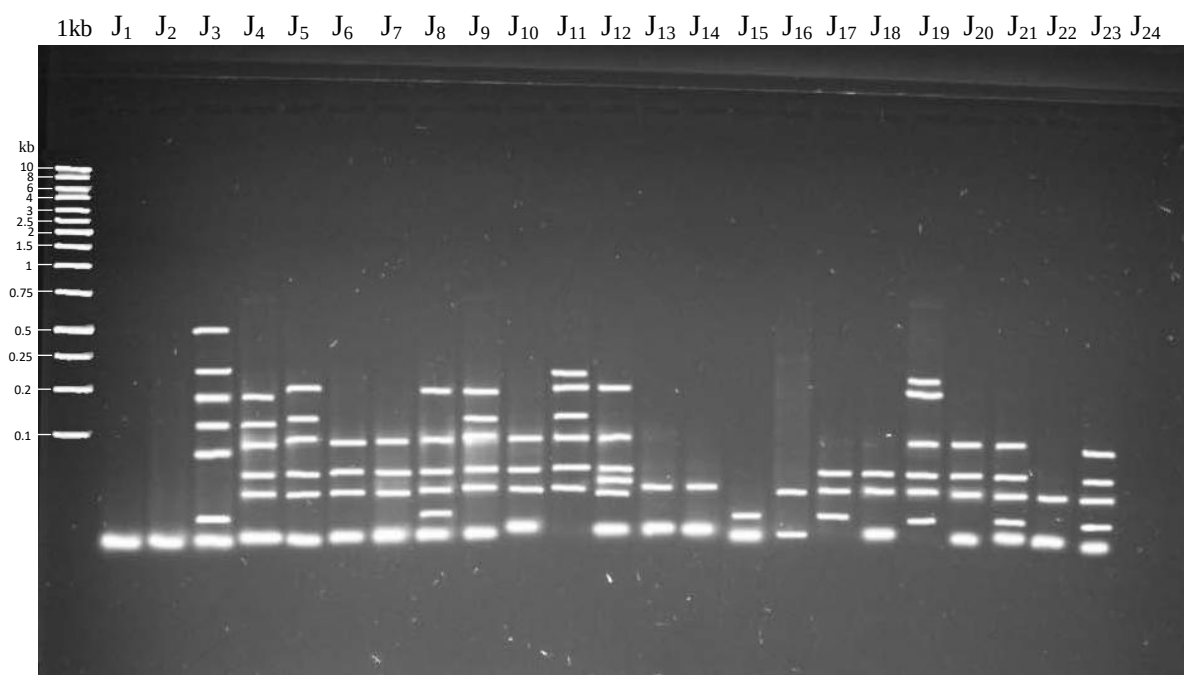


Plate 14. OPA-04 (5' AATCGGGCTG 3')

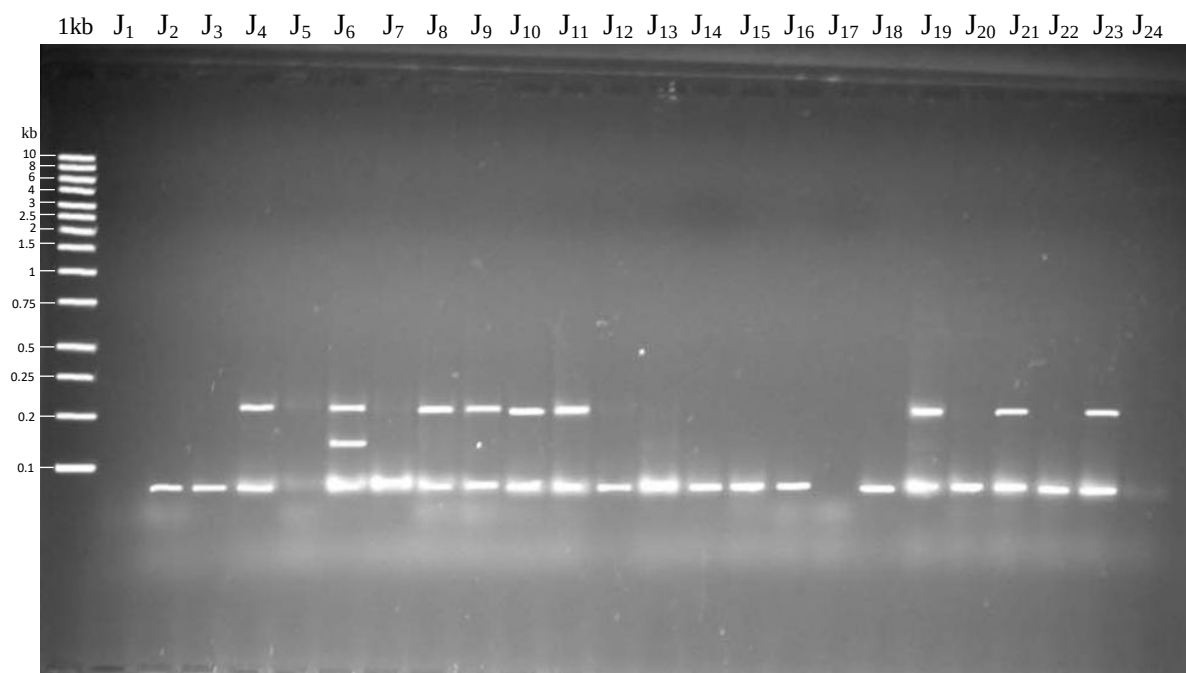


Plate 15. OPA-06 (5'GGTCCCTGAC3')

1kb J₁ J₂ J₃ J₄ J₅ J₆ J₇ J₈ J₉ J₁₀ J₁₁ J₁₂ J₁₃ J₁₄ J₁₅ J₁₆ J₁₇ J₁₈ J₁₉ J₂₀ J₂₁ J₂₂ J₂₃ J₂₄

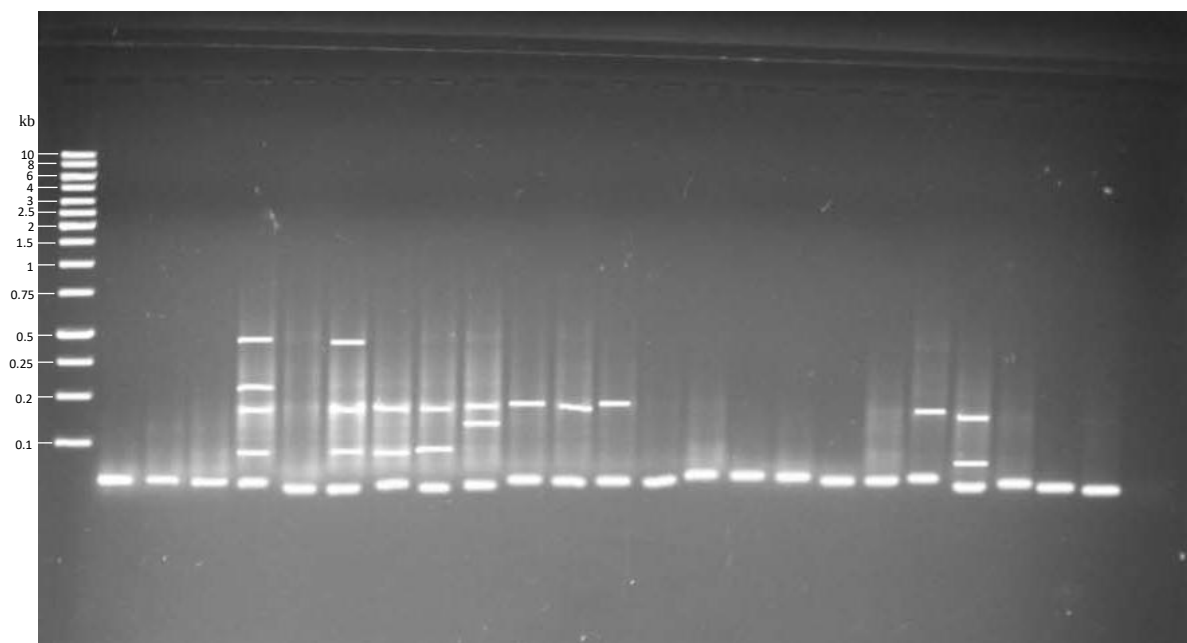


Plate 16. OPB-01 (5'GTTTCGCTCC3')

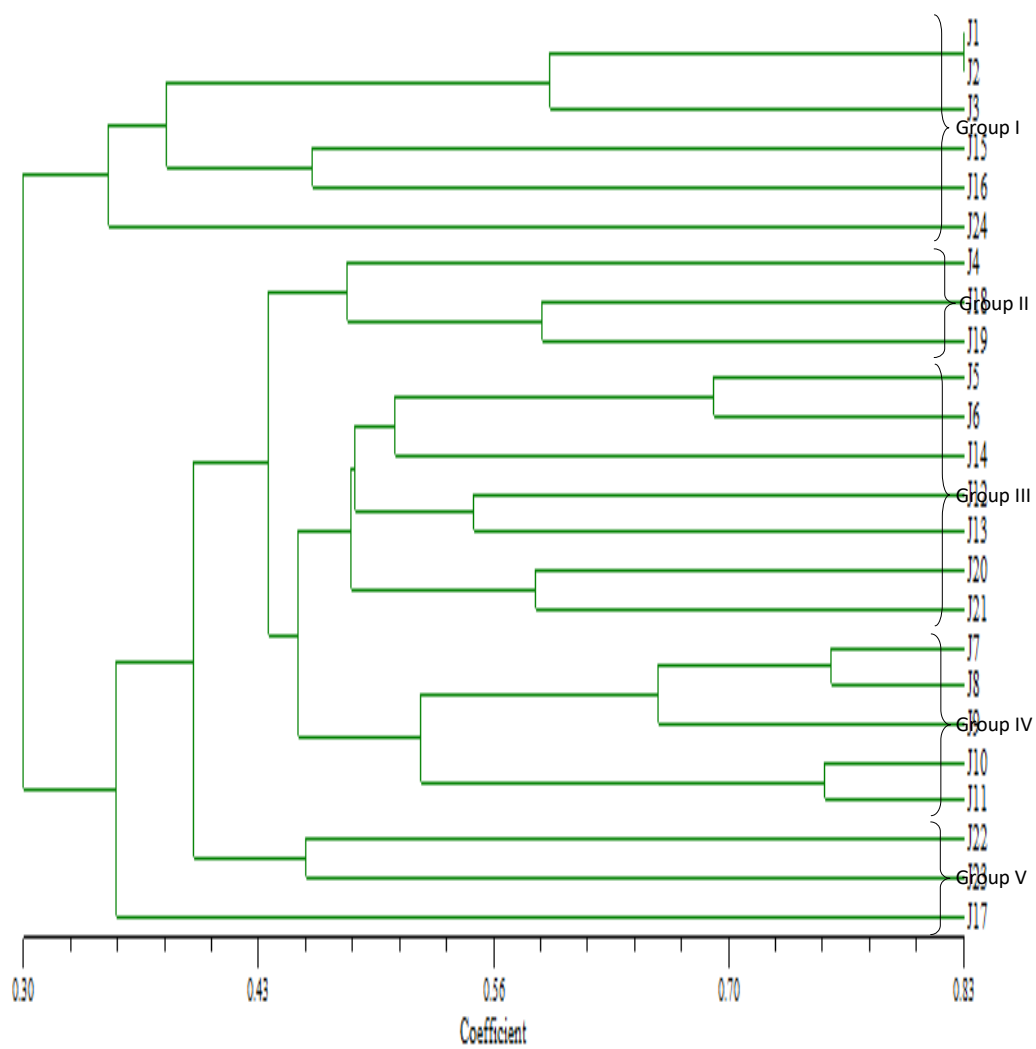


Fig. 38. RAPD dendrogram based on Jaccard's coefficient of similarity using NTSYS programme

4.12.7 Extraction of genomic DNA from *Enhydra* spp. (Helosi sak)

DNA purity has been implicated as one of the most important factor in RAPD reproducibility. And, as such it is desirable to optimize a method of DNA isolation that allows fast extraction of DNA ensuring uniformity of yield and purity of extracted DNA and with minimum shearing. The protocol employed in the present investigation could yield appreciable amount of good quality DNA with minimum shearing (Plate 17).

Spectrophotometric Analysis

The DNA extracted using the protocol described by Murray and Thomson (1980) showed good yield of DNA. The yield varied from 134.53 µg to 535.30 µg per gram of fresh leaves. The A_{260} to A_{280} ratio was also found to be less than 2 in all the samples. The least ratio was recorded as 1.03 and highest as 1.94. The A_{260} to A_{280} ratio was found 1.94 in Hs₁ which was highest and 1.03 in Hs₂₃ which was lowest. The spectral analysis data is shown in (Table 19).

Table 19. Quantification and purity determination of genomic DNA by spectral analysis

Sample	Purity % $A_{260/280}$	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
Hs ₁	1.94	1363.70	163.64
Hs ₂	1.92	1121.10	134.53
Hs ₃	1.35	4431.00	531.72
Hs ₄	1.62	4075.30	489.04
Hs ₅	1.44	4278.00	513.36
Hs ₆	1.75	3709.40	445.13
Hs ₇	1.53	4304.70	516.90
Hs ₈	1.86	3215.30	385.84
Hs ₉	1.87	3108.60	373.03
Hs ₁₀	1.87	2843.70	341.24
Hs ₁₁	1.76	3734.90	449.27
Hs ₁₂	1.76	3616.30	433.96
Hs ₁₃	1.50	4310.80	517.30
Hs ₁₄	1.14	4498.00	517.30

Sample	Purity % A _{260/280}	Concentration of DNA (ng/ µl)	Yield of DNA (µg /g of fresh leaves)
Hs ₁₅	1.25	4459.40	535.13
Hs ₁₆	1.23	4460.80	535.30
Hs ₁₇	1.52	4271.50	512.58
Hs ₁₈	1.62	4106.10	492.73
Hs ₁₉	1.20	4460.30	535.24
Hs ₂₀	1.18	4175.70	501.08
Hs ₂₁	1.17	4386.40	526.37
Hs ₂₂	1.19	3912.50	469.50
Hs ₂₃	1.03	4073.10	488.77
Hs ₂₄	1.16	4211.70	505.40

4.12.8 Polymorphism of *Enhydra* spp. (Helosi sak) land races revealed by RAPD

Twenty decamer random primers were used to generate RAPD finger prints of the studied land races. The PCR products were resolved in 1.5% agarose gel to separate the amplified DNA fragments according to molecular size and visualized under ultra violet after staining with ethidium bromide. The maximum level of polymorphism was generated by primer OPC-01 and was recorded as 100% followed by primer OPB-01 (80.00). Primer OPB-03 showed monomorphic band. The percentage of polymorphism generated is summarized in Table 20. The few RAPD banding patterns of *Enhydra* spp. land races generated by various primers are shown in (Plate 18, 19, 20, 21, 22, 23). Well resolved reproducible bands were considered for the analysis and scored as present (1) or absent (0) for further bioinformatics analysis to obtain Jacard's Correlation Coefficient Similarity matrix (Table 24) and the dendogram (Fig. 39). Similarity matrix value ranged from 0.16 (Hs₁, Hs₂₄) to 0.74 (Hs₁₀, Hs₁₁) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.16) was observed between Hs₁ and Hs₂₄ and lowest genetic distance (0.74) was observed between Hs₁₀ and Hs₁₁ among the land races. High genetic variability within land races and significant differentiation between land races indicates rich genetic resource of a species. In our RAPD analysis, significant genetic polymorphism was observed among the land races of *Enhydra fluctuans*.

From the dendrogram (Fig. 39) it is also clearly seen that the land races has high genetic diversity among themselves and in few cases two or more landraces could resolved into one group.

Group I (Hs₁ and Hs₂) has 29% similarity with rest of the population. Group II (Hs₃ and Hs₄) has 51% similarity with rest of the population. Group III (Hs₅, Hs₁₂, Hs₆, Hs₇, Hs₈, Hs₉, Hs₁₀ and Hs₁₁) has 54% similarity with rest of the population. Group IV (Hs₁₄, Hs₁₅, Hs₁₆ and Hs₁₃) has 57% similarity with rest of the population. Group V (Hs₁₇, Hs₁₈, Hs₂₂, Hs₂₃ and Hs₂₄) has 45% similarity with rest of the population. Group VI (Hs₁₉, Hs₂₀ and Hs₂₁) has 48% similarity with rest of the population.

The land race Hs₁ and Hs₂₄ contain the highest genetic variation, while land race Hs₁₀ and Hs₁₁ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race Hs₁ and Hs₂₄ could provide the opportunities for selecting as parental source in future breeding program to improve *Enhydra fluctuans*.

Table 20. Percent polymorphism revealed by RAPD of *Enhydra* spp. land races

Sl.No.	Primer	% Polymorphism
1`	OPA-01	45.00
2	OPA-02	56.00
3	OPA-03	60.00
4	OPA-04	75.00
5	OPA-05	80.00
6	OPA-06	66.67
7	OPA-07	46.24
8	OPA-08	56.00
9	OPA-09	45.15
10	OPA-10	55.00
11	OPB-01	80.00
12	OPB-02	38.35
13	OPB-03	0.00
14	OPB-04	40.00
15	OPB-05	60.00

16	OPC-01	100.00
17	OPC-02	60.00
18	OPC-03	47.00
19	OPC-04	49.50
20	OPC-05	65.17

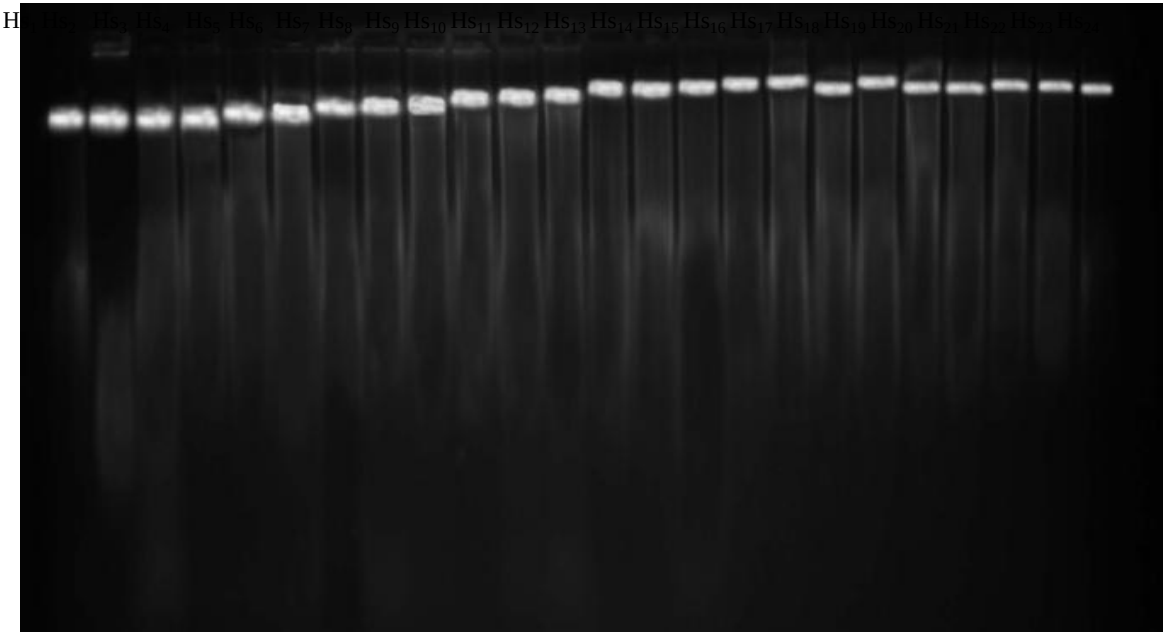


Plate 17. Genomic DNA of *Enhydra* spp. land races

1kb Hs1 Hs2 Hs3 Hs4 Hs5 Hs6 Hs7 Hs8 Hs9 Hs10 Hs11 Hs12 Hs13 Hs14 Hs15 Hs16 Hs17 Hs18 Hs19 Hs20 Hs21 Hs22 Hs23 Hs24

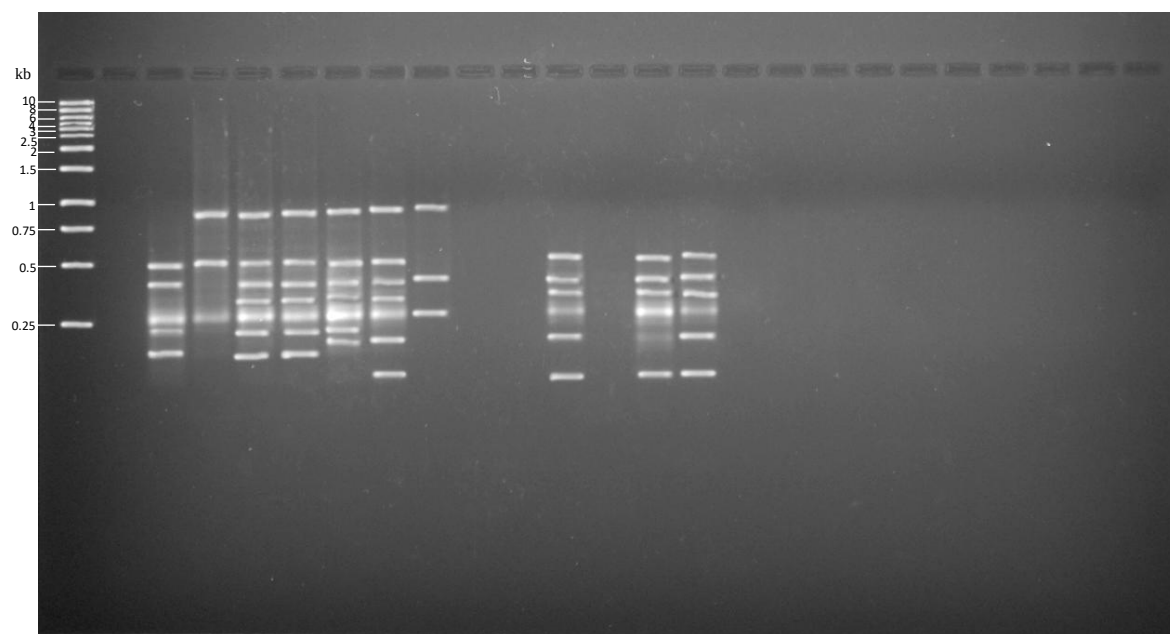


Plate 18. OPA-03 (5'AGTCAGCCAC3')

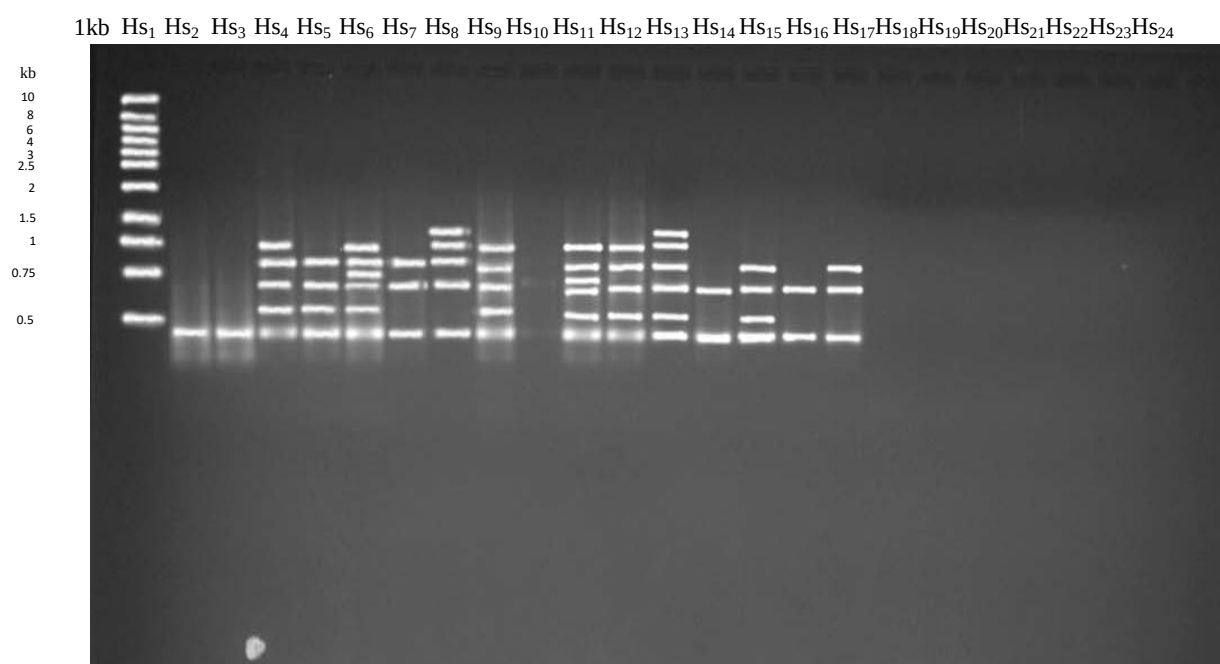


Plate 19. OPA-05 (5'AGGGGTCTTG3')

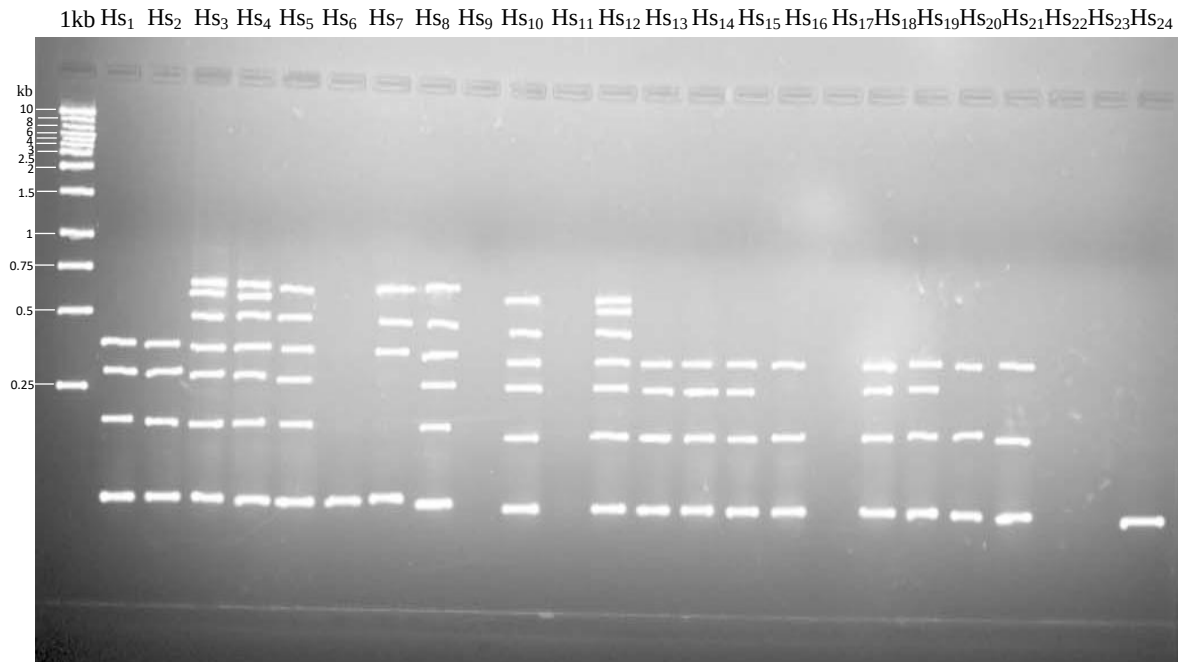


Plate 20. OPB-04 (5'GGACTGGAGT')

1kb Hs₁ Hs₂ Hs₃ Hs₄ Hs₅ Hs₆ Hs₇ Hs₈ Hs₉ Hs₁₀ Hs₁₁ Hs₁₂ Hs₁₃ Hs₁₄ Hs₁₅ Hs₁₆ Hs₁₇ Hs₁₈ Hs₁₉ Hs₂₀ Hs₂₁ Hs₂₂ Hs₂₃ Hs₂₄

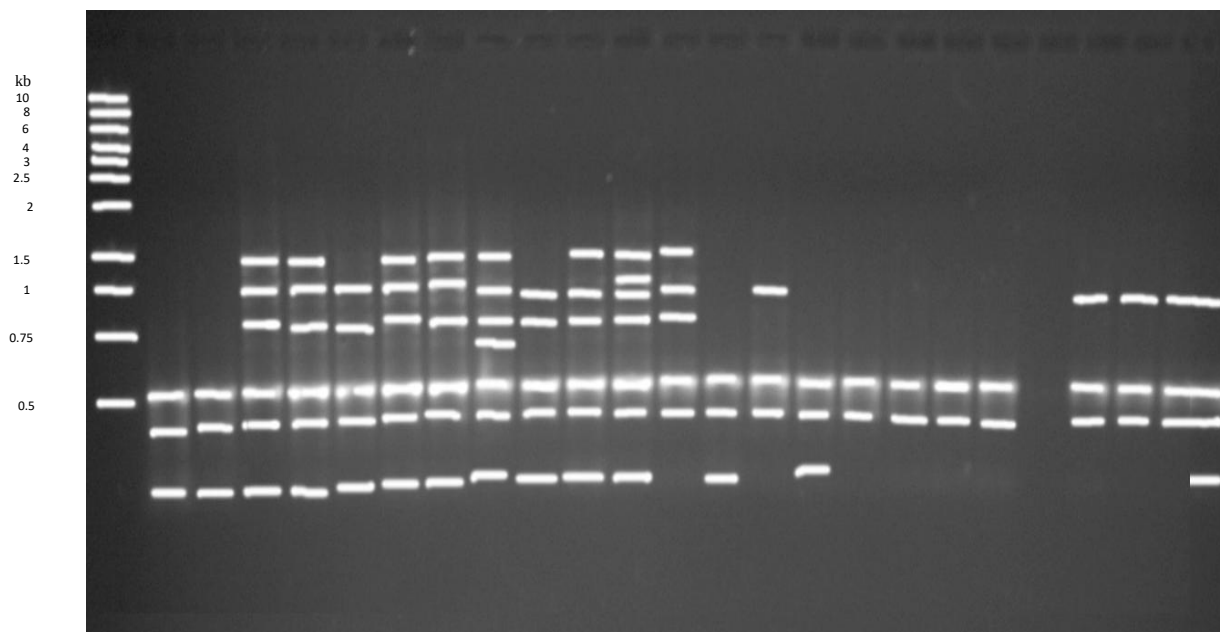


Plate 21. OPB-05 (5'TGCGCCCTTC3')

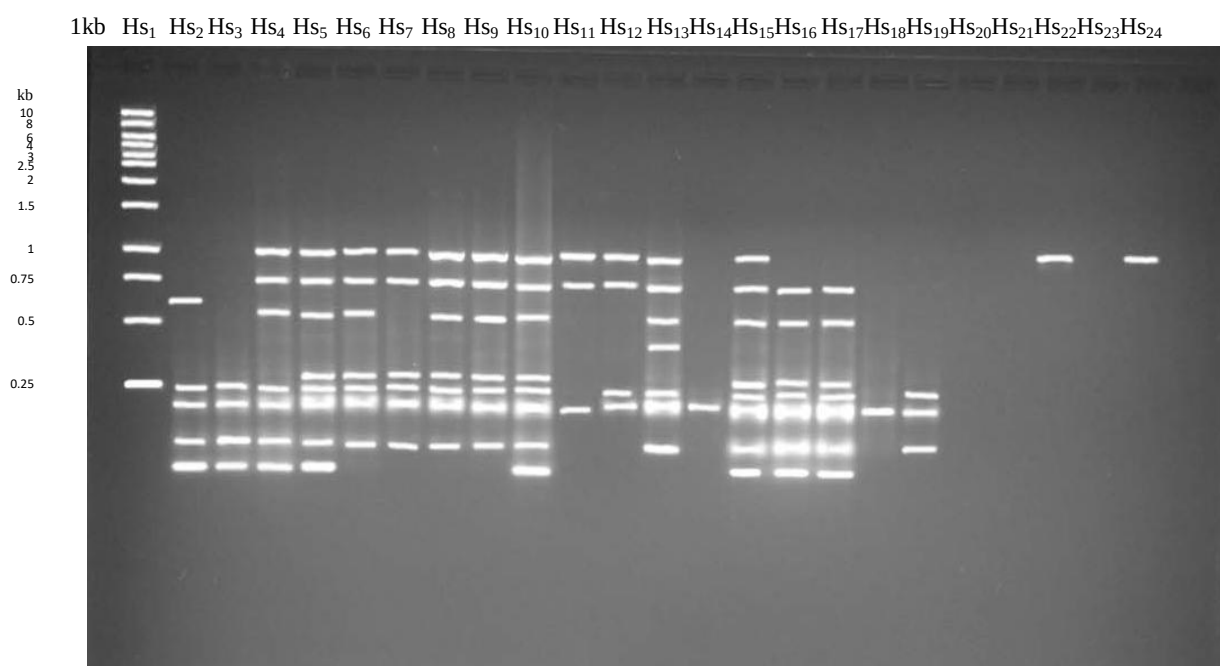


Plate 22. OPC-01 (5'TTCGAGCAAG3')

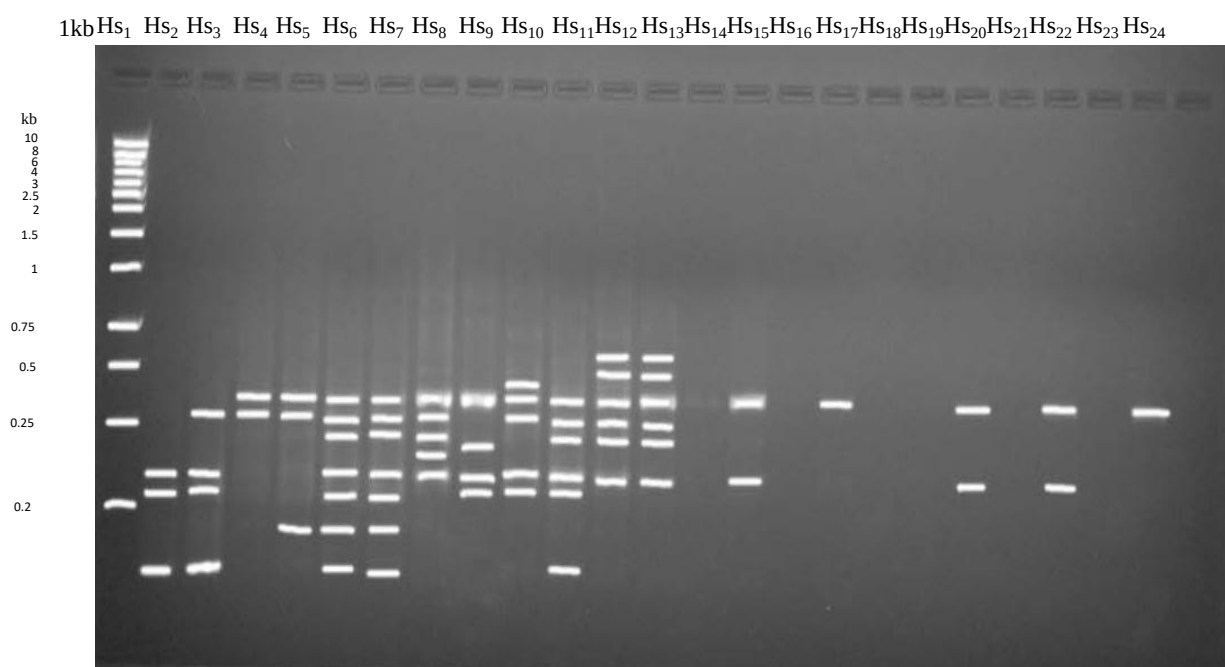


Plate 23. OPC-02 (5'GTGAGGGCTC3')

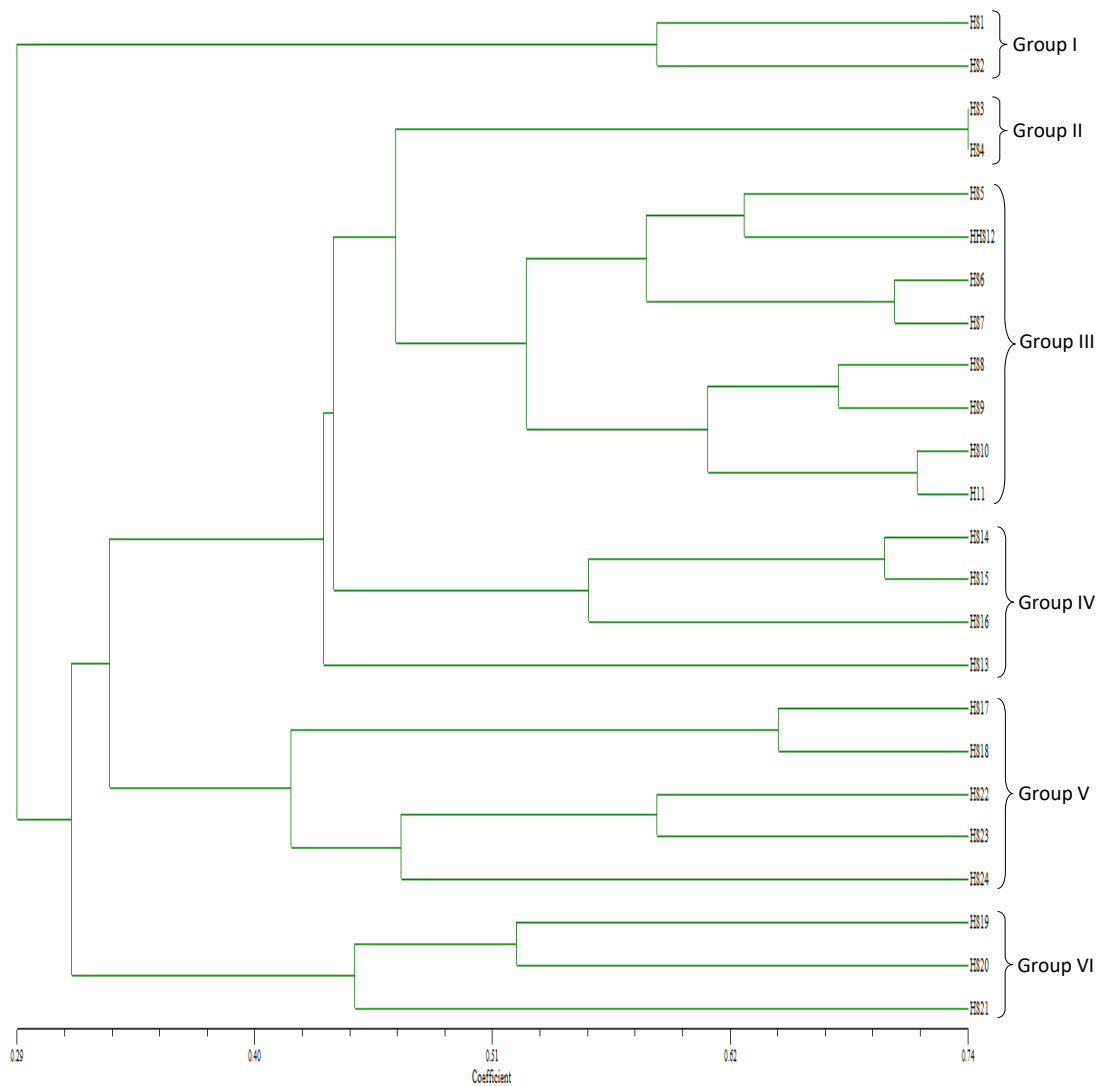


Fig. 39. RAPD dendrogram based on Jaccard's coefficient of similarity using NTSYS programme

Table 21. Correlation Coefficient Similarity Matrix of *Alternanthera* spp. land races based on RAPD banding pattern

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22	M23	M24
M 1 1	1																							
M 0.61 1 2 1111	0.61	1																						
M 0.31 0.49 1 3 1475 2064	0.31	0.49	1																					
M 0.22 0.35 0.59 1 4 2222 8209 1549	0.22	0.35	0.59	1																				
M 0.16 0.16 0.32 0.45 1 5 6667 6667 5 0704	0.16	0.16	0.32	0.45	1																			
M 0.14 0.16 0.25 0.31 0.50 1 6 5455 6667 3165 5069 8475	0.14	0.16	0.25	0.31	0.50	1																		
M 0.16 0.20 0.28 0.30 0.37 0.66 1 7 9492 2899 0488 7692 6812 0377	0.16	0.20	0.28	0.30	0.37	0.66	1																	
M 0.23 0.27 0.36 0.39 0.37 0.46 0.66 1 8 7288 9412 25 4737 5 0318 1017	0.23	0.27	0.36	0.39	0.37	0.46	0.66	1																
M 0.21 0.31 0.46 0.5 0.42 0.31 0.33 0.50 1 9 875 4286 1539 4658 0811 7662 7042	0.21	0.31	0.46	0.5	0.42	0.31	0.33	0.50	1															
M 0.19 0.27 0.39 0.39 0.47 0.30 0.28 0.34 0.62 1 10 403 027 759 5062 2222 2632 0488 5679 8571	0.19	0.27	0.39	0.39	0.47	0.30	0.28	0.34	0.62	1														
M 0.22 0.29 0.45 0.41 0.36 0.25 0.29 0.32 0.36 0.56 1 11 8571 8701 2381 6667 5854 0698 1839 3636 4103	0.22	0.29	0.45	0.41	0.36	0.25	0.29	0.32	0.36	0.56	1													
M 0.20 0.29 0.36 0.44 0.28 0.25 0.34 0.34 0.28 0.24 0.48 1 12 3704 5082 4865 1177 169 3731 3284 2857 5714 6914 6111	0.20	0.29	0.36	0.44	0.28	0.25	0.34	0.34	0.28	0.24	0.48	1												
M 0.25 0.40 0.5 0.47 0.28 0.29 0.38 0.38 0.33 0.26 0.40 0.60 1 13 4546 678 8261 3784 4118 2353 0282 7662 506 5063 7143	0.25	0.40	0.5	0.47	0.28	0.29	0.38	0.38	0.33	0.26	0.40	0.60	1											
M 0.28 0.36 0.36 0.38 0.38 0.37 0.38 0.38 0.35 0.33 0.29 0.29 0.44 1 14 5714 5079 7089 1579 0282 8788 5714 3562 8974 3333 5455 1667 7761	0.28	0.36	0.36	0.38	0.38	0.37	0.38	0.38	0.35	0.33	0.29	0.29	0.44	1										
M 0.32 0.33 0.30 0.25 0.33 0.37 0.33 0.31 0.35 0.39 0.31 0.20 0.24 0.51 1 15 3333 7692 3165 3333 0968 8235 9444 1351 726 7073 8333 6575 6129	0.32	0.33	0.30	0.25	0.33	0.37	0.33	0.31	0.35	0.39	0.31	0.20	0.24	0.51	1									
M 0.29 0.28 0.30 0.31 0.31 0.37 0.31 0.46 0.35 0.28 0.31 0.27 0.19 0.30 0.47 1 16 1667 8136 6667 9444 3433 2881 8182 7742 2113 9474 6456 6923 1781 4348 3684	0.29	0.28	0.30	0.31	0.31	0.37	0.31	0.46	0.35	0.28	0.31	0.27	0.19	0.30	0.47	1								
M 0.25 0.27 0.33 0.34 0.28 0.27 0.30 0.40 0.44 0.33 0.29 0.32 0.32 0.24 0.32 0.41 1 17 4902 8689 3333 7222 5714 6923 8824 9091 1177 3333 2683 8125 8358 3243 3077 3793	0.25	0.27	0.33	0.34	0.28	0.27	0.30	0.40	0.44	0.33	0.29	0.32	0.32	0.24	0.32	0.41	1							
M 0.17 0.27 0.37 0.30 0.30 0.33 0.39 0.43 0.40 0.33 0.27 0.39 0.44 0.33 0.22 0.25 0.50 1 18 6471 5862 1429 9859 303 8983 3443 5484 2985 3333 5 6552 0678 3333 3881 8065 9434	0.17	0.27	0.37	0.30	0.30	0.33	0.39	0.43	0.40	0.33	0.27	0.39	0.44	0.33	0.22	0.25	0.50	1						
M 0.18 0.25 0.32 0.37 0.39 0.39 0.40 0.46 0.37 0.32 0.44 0.47 0.42 0.42 0.28 0.22 0.25 0.37 1 19 9655 7576 9114 8378 7059 6825 2985 2687 3333 9114 1558 541 4242 6471 169 5352 3521 0968	0.18	0.25	0.32	0.37	0.39	0.39	0.40	0.46	0.37	0.32	0.44	0.47	0.42	0.42	0.28	0.22	0.25	0.37	1					
M 0.22 0.27 0.32 0.41 0.39 0.41 0.37 0.47 0.40 0.41 0.47 0.42 0.39 0.44 0.39 0.35 0.28 0.24 0.66 1 20 807 2727 5 0959 1304 2698 6812 7612 5405 3333 3684 1875 7059 1177 3939 3846 5714 6377 6667	0.22	0.27	0.32	0.41	0.39	0.41	0.37	0.47	0.40	0.41	0.47	0.42	0.39	0.44	0.39	0.35	0.28	0.24	0.66	1				
M 0.26 0.35 0.39 0.35 0.27 0.32 0.31 0.39 0.38 0.33 0.38 0.42 0.46 0.37 0.37 0.32 0.29 0.29 0.35 0.50 1 21 0877 4366 2113 1429 2581 3433 3939 5714 7838 1579 3729 6667 8788 0968 7869 6875 5082 3846 8475	0.26	0.35	0.39	0.35	0.27	0.32	0.31	0.39	0.38	0.33	0.38	0.42	0.46	0.37	0.37	0.32	0.29	0.29	0.35	0.50	1			
M 0.28 0.33 0.36 0.33 0.25 0.28 0.25 0.33 0.39 0.32 0.28 0.25 0.36 0.28 0.31 0.35 0.34 0.32 0.16 0.27 0.50 1 22 8889 3333 2319 8235 3731 3333 7576 8462 3939 3944 2051 3968 0656 3582 1475 7143 4828 1429 9014 2727 9804	0.28	0.33	0.36	0.33	0.25	0.28	0.25	0.33	0.39	0.32	0.28	0.25	0.36	0.28	0.31	0.35	0.34	0.32	0.16	0.27	0.50	1		
M 0.19 0.31 0.27 0.34 0.26 0.23 0.24 0.28 0.33 0.29 0.32 0.36 0.34 0.25 0.27 0.29 0.35 0.35 0.28 0.27 0.29 0.47 1 23 6078 579 6316 2857 087 0769 6377 5714 8028 3333 0513 6667 375 3521 6923 5082 0877 3582 9412 0323 0588	0.19	0.31	0.27	0.34	0.26	0.23	0.24	0.28	0.33	0.29	0.32	0.36	0.34	0.25	0.27	0.29	0.35	0.35	0.28	0.27	0.29	0.47	1	
M 0.20 0.24 0.22 0.32 0.21 0.29 0.28 0.28 0.24 0.20 0.25 0.25 0.30 0.21 0.23 0.27 0.38 0.28 0.26 0.23 0.18 0.37 0. 1 24 9302 5283 2222 8125 875 0909 3333 5714 6377 548 3333 8621 5085 2121 3333 2727 4615 3019 2295 8095 3333 5 5	0.20	0.24	0.22	0.32	0.21	0.29	0.28	0.28	0.24	0.20	0.25	0.25	0.30	0.21	0.23	0.27	0.38	0.28	0.26	0.23	0.18	0.37	0. 1	

Table 22. Correlation Coefficient Similarity Matrix of *Amaranthus* spp. land races based on RAPD banding pattern

	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24
H 1 1	1																							
H 0.48 1 2 8889	0.48	1																						
H 0.46 0.59 1 3 4286 0164	0.46	0.59	1																					
H 0.18 0.37 0.52 1 4 9655 7049 3077	0.18	0.37	0.52	1																				
H 0.36 0.36 0.45 0.43 1 5 2069 2319 3333 2836	0.36	0.36	0.45	0.43	1																			
H 0.29 0.37 0.46 0.36 0.64 1 6 7297 037 5116 1446	0.29	0.37	0.46	0.36	0.64	1																		
H 0.29 0.37 0.45 0.29 0.52 0.81 1 7 5775 1795 2381 7619 5641 3333	0.29	0.37	0.45	0.29	0.52	0.81	1																	
H 0.27 0.33 0.48 0.34 0.38 0.56 0.65 1 8 2727 7838 052 6667 75 0976 3333	0.27	0.33	0.48	0.34	0.38	0.56	0.65	1																
H 0.25 0.32 0.41 0.28 0.35 0.47 0.55 0.74 1 9 7576 4324 25 2051 8025 6744 6962 2424	0.25	0.32	0.41	0.28	0.35	0.47	0.55	0.74	1															
H 0.1 0.28 0.29 0.48 0.25 0.27 0.26 0.38 0.42 1 1 5714 7297 2143 6757 907 1905 0282 6471 0	0.1	0.28	0.29	0.48	0.25	0.27	0.26	0.38	0.42	1														
H 0.29 0.42 0.59 0.51 0.42 0.45 0.39 0.51 0.52 0.56 1 1 6875 029 1549 5152 8571 977 7727 3158 4516 1	0.29	0.42	0.59	0.51	0.42	0.45	0.39	0.51	0.52	0.56	1													
H 0.23 0.29 0.43 0.47 0.36 0.40 0.34 0.4 0.33 0.45 0.6 1 1 7288 4118 0556 541 9863 9639 5238 3333 2	0.23	0.29	0.43	0.47	0.36	0.40	0.34	0.4	0.33	0.45	0.6	1												
H 0.36 0.31 0.44 0.32 0.37 0.36 0.33 0.36 0.35 0.28 0.54 0.61 1 1 746 1177 8125 6812 5854 3333 9863 6164 125 6875 8182 3	0.36	0.31	0.44	0.32	0.37	0.36	0.33	0.36	0.35	0.28	0.54	0.61	1											
H 0.22 0.31 0.34 0.37 0.29 0.28 0.25 0.31 0.28 0.29 0.35 0.42 0.55 1 1 449 579 3284 5 8508 3951 4286 169 8246 8209 1053 102 4	0.22	0.31	0.34	0.37	0.29	0.28	0.25	0.31	0.28	0.29	0.35	0.42	0.55	1										
H 0.16 0.23 0.30 0.41 0.35 0.36 0.31 0.32 0.26 0.33 0.37 0.36 0.39 0.58 1 1 0714 0769 137 3793 2941 25 25 8767 3158 8983 1429 5079 6552 6957 5	0.16	0.23	0.30	0.41	0.35	0.36	0.31	0.32	0.26	0.33	0.37	0.36	0.39	0.58	1									
H 0.24 0.26 0.33 0.36 0.43 0.35 0.34 0.36 0.30 0.35 0.43 0.33 0.34 0.37 0.59 1 1 2295 3333 2069 5484 8974 2105 2319 9859 7143 0769 871 4828 2549 5745 6	0.24	0.26	0.33	0.36	0.43	0.35	0.34	0.36	0.30	0.35	0.43	0.33	0.34	0.37	0.59	1								
H 0.22 0.32 0.36 0.40 0.42 0.42 0.41 0.36 0.34 0.35 0.48 0.33 0.36 0.27 0.50 0.53 1 1 807 8125 9863 3226 6471 5 0256 6667 4839 5294 8235 5079 8689 9091 8462 7	0.22	0.32	0.36	0.40	0.42	0.42	0.41	0.36	0.34	0.35	0.48	0.33	0.36	0.27	0.50	0.53	1							
H 0.23 0.33 0.37 0.45 0.41 0.44 0.45 0.46 0.36 0.33 0.40 0.34 0.34 0.37 0.36 0.33 0.52 1 1 2143 3333 5 7627 1765 8718 3333 3768 9863 871 8451 3284 9206 5 6667 8983 6316 8	0.23	0.33	0.37	0.45	0.41	0.44	0.45	0.46	0.36	0.33	0.40	0.34	0.34	0.37	0.36	0.33	0.52	1						
H 0.20 0.28 0.32 0.37 0.45 0.4 0.40 0.40 0.43 0.28 0.39 0.35 0.37 0.31 0.39 0.30 0.40 0.53 1 1 9677 5714 9114 3134 7143 2439 7895 2432 9855 4737 2113 8788 746 6825 7692 9091 3333 9	0.20	0.28	0.32	0.37	0.45	0.4	0.40	0.40	0.43	0.28	0.39	0.35	0.37	0.31	0.39	0.30	0.40	0.53	1					
H 0.20 0.36 0.28 0.30 0.44 0.36 0.35 0.39 0.41 0.34 0.41 0.34 0.37 0.41 0.35 0.27 0.34 0.44 0.58 1 2 7547 2069 7671 6452 4444 7089 0649 1304 791 4828 791 9206 931 1765 0877 5862 4262 6429 1818 0	0.20	0.36	0.28	0.30	0.44	0.36	0.35	0.39	0.41	0.34	0.41	0.34	0.37	0.41	0.35	0.27	0.34	0.44	0.58	1				
H 0.28 0.37 0.34 0.35 0.30 0.28 0.23 0.3 0.32 0.30 0.42 0.37 0.47 0.48 0.44 0.27 0.37 0.33 0.38 0.47 1 2 2609 037 8485 7143 303 75 75 3529 3571 8571 931 0588 8889 7778 5 3333 9831 9167 1	0.28	0.37	0.34	0.35	0.30	0.28	0.23	0.3	0.32	0.30	0.42	0.37	0.47	0.48	0.44	0.27	0.37	0.33	0.38	0.47	1			

H	0.34	0.35	0.39	0.29	0.37	0.27	0.22	0.28	0.30	0.30	0.43	0.33	0.37	0.32	0.29	0.36	0.28	0.25	0.24	0.27	0.44	1		
2	8837	1852	6825	3103	0968	5	5	5714	8824	9091	5484	8983	037		0909		8136		6154	2727	4444			
2																								
H	0.17	0.25	0.37	0.39	0.24	0.25	0.20	0.28	0.23	0.30	0.39	0.29	0.27	0.26	0.26	0.33	0.33	0.31	0.24	0.2	0.24	0.42	1	
2	3913	4546	7049	2157	6154	641	5128	3582	1884	7692	3443	3103	2727	5306	4151	3333	3333	4815	1936		4898	8571		
3																								
H	0.17	0.23	0.27	0.35	0.20	0.16	0.17	0.17	0.16	0.29	0.27	0.27	0.30	0.30	0.29	0.23	0.34	0.3	0.26	0.17	0.25	0.31	0.47	1
2	0732	5294	8689	4167	9677	4557	3333	6471	1765	1667	4194	7778	6122	2326	7872	4043	6939		7857	6471		7073	0588	
4																								

Table 23. Correlation Coefficient Similarity Matrix of *Chenopodium* spp. land races based on RAPD banding pattern

	J1	J2	J3	J4	J5	J6	J7	J8	J9	J10	J11	J12	J13	J14	J15	J16	J17	J18	J19	J20	J21	J22	J23	J
																								2
																								4
J	1																							
1																								
J	0.82	1																						
2	8571																							
J	0.55	0.64	1																					
3		1026																						
J	0.22	0.25	0.31	1																				
4	4138	8621	4815																					
J	0.24	0.2	0.18	0.51	1																			
5	2424		8406	6667																				
J	0.22	0.21	0.18	0.42	0.68	1																		
6	2222	6216	9189	6471	75																			
J	0.24	0.29	0.26	0.30	0.41	0.57	1																	
7	6377	4118	4706	137	3333	7465																		
J	0.2	0.24	0.25	0.37	0.42	0.54	0.75	1																
8		3243		5	8571	6667	3846																	
J	0.25	0.28	0.28	0.39	0.48	0.5	0.59	0.71	1															
9	3333		7671	1892	052		4595	831																
J	0.26	0.26	0.26	0.46	0.47	0.54	0.47	0.50	0.67	1														
10	8657	087	8657	875	8873	1667	2973	6667	1429															
J	0.28	0.27	0.24	0.37	0.45	0.5	0.45	0.44	0.58	0.75	1													
11	125	2727	2424	8788	7143		2055	7368	3333															
J	0.29	0.34	0.33	0.41	0.48	0.59	0.5	0.51	0.50	0.52	0.55	1												
12	8508	8485	8462	1765	6111	1549		3158	6329	7778	0725													
J	0.27	0.32	0.36	0.39	0.41	0.43	0.49	0.46	0.46	0.37	0.33	0.55	1											
13	4194	7869	2069	6825	4286	8356	2754	5753	0526	8378	7838	2239												
J	0.32	0.33	0.36	0.45	0.50	0.50	0.32	0.37	0.39	0.34	0.30	0.45	0.53	1										
14	2034	3333	8421		7692	7246	4675	6623	2405	6667	6667	0704	2258											
J	0.44	0.45	0.41	0.28	0.33	0.36	0.31	0.31	0.35	0.33	0.25	0.27	0.30	0.44	1									
15	6809	8333	6667	3333	3333	2319	4286	5069	1351	8235	7143	3973	7692	8276										
J	0.34	0.33	0.27	0.21	0.40	0.43	0.34	0.32	0.37	0.38	0.38	0.33	0.22	0.32	0.46	1								
16	6154	3333	2727	5385	625	2836	2857	4324	8378	806	4615	8028	5352	3077	1539									
J	0.25	0.26	0.18	0.27	0.34	0.37	0.42	0.44	0.39	0.34	0.36	0.31	0.27	0.22	0.22	0.41	1							
17		3158	6441	4194	3284	1429	4242	1177	726	7826	3636	9444	9412	8571	5807	8182								

[illegible]

Table 24. Correlation Coefficient Similarity Matrix of *Enhydra* spp. land races based on RAPD banding pattern

	HS1	HS2	HS3	HS4	HS5	HS6	HS7	HS8	HS9	HS10	H11	HHS12	HS13	HS14	HS15	HS16	HS
HS1	1																
HS2	0.589744	1															
HS3	0.307692	0.530303	1														
HS4	0.228571	0.39726	0.736111	1													
HS5	0.197368	0.289157	0.529412	0.617284	1												
HS6	0.171429	0.240506	0.406977	0.46988	0.671053	1											
HS7	0.238095	0.342857	0.426829	0.372093	0.5375	0.701493	1										
HS8	0.257143	0.316456	0.45977	0.406593	0.564706	0.589744	0.666667	1									
HS9	0.242857	0.373333	0.465116	0.426966	0.517241	0.5	0.506329	0.675325	1								
HS10	0.242857	0.337662	0.448276	0.443182	0.517241	0.447059	0.383721	0.573171	0.662338	1							
H11	0.217391	0.31579	0.430233	0.458824	0.554217	0.538462	0.45	0.575	0.644737	0.712329	1						
HHS12	0.277778	0.402597	0.541177	0.571429	0.630952	0.6	0.530864	0.614458	0.494382	0.528736	0.585366	1					
HS13	0.285714	0.333333	0.405063	0.4	0.409639	0.402597	0.424658	0.48052	0.378049	0.4125	0.428571	0.594595	1				
HS14	0.272727	0.408451	0.5	0.512195	0.573171	0.5	0.414634	0.431818	0.453488	0.453488	0.402299	0.604938	0.527778	1			
HS15	0.275862	0.451613	0.467533	0.561644	0.4875	0.375	0.325	0.333333	0.419753	0.4375	0.365854	0.463415	0.408451	0.69697	1		
HS16	0.352941	0.416667	0.367089	0.3625	0.407407	0.4	0.346667	0.460526	0.447368	0.410256	0.371795	0.474359	0.357143	0.550725	0.564516	1	
HS17	0.230769	0.333333	0.355263	0.333333	0.3625	0.408451	0.391304	0.394737	0.478873	0.458333	0.457143	0.325301	0.323529	0.36	0.393939	0.45	1
HS18	0.222222	0.344262	0.418919	0.394737	0.405063	0.307692	0.306667	0.384615	0.407895	0.445946	0.350649	0.365854	0.415385	0.444444	0.46875	0.412698	0.647
HS19	0.2	0.245902	0.337838	0.369863	0.381579	0.315069	0.314286	0.39726	0.328947	0.364865	0.324324	0.394737	0.387097	0.361111	0.294118	0.238806	0.392
HS20	0.219512	0.269231	0.271429	0.216216	0.25	0.228571	0.223881	0.277778	0.28169	0.28169	0.257143	0.315069	0.461539	0.313433	0.258065	0.237288	0.259
HS21	0.325	0.326923	0.277778	0.24	0.225	0.308824	0.307692	0.283784	0.305556	0.342857	0.358209	0.394366	0.436364	0.318841	0.265625	0.245902	0.267
HS22	0.186047	0.240741	0.232877	0.181818	0.230769	0.279412	0.31746	0.256757	0.295775	0.333333	0.308824	0.24359	0.305085	0.328358	0.338983	0.321429	0.408
HS23	0.1875	0.258621	0.28	0.25974	0.275	0.347826	0.328358	0.337838	0.361111	0.380282	0.376812	0.2875	0.276923	0.30137	0.307692	0.454546	0.595
HS24	0.162791	0.245283	0.236111	0.216216	0.217949	0.283582	0.223881	0.260274	0.28169	0.3	0.275362	0.230769	0.225807	0.294118	0.258065	0.403846	0.416

CHAPTER V

SUMMARY AND CONCLUSION

The present investigation was conducted in the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat-785013 during the period 2010-2014. Twenty five indigenous plant materials viz. *Bacopa monnieri*, *Leucas longifolia*, *Enhydra fluctuans*, *Erynginum foetidum*, *Amaranthus spinosus*, *Pteridium aquilinum*, *Amaranthus viridis*, *Hydrocotyl rotundifolia*, *Alternanthera sessilis*, *Paederia foetida*, *Eclipta alba*, *Houttuynia cordata*,

Talinum triangulare, *Polygonum chinense*, *Oxalis corniculata*, *Oldenlandia corymbosa*, *Malva sylvestris*, *Chrysopagon coronarium*, *Trigonella foenum-graecum*, *Basella rubra*, *Chenopodium album*, *Stellaria media*, *Basella alba*, *Centalla asiatica* and *Oxalllis acetosella* were collected from various places of Jorhat district on the basis of popularity of use by the people of Jorhat district. These plants were planted in pot in the Department of Biochemistry and Agricultural Chemistry, Assam Agricultural University, Jorhat- 875013 for investigation. Young tender edible parts of these plants species were collected and dried under sunshine and after drying, milling were done in wily mill. The dry powder was used for biochemical and selected plants were used for molecular investigation. The following observations were recorded during the course of investigation.

It was seen that Puroi (green) (92.77%) has the highest moisture content followed by Pirali paleng (92.47%) and Bonjaluk (76.40) has the lowest moisture content. Puroi (green) (17.70%) has the highest ash content followed by Khotora and Jilmil (14.60%) and Bhedailata (7.80%) has the lowest ash content. Bonjaluk (26.10 g/100 g) has the highest crude fibre content followed by Bhedailata (20.40%) and Puroi (green) (2.50%) has lowest crude fibre content. Puroi (red) (7.10%) has the highest crude fat content followed by Saru Tengesi (6.20%) and Bor Manimoni (1.13%) has lowest crude fat content. Dhekia (39.38%) has the highest crude protein content followed by Jilmil (36.76%) and Bor Manimoni (15.77%) has lowest crude protein content. Dhekia (992.00 mg %) has the highest total free amino acid content followed by Saru Manimoni (970.72 mg %) and Bor Manimoni (129.27 mg %) has lowest total free amino acid content. Pirali paleng (4.13%) has the highest soluble protein content followed by Madhuholeng (3.51%) and Mandhanian (1.10%) has the lowest soluble protein content.

Madhuholang (603.00 mg %) has the highest phosphorous content followed by Saru Tengesi (142.00 mg %) and Bor Tangeshi (38.00 mg %) has the lowest phosphorous content. Pirali paleng (9.58%) has the highest potassium content followed by Helosi sak (6.06%) and Puroi (red) has the lowest (0.64%) potassium content. Helosi sak (233.00 mg %) has the highest sodium content followed by Brahmi (231.00 mg %) and Bhedailata (52.00 mg %) has the lowest sodium content. Bhedailata (2.10%) has the highest calcium content followed by Jilmil (1.70%) and Brahmi (0.20%) has the lowest calcium content. Khotora (401.00 mg %) has the highest iron content followed by Matikandori (139.00 mg %) and Helosi sak, Hatikhutora, Dhekia

and Puroi (red) (31.00 mg %) has lowest iron content. Helosi sak, Madhoholeng (0.020%) has the highest copper content followed by Khotora (0.01%) and Doron (0.003%) has the lowest copper content. Puroi (green) (7.55 mg %) has the highest zinc content followed by Keheraj (6.29 mg %) and Bhedailata (0.013 mg %) has lowest zinc content. Baburi (132.00 mg %) has the highest manganese content followed by Pirali paleng (84.67 mg %) and Matikandori (12.00 mg %) has the lowest manganese content. Puroi (green) (1.70 mg %) has the highest arsenic content followed by Brahmi, Bhedailata (1.20 mg %) and Hatikhutura, Dhekia, Matikandori, Keheraj, Masundari, Saru tengeshi, Puroi (red), Morolia, Bor manimoni, Bor tengeshi (0.0 mg %) has the lowest arsenic content.

Hatikhutura (56.42%) has the highest starch content followed by Laffa (41.69%) and Madhuholang (18.78%) has the lowest starch content. Saru Manimoni (21.69%) has the highest total soluble sugar content followed by Masundori (18.37%) and Bor Tangeshi (7.37%) has lowest total soluble sugar content. Saru Manimoni (18.58%) has the highest reducing sugar content followed by Laffa (15.34%) and Puroi (red) (4.19%) has the lowest reducing sugar content. Helosi sak (119.67 mg %) has the highest vitamin C content followed by Saru Manimoni (119.33 mg %) and Masundori (39.00 mg %) has the lowest vitamin C content. Khotora (8.80 mg %) has the highest riboflavin content followed by Bhadailata (8.10 mg %) and Brahmi, Helosi sak (0.17 mg %) has lowest riboflavin content. Baburi (0.08 mg %) has the highest thiamine content followed by Doron (0.071 mg %) and Bor tangeshi (0.00 mg %) has lowest thiamine content. Masundori (2.63 mg %) has the highest niacin content followed by Laffa (2.60 mg %) and Khotora (0.15 mg %) has the lowest niacin content.

Pirali paleng (0.74%) has the highest saponin content followed by Methi (0.61%) and Mandhania (0.23%) has the lowest saponin content. Bhedailata (2.82%) has the highest tannin content followed by Dhekia (2.38%) and Morolia (0.37%) has lowest tannin content. Doron (0.32%) has the highest phytic acid content followed by Helosi sak, Keheraj (0.27%) and Morolia (0.03%) has the lowest phytic acid content. Puroi (green), Laffa (0.13%) has the highest oxalate content followed by Puroi (red) (0.12%) and Pirali paleng, Bonjaluk (0.02%) has the lowest oxalate content.

Hatikhutura (3.23%) has the highest total alkaloid content followed by Saru Manimoni (3.10%) and Madhuholang (0.13%) has the lowest total alkaloid content. Brahmi (2.2%) has the highest total phenol content followed by Doron (2.1%)

and Bor Manimoni (0.25%) has the lowest total phenol content. Jilmil (3.40%) has the highest total flavanoid content followed by Bhedailata (2.94%) and Bor Manimoni (0.86%) has total flavanoid content. Morolia (1.63 mg %) has the highest total flavanol content followed by Puroi (red) (1.39 %) and Bor Tangeshi (0.33%) has the lowest total flavanol content.

Bonjaluk (91.72%) has the highest free radical scavenging activity followed by Helosi sak (91.35%) and Puroi (green) (74.81%) has free radical scavenging activity. Dhekia, Keheraj (0.24 unit min⁻¹ mg⁻¹protein) has the highest super oxide dismutase activity followed by Bhedailata (0.23 unit min⁻¹ mg⁻¹protein) and Saru Tangeshi, Methi, Morolia (0.11 unit min⁻¹ mg⁻¹protein) has the lowest super oxide dismutase activity. Bhrami (0.13 unit min⁻¹ mg⁻¹protein) has the highest catalase activity followed by Bonjaluk (0.053 unit min⁻¹ mg⁻¹protein) and Helosi sak, Dhekia (0.005 unit min⁻¹ mg⁻¹protein) has the lowest catalase activity. Saru Manimoni, Madhohhleng (0.08 unit min⁻¹ mg⁻¹protein) has the highest peroxidase activity followed by Mandhanian (0.07 unit min⁻¹ mg⁻¹protein) and Puroi (green) (0.01 unit min⁻¹ mg⁻¹protein) has the lowest peroxidase activity.

The RAPD analysis of *Alternanthera* spp. land races revealed that the genetic diversity exist among the land races. The maximum level of polymorphism was generated by primer OPA-04 and was recorded as 100% followed by primer OPB-05 (87.50%). Primer OPB-06 showed monomorphic banding pattern. Similarity matrix value ranged from 0.14 (M₁, M₆) to 0.67 (M₁₉, M₂₀) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.14) were observed between M₁ and M₆ and lowest genetic distance were observed between M₁₉ and M₂₀ (0.67) among the land races. The land race M₁ and M₆ contain the highest genetic variation, while land race M₁₉ and M₂₀ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race M₁ and M₆ could provide the opportunities for selecting as parental source in future breeding program to improve *Alternanthera sessilis*.

The RAPD analysis of *Amaranthus* spp. land races revealed that the genetic diversity exist among the land races. The maximum level of polymorphism was generated by primer OPB-01 and was recorded as 71.42% followed by primer OPB-

07(66.67%). Primer OPA-09 and primer OPB-03 has monomorphic banding pattern. Similarity matrix value ranged from 0.10 (H₁, H₁₀) to 0.74 (H₈, H₉) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.10) was observed between H₁ and H₁₀ and lowest genetic distance (0.74) were observed between H₈ and H₉ among the land races. The land race H₁ and H₁₀ contain the highest genetic variation, while land race H₈ and H₉ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race H₁ and H₁₀ could provide the opportunities for selecting as parental source in future breeding program to improve *Amaranthus spinosus*.

The RAPD analysis of *Chenopodium* spp. land races revealed that the genetic diversity exist among the land races. The maximum level of polymorphism was generated by primer OPA-04 and was recorded as 100% followed by primer OPB-02 (77.21%). Primer OPA-02 and primer OPA-05 has showed monomorphic band. Similarity matrix value ranged from 0.19 (J₃, J₁₇) to 0.75 (J₇, J₈) indicating high degree of genetic diversity among the landraces studied. The highest genetic distance (0.19) were observed between J₃ and J₁₇ and lowest genetic distance (0.75) were observed between J₇ and J₈ among the land races. The land race J₃ and J₁₇ contain the highest genetic variation, while land race J₇ and J₈ contain the lowest genetic variation, among the land races used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race J₃ and J₁₇ could provide the opportunities for selecting as parental source in future breeding program to improve *Chenopodium album*.

The RAPD analysis of *Enhydra* spp. land races revealed that the genetic diversity exist among the land races. The maximum level of polymorphism was generated by primer OPC-01 and was recorded as 100% followed by primer OPB-01 (80.00). Primer OPB-03 showed monomorphic band. Similarity matrix value ranged from 0.16 (Hs₁, Hs₂₄) to 0.74 (Hs₁₀, Hs₁₁) indicating higher degree of genetic diversity among the landraces studied. The highest genetic distance (0.16) was observed between Hs₁ and Hs₂₄ and lowest genetic distance (0.74) was observed between Hs₁₀ and Hs₁₁ among the land races. The land race Hs₁ and Hs₂₄ contain the highest genetic variation, while land race Hs₁₀ and Hs₁₁ contain the lowest genetic variation, among the land races

used in this study. Land races having close proximity in their origin, breeding strategy and morphological traits are likely to have less genetic distance from each other. Hence, the results of this study suggested that the land race Hs₁ and Hs₂₄ could provide the opportunities for selecting as parental source in future breeding program to improve *Enhydra fluctuans*.

So it can be concluded that these plants are rich in moisture, ash, mineral, fibre, fat, crude protein and carbohydrates. So these plants could act as a cheapest source of nutrient for the rural and urban people to fulfill their nutritional requirement.

These plants are rich in vitamins. These plants could act as a cheapest source of vitamins for the rural and urban community to fulfill their vitamins requirement.

These plants are poor in anti-nutritional factors. So these plants are healthy for the people of Assam.

These plants are rich in antioxidants. So these plants could act as a cheapest source of antioxidants for the rural and urban poor people to fulfill their antioxidants requirement.

After RAPD analysis it could be concluded that among the land races of these plant species genetic variation exist in molecular level, though no morphological variation was seen, so improvement of these plant species is possible by breeding programme by the utilization of superior land races.

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