MORPHOLOGICAL AND ANTIOXIDANT ACTIVITIES OF *ALOE* SPECIES OF PALAMPUR AND ITS SURROUNDING AREA

THESIS

By

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(S-2010-30-22)

Submitted to

CHAUDHARY SARWAN KUMAR
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partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE IN BASIC SCIENCES
(DEPARTMENT OF BIOLOGY AND ENVIRONMENTAL SCIENCES)
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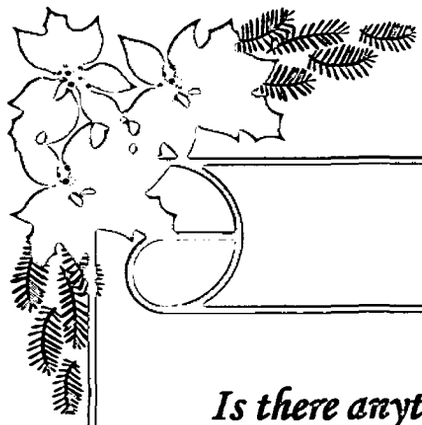

Is there anything I can say,

anything I can give

or do for you.....

Because all that I'm

all that I have

I owe to you.....

Affectionately Dedicated

to my

Revered Parents

&

beloved Brothers

Who have always sacrificed

their present

to make my future better

Dr. (Mrs.) Anita Singh
Professor

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Palampur – 176 062 (H.P.) India

CERTIFICATE – I

This is to certify that the thesis entitled "**(Morphological and Antioxidant activities of *Aloe* species of Palampur and its Surrounding Area)**"submitted in partial fulfillment of the requirements for the award of the degree of **Master of Science (Basic Sciences)** in the discipline of **(Biology)** of CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur is a bonafide research work carried out by **Ms. Shalini Rana (S-2010-30-22)** daughter of **Shri Amar Singh** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

(Dr. Anita Singh)
Major Advisor

Place: Palampur
Dated: July, 2012

CERTIFICATE- II

This is to certify that the thesis entitled **"Morphological and Antioxidant activities of *Aloe* species of Palampur and its Surrounding Area"** submitted by **Ms. Shalini Rana (S-2010-30-22)** daughter of **Shri Amar Singh** to the CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur in partial fulfillment of the requirements for the degree of **Master of Science (Basic Sciences)** in the discipline of **Biology** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

17.11.12

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Needless to say, all omissions and errors are mine

Place: Palampur
Dated: 16th July, 2012

Shalini Rana.
(Shalini Rana)

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LIST OF ABBREVIATIONS USED

e.g.	Example gratia (for example)
et al.	et alli (and others)
i.e	idest (that is)
abstr.	Abstracts
A	Absorbance
Anon.	Anonymus
%	Per cent
cm	Centimeter
CD	Critical difference
cm^2	Centimeter square
g	gram
hrs	hours
p.	Page
pp.	Pages
$\mu g/g$	microgram per gram
mg	milligram
min	minute
ml	milliliter
nm	nanometer
Wt	weight
D.M.S.O	Dimethyl sulphoxide
ANOVA	Analysis of variance
°c	Degree centigrade
L^{-1}	Per liter
FYM	Farm yard manure
DAP	Days after plantation
DPPH·	2, 2-Diphenyl-1-picrylhydrazyl radical
ROS	Reactive oxygen species
T	Treatments

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LIST OF PLATES

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Title of the Thesis	:	Morphological and Antioxidant activities of *Aloe* species of Palampur and its Surrounding Area.
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ABSTRACT

The present study was carried out in the Department of Biology & Environmental Sciences, COBS, CSKHPKV, Palampur during the year 2011-2012 to evaluate different morphological, physiological parameters, and to determine and compare the antioxidant activity of two *Aloe* species grown in different treatments. Maximum plant height, number of leaves, leaf area and fresh weight in both the species of *Aloe* were recorded in the pots treated with the T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM). Plant height, no. of leaves, leaf area and fresh weight of *Aloe vera* was found to be more than that of *Aloe perryi.* Chlorophyll content of *Aloe* species was maximum with the application of T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+FYM). Chlorophyll content was found more in *Aloe perryi* than *Aloe vera.* Ascorbic acid content in both the species was almost equal but *Aloe vera* possessed slightly more amount of ascorbic acid than *Aloe perryi.* T_6 (Soil+Sand+Vermicompost) proved to be a better treatment for obtaining maximum amount of ascorbic acid. Both the species were found rich in iron and low in copper. The antioxidant activities of the extracts were investigated with DPPH·method. *Aloe vera* exhibited the strongest activity as a DPPH scavenger than *Aloe perryi* . Least antioxidant activity was shown by control pot and maximum by pots treated with T_6 (Soil+Sand+Vermicompost).The results of this study indicate that the genus *Aloe* is favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit reactive oxygen species attacks on biological and food systems.

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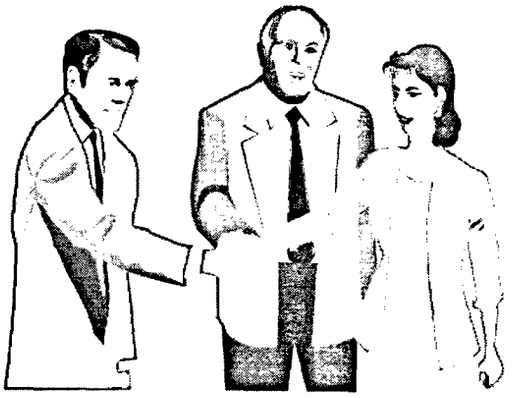


INTRODUCTION

1. INTRODUCTION

An estimate says the demand for medicinal crops figures to be US$60 billion. With the increasing interest in 'natural' products they may arose to 5 trillion by 2050. *Aloe* is among the few medicinal plants which enjoy a major chunk of the market across the globe. *Aloe* origned from arab words "allcoh", a traditional herb from ancient Egypt, its effects were identified by people of ancient Egypt and called as Secret Plant.

The major markets for *Aloe* and its extracts are Australia, US and the entire Europe. *Aloe* genus, family Liliaceae consists of at least 400 known species, many of which have been used in many countries for centuries (Okamura *et al.* 1996). Among 400 species, *Aloe vera* is considered as an important medicinal plant in many countries (Hasanuzzaman *et al.* 2008 and Reynolds 2004). *Aloe* has its origin in the African continent. It is also grown as an ornamental plant. In India, it is grown in states like Chattisgarh, Maharashtra, Madhya Pradesh, Gujarat and Andhra Pradesh and is commonly known as Ghrit kumari or first aid plant. It is a perennial herb with short, stout and thick stem. Leaves are sessile, crowded, lanceolate, pale green in color , fleshy and with spiny margins. Flowers are cylindric, pendulous and yellow in color, born on leafless scape, which is longer than the leaves (Martinez *et al.* 1996). Its thick leaves contain water supply for the plant to survive longer periods of drought. These leaves have a high capacity of retaining water even in warm and dry climates and therefore this plant can survive very harsh circumstances where most other vegetation disappears. When a leaf is cut, an orange yellow sap drips from the open end. As a drink , this bitter sap has a very strong laxative effect. Species of *Aloe* which have been used as folk medicine includes: *Curacao Aloe* (*Aloe vera*), *Cape Aloe* (*Aloe ferox*) and *Socotra Aloe* (*Aloe perryi*). This plant species can be easily propagated from cuttings and is probably the most widely cultivated species of this genus in the world. It is widely used, not only as a folk remedy for gastrointestinal complaints, skin injuries and burns, but also as an ingredient in health foods and cosmetics (Capasso *et al.* 1998). *Aloe* gel, among other things, enhance immunity, improves liver functions, prevents asthma and has anti-inflammatory, anti-ulcerous, anti-diabetic and antihypertensive properties (Dagne *et al.*

2000). Also epidemiological data suggests that the intake of *Aloe vera* prevents human lung cancer (Sakai 1989).

Aloe is also one of the promising herbs having the unique quality to repel termites. In rat infested fields, the farmers plant *Aloe* to attract the snakes (prescence of *Aloe* invites snakes) for rat management. In areas where soil erosion is a problem *Aloe* is planted, it helps a lot in soil binding. The traditional healers of India use fresh and dry leaves of *Aloe vera* in treatment of many common and complicated diseases. All *Aloe* species contain anthraquinone glycosides. Barbaloin is the major active constituent. The prescence of various anthraquinones (isobarbaloin, *aloe* emodin, aloin, aloetic acid, chrysophanic acid, chrysamminic acid, ethereal oils, resistannols have been reported in *Aloe vera* (Reynolds T and Dweck AC 1999 ; Meadows 1980 ; Geetha 2004) and galactouronic acid, choline salicylate, saponins, mucopolysacchrides, glucosamines, hexuranic acid, coniferyl alcohol are also reported (Erazo *et al.*1985). Aloin is the principal active constituent of *Aloe vera* (Grun *et al.*1980). The characteristic odour of gel is due to the traces of essential oils (Anon 1959).The cultivation of *Aloe* has acquired great commercial importance for medicinal products and cosmetics processing but information are scarce about agronomic management of this crop. It flourishes in a variety of climate and can withstand drought. It grows very well in temperate and low rainfall. Driest and poorest soils are good for economic yield of *Aloe*. No chemical fertilizer is recommended for its cultivation. It thrives well when grown with FYM. In India, *Aloe* is raised organically and only farmyard manure is applied @12-15 tones/ha. In standing crop, cow dung solution is applied for nutrient and pest management. However, for better results, the soil should be supplemented with ammonium nitrate every year. The crop is not irrigated normally and this can come up very well in dry areas. Therefore, there is no recommendation on irrigation scheduling. However, it requires about 150 mm of water every month for its quality crop production. *Aloe* is very useful because it contains numerous antioxidants. Antioxidants are substances that delay or prevent the decline in energy and endurance or prevent the oxidation of cellular oxidizable substrates. They exert their effect by scavenging reactive oxygen.

In recent years, there has been an increasing interest in finding natural antioxidants, which can protect the human body from free radicals and retard the progress of many chronic diseases. In an aerobic environment, all animals and plants require oxygen and hence reactive oxygen species (ROS) are ubiquitous. It is already established

that excessive generation of ROS is involved with structural alterations of cellular molecules leading to cytotoxicity and cell death. This eventually results in a variety of biological phenomena such as mutation, carcinogenesis, radiation or UV exposure, inflammation and neurodegenerative disorders (Yoshikawa *et al.* 2000). Antioxidants play a significant role in the prevention of diseases and do have a capacity to reduce oxidative stress by chelating trace elements or scavenging free radicals and protecting antioxidant defenses (Banerjee and Dasgupta 2005 and Govindarajan *et al.* 2005).

Natural antioxidants such as α-tocopherol and ascorbic acid are widely used because they are regarded as safer and causing fewer advxidaterse reactions but their antioxidant activities are lower than the synthetic antioxidants such as butylated hydroxyanisol (BHA) and butylated hydroxytoluene (BHT) which have been restricted by legislative rules because they are suspected to have some toxic effects and as possible carcinogens . Therefore, there is a considerable interest in finding new and safe antioxidants from natural sources to replace these synthetic antioxidants. Recently, natural plants have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids which prevent free radical damage, reducing risk of chronic diseases. Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular disease. The search for newer natural antioxidants, especially of plant origin has ever since increased.

Therefore, research on **"Morphological and Antioxidant activities of *Aloe* species of Palampur and its Surrounding Area"** was carried out at Herbal Garden, Department of Biology and Environmental Sciences, COBS, CSK HP Krishi Vishvavidyalaya Palampur during the year 2011-2012 to study their effects with following objectives:-

i Collection and identification of different *Aloe* species.

ii To study morphological and physiological parameters of *Aloe* species.

iii To determine and compare the antioxidant activity of different *Aloe* species.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

In recent times, focus on plant research has increased all over the world and a number of evidences has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period. There are 2,50,000 higher plant species on earth. More than 80,000 are medicinal. In india, there are 45,000 different plant species. Of these , about 15,000-20,000 species have good medicinal value. However only 7,000-7,500 species are used for their medicinal values by traditional communities (Asparagus sp. and Berginia sp. etc.). At present medicinal plants are important for the global economy, as approximately 80 per cent of traditional medicinal preparations involve the use of the plant or plant extract (Dhyani and Kala 2005). *Aloe* is one of them.

2.1 Plant Description

2.1.1 General Description

The word *Aloe* is derived from the Arabic word "Alloeh", means 'bitter and shiny substance'. *Aloe vera,* often called the "Natural healer", "Lily of the desert" or the "Plant of immortality", is a member of family Liliaceae, the family of perennial tropical plants of African origin. More than 400 species are known worldwide. Records of the use of *Aloe vera* as folk medicine date to antiquity with an early account from around 1500 B.C. The exudates of *Aloe vera* are used for numerous medical and cosmetic applications since ancient times (Morton 1961).

Aloe vera has thick fleshy leaves covered with fleshy spikes and bears hanging and tubular yellow flowers (Martinez *et al.* 1996). *Aloe* is native to North Africa but found abundantly throughout the warmer part of the world. *Aloe vera* is now planted under all warm climates of the world. *Aloe vera* has been used for centuries in China and India. Legend suggests that "Aristotle" convinced "Alexander The Great" to capture the "Island of Socotra" in the "Indian ocean" for the supply of *Aloe vera* to heal wounded soldiers. The virtues of *Aloe vera* have been recorded by many ancient civilizations, those of Iran (Persia) , Egypt , Greece, Italy and India. In India , only 4 species are reported to occur and among these *Aloe barbadensis* is most widely naturalized (Anon 1962). The

plant is reported to grow to a huge tree in Africa (its native home) but normally it grows 1-2 feet high and 2-3 feet wide. *Aloe vera* has two major liquids present in it, yellow latex (exudates) and a clear gel (mucilage). Thus, there are three portions of *Aloe vera* leaves, yellow sap containing mainly anthroquinones, internal gel matrix (mucilage) or the fillet and the rind which consists of outer rinds, tips, bases and thorns (Chauhan *et al.* 2007).

In India , the use of different parts of several medicinal plants to cure specific ailments has been vogue from ancient times. The indigenous system of medicine namely Ayurvedic, Jidda and Unani have been in existence for several centuries. There is a growing tendency all over the world to shift from synthetic to natural based products including medicinal plants. Commercial exploitation of *Aloe vera* gel has been carried out for at least 50 years. Various companies in the USA act as primary growers and processors of the plant and manufacture bulk supplies of the gel for domestic and export market. Many other companies are secondary processors of *Aloe vera* products, and cosmetics firms and chain store often buy the gel for incorporation into their own brand name products (Grindlay and Reynolds 1986).

In food industry also it is being used as an ingredient for functional foods. Though the exact data are not available about area and production of *Aloe vera* in India, but as an estimate it has occupied an area of about 75 ha with an annual production of 1400 tonnes (Saroj *et al.* 2004). The commercial farming of *Aloe* is still in its primary stages but in the recent past its cultivation in the semi arid regions of Rajasthan, Gujarat, Andhra Pradesh, Madhya Pradesh , Maharashtra, Tamil Nadu and Karnataka has increased. In Himachal Pradesh some production has been reported in Kullu, Chamba, Mandi, Shimla, Solan, Kangra and Una districts, as the agro climatic conditions for *Aloe vera* cultivation are favorable (nccam.nih.gov.2006). In fact *Aloe* has been identified as a promising plant for diversification and income generation in Himachal Pradesh because of its multidimensional uses.

2.1.2 Physical Characteristics

1. Number and Weight of Leaves

Lele (2007) studied 100 mature *Aloe vera* plants for number and weight of leaves and concluded that each plant of *Aloe vera* contains 12-16 leaves with a mean weight of 1.36 kg.

Mishra (2008) concluded from her studies that the value for number of leaves per plant of *Aloe vera* ranged from 10.0-17.0 with a mean number of 14±4 leaves/plant and the value for their weight ranged from 80-260 g/leaf.

Rodriguez *et al.* (2008) studied *Aloe vera* plant for its weight and reported that plant weight may vary from 850 - 2,500 g.

Hazrati *et al.* (2012) studied the effect of nitrogen fertilization on number and weight of leaves of *Aloe vera* plant and concluded that each plant of *Aloe vera* contains 20 to 22 leaves with a mean weight of 262.36 to 317.52 g.

2. Length, Width and Shape of Leaves

Bently (2000) studied mature leaves of *Aloe vera* and reported that on an average leaves are 20-24 inches in height and 4-6 inches across the base, tapering towards a point at the end.

Anon (2004) described *Aloe vera* leaves as fleshy, sword–shaped which are grey-green in colour and grow upto 80 cm long.

Bhattacharjee (2004) reported the mean length and width of *Aloe vera* leaves as 50 cm and 80 cm respectively.

Hasanuzzman *et al.* (2008) studied plant characteristics , growth and leaf yield of *Aloe vera* as affected by organic manure in pot culture and reported mean no. of leaves/plant as 8.67 to 12 and fresh weight as 393.33 to 993.33 g

Mishra (2008) reported the length of each leaf ranged from 16.50-14.50 cm with a mean length of 32.17±7.14.

2.2 Chemical Characteristics.

1. Moisture Content

Djeraba *et al.*(2000) found that the gel consists primarily of water 98 per cent, and very little dry matter 2 per cent. Whereas Paul (2003) reported the moisture content of *Aloe vera* gel varying from 97 to 99 per cent.

Vega *et al.* (2005) studied the gel of the plant and concluded that water is retained in the gel to facllitate adaptation to xerophytic climate. The each gram of dense gel was reported to contain 0.985 g water.

Bhatia (2007) quoted that on an average *Aloe vera* gel contains 98 per cent water.

Chauhan *et al.* (2007) also studied the moisture content of *Aloe vera* and reported the value to vary from 99-99.5 per cent.

Mishra (2008) concluded from her study that gel of *Aloe vera* contained 97.85 per cent moisture content.

2. Mineral Contents

Gautam and Awasthi (2007) reported that among various minerals elements analyzed, contents of copper, zinc, calcium, and iron in leaf powder as 0.81±0.09, 2.3±0.18, 22.9±1.00, 64.8±0.009 mg/100 g and in fresh leaves as 0.025±0.00, 0.007±0.01, 0.73±0.02, 2.1±0.18 mg/100g respectively.

Ravindra *et al.* (2007) analysed *Aloe vera* gel powder for inorganic mineral matter and reported an ash yield of 3.25 g/100g of powder.

Rodriguez *et al.* (2008) stated that the elemental analysis values of *Aloe vera* gel for sodium and mangenese were equal to those reported by others. However , the calcium iron, magnesium and phosphorous contents were different.

Mishra (2008) reported that gel of *Aloe vera* was found to be rich in potassium and phosphorous (138 and 195 mg/100 ml), low in magnesium, (155 mg/100 ml). Among the macro minerals, *aloe vera* gel was rich in iron (16.1±2.0 mg/100 ml) and poor in copper (3.15±0.5 mg/100 ml).

3. pH

Some authors have studied the *Aloe vera* gel for its pH values varying from 4.4 to 4.8 (Paul 2003 and Chauhan *et al.* 2007).

Gautam and Awasthi (2007) prepared *Aloe vera* leaf powder (AVLP) and reported its average pH values as 4.8.

Mishra (2008) reported pH of *Aloe vera* gel as 4.30 ± 0.17 .

4. Ascorbic Acid

According to Jacques (1999) age related cataract and age related ocular degeneration are delayed by consumtion of antioxidant nutrients such as vitamin C and E.

Paul (2003) reported that *Aloe vera* contains many vitamins including important antioxidant vitamins- vitamin A and C and carotenoids but has not specified the exact value.

Gautam and Awasthi (2007) standardized the *Aloe vera* leaf powder (AVLP) and concluded from their study that the *Aloe vera* leaf powder contained 27.0 mg/100 g ascorbic acid whereas fresh *Aloe vera* leaf contained 0.87 mg/100 g ascorbic acid.

Mishra (2008) concluded the ascorbic acid content in *Aloe vera* was very less as 1.06±0.12 mg/100 ml

5. Crude Fibre

Sharma & Pareek (2003) conducted a research on *Aloe vera* leaf and conducted from their findings that crude fibre content in fresh leaves is as high as 18.2 % on wet weight basis.

Gautam and Awasthi (2007) reported that fresh *Aloe vera* leaf contains 0.59 percent crude fibre, whereas the dehydrated *Aloe vera* contained 18.5 per cent crude fibre.

Mishra (2008) reported that the crude fibre content of *Aloe vera* gel is 0.61 per cent on fresh weight bases and 26.82 per cent on dry weight bases.

6. Carbohydrates

Green (1996) and Paul (2003) analysed mucilage layer of the *Aloe vera* for its sugar content and reported that sugars formed 25 per cent of the solid fraction and comprised of glucose, mannose and some polysaccharides.

Gautam and Awasthi (2007) reported that the mean carbohydrate content of *Aloe vera* leaf powder as 48.0±0.60 per cent on dry matter basis and 1.54±0.17 per cent on fresh weight basis. The reducing and non-reducing sugars in fresh *Aloe vera* leaf were reported to be almost equal (24.3 mg/100 g). The leaf powder was also reported to contain equal amounts of both the sugars (76.0 ± 0.57 mg/100 g).

Wendell (2007) studied *Aloe vera* gel for various sugar constituents and stated that the most prominent monosaccharide in *Aloe vera* gel is mannose-6-phosphate and the most common polysaccharide are aluco-mannans (beta-1,4) acetylated mannan.

Mishra (2008) concluded that the digestible and total carbohydrate in *Aloe* gel was 37.13 and 17.13 per cent.

2.3. Other Constituents

Adamski *et al.* (1976) studied chemical contents in the leaves of three year old *Aloe arborescens*. He found the prescence of 9.74 % total protein per dry weight, 7.94% active protein and 1.80 % non-protein nitrogenous substances.

Erazo *et al.* (1985) studied evaluation of humectants properties of *Aloe perryi* Baker. Chemical analysis revealed the prescence of saponins, mucilages, free sugars and anthraquinones, but the absence of tannins and alkaloids.

Dagne *et al.* (1994) studied the prescence of anthraquinones, pre anthraquinones and isoeleutherol in the roots of *Aloe* species. Technique of Thin layer chromatography was used for this purpose.

Djeraba *et al.* (2000) concluded that the gel consists primarily of water (> 98%) and polysaccharides (pectins, cellulose, hemicelluloses) and glucomannan , acemannan and mannose derivatives.

Lee *et al.* (2001) studied the acemannan compound and considered it as the main functional component of *Aloe vera* which is composed of a long chain of acetylated mannose.

Succu *et al.* (2001) studied the *Aloe vera* latex (or *aloe* juice) which is the bitter yellow exudates from the pericyclic tubules in the outer skin of the leaves. The major and active constituents of *aloe* latex were reported to be hydroxyl anthracene derivatives (15-40 %) such as the anthraquinone glycosides aloin A & B.

Vega *et al.* (2005) concluded that the gel of *Aloe vera* is protected by a film of a substance known as *aloe*, composed mainly of resins and aloins.

Gautam and Awasthi (2007) reported the water insoluble dietary fiber in *Aloe vera* leaf powder as 16.9±0.01 per cent and in fresh leaves as 0.54±0.01 per cent, whereas the water soluble fibre contents in *Aloe vera* leaf powder and fresh leaf were reported as 4.4 ±0.01 per cent and 0.14 ± 0.00 per cent respectively.

Huaxue *et al.*(2007) studied the prescence of aloin in *Aloe* with the technique of reversed-phase high performance liquid chromatography. Aloin was successfully separated on a discovery column using methanol-water as mobile phase.

Saradhi *et al.*(2007) studied effect of NPK fertilizers on chemical constituents of *Aloe vera* leaves. Free o-glycones and o-glycosides were not affected by levels of NPK nutrients applied, but c-glycosides, anhydrous barbaloin and hydroxy anthroquinone derivatives were significantly affected with application of NPK fertilizers at 40:40:40 or 80:80:80 $N:P_2O_5:K_2O$ kg/ha.

Wendell (2007) studied *Aloe vera* and stated that the gel of *Aloe vera* contains glycoprotein with mannan named acemannan which has been isolated and is being marketed as "Carrisyn". In addition to it he mentioned in his study that recently a novel anti-inflammatory compound C-glucosyl chromosome has been isolated from *Aloe vera* gel. Further he reported that *Aloe* gel contains lignin, salicylic acid, saponins, sterols, triterpenoids along with proteolytic enzyme, caboxypeptidase, and gluthione peroxidase as well as several isozymes of super oxido dismutase.

Alemdar *et al.*(2009) studied investigation of in vitro antimicrobial activity of *Aloe vera* juice. The disc diffusion method was used to test the antimicrobial activity. The study showed that *Aloe vera* juice has antimicrobial activity against *M. smegmatis*, *K. pneumoniae*, *E. faecalis*, *M. luteus*, *C. albicans* and *B. sphericus*, but has no inhibitory effect against the other bacterial strains.

2.4. Medicinal , Therapeutic and Cosmetic Uses

Aloe has numerous uses in medical science it has a long history as a skin lotion. The bulk of the *Aloe* leaf is filled with gel, 96% water with the other 4% containing 75 known substances. Applied to wounds, *Aloe* gel is a mild anesthetic, relieving itching, swelling and pain. It is antibacterial and antifungal, increases blood flow to wounded areas, and stimulates fibroblasts, the skin cells responsible for wound healing. The gel is used in many formulations for medicinal and cosmetic purposes. It is probably one of the most discussed medicinal plant in history.

Waller *et al.*(1978) studied the natural products from *Aloe barbadensis* Miller. Seventeen amino acids D-glucose and D-mannose, cholesterol, campesterol, beta-sitosterol, lupeol and unknown alkaloid were isolated from *Aloe barbadensis* leaves.

Aloe vera's chemical and therapeutic properties have been reviewed in detail by Coats & Ahola (1979) whereas Gupta *et al.*(1981) conducted a study on *Aloe vera* and concluded that the prevalent use of orally ingested *Aloe vera* gel juice is useful for ulcerous , gastrointestinal and kidney problems.

Aggarwal (1986) reported that *Aloe* had virtually eliminated effect of heart disease stress related disorders and diabetes in majority of the experimental subjects who were instructed to take approximately 4 oz of fresh *Aloe* juice and ¾ oz of the husk of isabgol mixed with wheat flour to make a loaf of bread. Treatment considered eating of loaf of *Aloe* bread per day.

Kirtikar and Basu (1989) described various properties uses and health benefits of *Aloe*. The author have reported that fresh *Aloe vera* juice is catharatic and cooling. Squash acts as tonic. The strained mixture of juice and rose water is used as eyewash for catarrhal and purulent of opthalmia and other eye disorders.

Anon (1992) described that *Aloe vera* was also used extensively by ancient Indian civilizations for its cathartic, emmemagognic (promotion of menstrual flow) and anti-helmintic (de-worming) properties.

Davis *et al.* (1994) studied Mannose-6-phosphate, a sugar constituent of the *Aloe vera* gel which was demonstrated to have wound healing properties.

Ishii *et al.* (1994) conducted a study on anthroquinones present in *Aloe vera* latex and studied their functions on laxative stimulation . It was concluded from the study that *Aloe vera* latex increased intestinal water content , stimulated mucus secretion and increased intestinal peristalisis.

Udupa *et al.*(1994) studied the anti-inflammatory and wound healing properties of *Aloe vera.*

Anon (1996) recommended through his study the use of *Aloe vera* juice for treating ulcer, application of gel liberally twice a day for burns and eczema, and use of 5 drops of tincture in water before meal for stimulation of appetite.

Choii *et al.* (2001) concluded from their study that a number of glycoproteins present in *Aloe vera* gel have been reported to have an anti tumour anti ulcer effects and to increase proliferation of normal human dermal cells.

Neall (2004) reported that the fresh juice obtained from the cut bases of the leaves is catharatic and cooling and used to treat liver, spleen and muscular pain.

Panda (2004) recognized *Aloe vera* as one of the best agents to promote weight loss. He stated that *Aloe vera* gel stabilizes BMI, and it also stimulates the metabolic rate in liver cells, helping to burn more energy.

Vibha (2004) concluded from a study that the active principles of *Aloe vera* can not only detoxify the body of harmful toxins , but also rid the body of excess adipose tissues in a harmonious way.

Devi *et al* .(2005) studied cosmeceutical applications of *Aloe* gel. The chemistry, cosmetic and medical applications of *Aloe* gel as well as its adverse effects were discussed. Therapeutic properties of *Aloe* gel includes its healing effects on wounds, on burns, frostbite damage and on inflammation.

Subramanian *et al.* (2006) studied wound healing potential of *Aloe vera* leaf gel in experimental rabbits. The ethanolic extract in the form of an ointment was tested in an excision wound model. The prophylactic effect elicited by the extract in terms of wound contraction and related biochemical parameters strongly support the healing and sealing properties of *Aloe vera* leaf gel.

Subramanian *et al.*(2006) studied in vitro antibacterial and antifungal activities of ethanolic extract of *Aloe vera* leaf gel. In vitro antimicrobial properties of ethanolic extract of *Aloe vera* leaf gel were investigated against various common pathogenic becteria and fungi. The well and disc-diffusion method showed significant zone of inhibition against all the pathogens studied .

Lele (2007) described that externally *Aloe vera* is used as a base for aromatherapy facial cosmetic products especially during troubled oily periods. After washing and tonning the face, light massage with cold *Aloe vera* gel for a few moment blots off the excess oil and tightens the pore. It was concluded from this study that *Aloe vera* has proved best in the development of shampoos and creams.

2.5. Morphological Parameters:-

In general, Variation in leaf area was the main factor determining difference in yield. Variation in NAR was of minor importance (Watson 1947).

Short day produced greater leaf area than long day and the determining factor was the total amount of high intensity light rather than duration of illumination per day (Duncan 1959).

Basuchaudhari and Das Gupta (1982) reported that the increased plant height is generally accompanied by greater leaf number per plant leading to increased LAI and dry matter content.

Chlorophyll content has been reported to be positively correlated with rate of photosynthesis and yield (Khenyon and Turner 1990).

Chlorophyll is main pigment involved in photosynthesis process which is responsible for the production of dry matter. Chlorophyll content of leaf tissue varies with cultivars, age and the crop growth stages, light and temperature (Kumar and Singh 1996).

Rosati (2002) reported application of large amount of organic manure may be beneficial in improving soil parameters but in the absence of soil pathogens it is likely to cause them to proliferate.

Saha *et al.*(2005) conducted an effects of organic and inorganic sources of fertilizer on performances of *Aloe vera* .The organic source of fertilizer was liquid vermiwash, while inorganic N and K fertilizers were urea and muriate of potash in solution form. The result revealed that there was significant increase in biological and gel yields, plant height, number of leaves per plant and chlorophyll content with application of fertilizer as compared to no fertilizer treatment.

Kaur *et al.* (2010) studied somaclonal variation in plants regenerated from cultures of two morphologically distinct accessions of *Aloe vera* linn and found chlorophyll content in clonal and regenerated plants of accession HPM1, as 3.03 ± 0.006 and 3.08±0.019 *u*g/mg and in clonal and regenerated plants of accession PBL3 chlorophyll content was found to be 5.34 ± 0.078 and 4.06 ± 0.016 *u*g/mg respectively.

2.6. Antioxidant

Lee *et al.* (2000) isolated and identified a phenolic antioxidant from methanolic extract of *Aloe barbadensis* using a combination of column and thin-layer chromatography. Rajeskaran *et. al* (2005) evaluated the presence of antioxidant property in the alcoholic extract of *Aloe vera* leaf gel. Oral administration of *Aloe vera* leaf gel extract at a conc. of 300 mg/kg to diabetic rats significantly decreased the levels of blood glucose, glycosylated hemoglobin and increased haemoglobin.

Yagi *et al.* (2002) determined antioxidant, free radical scavenging and anti-inflammatory effects of aloesin derivatives in *Aloe vera.* The DPPH radical and superoxide anion scavenging activities were determined.

Yun *et al.* (2003) determined the polysaccharide and flavonoid concentrations of two- three, and four-year-old *Aloe vera* , and their antioxidant activities were evaluated compared to BHT and alpha -tocopherol by the DPPH radical scavenging method and the linoleic acid system at 100 micro g of soluble solids per ml of ethanol. The results showed that three-year-old *Aloe vera* contained significantly higher levels of polysaccharides and flavonoids than two- and four-year-old *Aloe vera*, and no significant differences in flavonoid levels were found between three and four-year-old *Aloe vera.* All the *Aloe* extracts showed significant antioxidant activity. The antioxidant activity of *Aloe vera* extracts and reference compounds followed the order: three-year-old *Aloe vera* > BHT > four-year-old *Aloe vera* > alpha -tocopherol > two-year-old *Aloe vera.* The three-year-old extract exhibited the strongest radical scavenging activity of 72.19%, which is significantly higher than that of BHT at 70.52% and alpha -tocopherol at 65.20%. These data suggest that the growth stage plays a vital role in the composition and antioxidant activity of *Aloe vera.*

Qiuhui *et al.* (2005) evaluated free radical scavenging activities of extracts of *Aloe vera* leaf skin by supercritical CO_2 extraction and solvent extraction. The inhibition percentage of extracts of *Aloe vera* and reference antioxidants followed the decreasing order: Trolox (76.8 %) > ethanol extracts of *Aloe vera* skin (39.7%) > BHT (35.9%) > the extracts of supercritical carbon dioxide extraction (33.5%) > a-tocopherol (25.6%) > ethanol extracts of *Aloe vera* pulp (14.2 %). Compared to BHT and a-tocopherol , the

extracts of *Aloe vera* skin, by supercritical carbon dioxide extraction and ethanol, showed stronger antioxidant activities. Component in the rind of *Aloe vera* are responsible for the highest antioxidant activity of *Aloe vera* extracts.

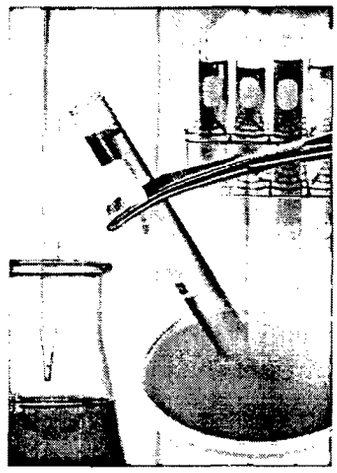
Miladi *et al.* (2008) evaluated the total antioxidant capacity of *Aloe vera.* The antioxidant capacity of the fruit extracts of *AVLS* (*Aloe vera* leaf skin) was found to decrease in this order: hexane extract>ethyl acetate extract > chlorophorm-ethanol extract > butanol extract.

Margarita *et al.*(2009) studied the influence of temperature on the drying kinetics, physicochemical properties, and antioxidant capacity of *Aloe vera* . A drying temperature of 80^0C and 90^0C resulted in significant variation in and/ or loss of the physicochemical and nutritional properties of the gel ; in addition the antioxidant capacity of the gel was decreased at these temperatures.

Saritha *et al.* (2010) studied antioxidant and antibacterial activity of *Aloe vera* gel extracts. The scavenging effect of the *Aloe vera* gel extracts and BHA using the DPPH radical were in the following order: BHA (94.56%)>MEAG (93.14%) >AEAG (74.03%) >CEAG(57.68%)>EEAG(57.21%)>HEAG(23.59%). The methanol extract showed better radical scavenging activity than the other extracts.

Kumar *et al.* (2011) studied *Aloe vera* and nine other plants and their possible constituents responsible for its antioxidant property and were compared by reducing power, 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity method. The % scavenging activity of *Aloe vera* was found higher in higher concentrations.

Tin AK (2011) evaluated antioxidant and antifungal activities of the leaf extract of *Aloe vera.* The scavenging activity of *Aloe vera* was found to be 58.36 *u*g/ml. He concluded that although *Aloe* extracts was not as high as the positive control, and ascorbic acid, it has also the higher antioxidant activity and *Aloe vera* gel made in methanol and ethanol possessed maximum DPPH radical scavenging activities.



MATERIALS AND METHODS

3. MATERIALS AND METHODS

An appropriate selection and application of scientific methodology is pre-requisite for conducting any investigation as it adds to precision, reliability and validity of the findings/ facts relating to the research problem and the present study is no exception. Therefore, in this chapter the methodology used in the present study is explained. The present investigation was conducted in the Herbal Garden, Department of Biology and Environmental Sciences, COBS, CSK HP Krishi Vishvavidyalaya, Palampur during the year of 2011-2012. The details of the material used and experimental method followed in the study are presented as under.

3.1. Materials

The experimental material mainly consisted of two *Aloe* Species i.e *Aloe vera, Aloe perryi* was collected from the Herbal Garden, Department of Biology and Environmental Sciences, COBS, CSK HP Krishi Vishvavidyalaya, Palampur and Institute of Himalayan Bio resource technology (IHBT), Palampur and experimental pots were procured from Department of Biology & Environmental Sciences, COBS, CSK HP Krishi Vishvavidyalaya, Palampur

3.2. Experimental Site

The pot experimental was conducted in the Herbal Garden, Department of Biology and Environmental Sciences, COBS, CSK HP Krishi Vishvavidyalaya, Palampur and is situated at 32°6′ N latitude and 72°3′ E longitudes and at an altitude of 1,290 m above mean sea level. This area falls in humid and wet temperate zone in the mid hills of Shivalik region of Himalayas. The soil is clay loam in texture.

3.3. Experimental Details

Whole experimental set up was conducted in pots and the pots were prepared by filling the mixture of different treatments and their combinations:-

Treatments	Symbols
Sand	T_1
Soil	T_2
Soil+Sand	T_3
Soil+FYM	T_4
Soil+Sand+FYM	T_5
Soil+Sand+Vermicompost	T_6

Aloe species were transplanted in 36 pots of 20cm diameter and 18 cm height, these 36 pots were used to record morphological observations at 15 days interval till harvest and also used for plants sampling at different time intervals i.e 40 D, 80 D, 120 D and 150 D. The plants were irrigated when needed. The experiment was set up on 25th September , 2011 by planting plantlets of different *Aloe* species.

Total number of pots : 36

Aloe species:-

(i) *Aloe vera*

(*ii*) *Aloe perryi*

To achieve the objectives of the study, observations on various physiological studies were undertaken both, in pots and in the laboratory on following parameters.

3.4. Morphological Parameters

Various morphological characters studied during experimentation include shape of leaves, plant height (cm), number of leaves, leaf area(cm^2), fresh weight of leaves (gm).

3.4.1 Shape of the leaves

Shape of the leaves of different *Aloe* species were observed visually.

3.4.2 Plant height (cm)

The plant height was recorded at fortnight interval. It was measured in cm from soil level up to the tip of longest leaf with the help of inch tape and their mean value was used for statistical analysis.

3.4.3 No. of leaves

The no. of leaves were counted manually in the field upto harvest and their mean values were used for statistical analysis.

3.4.4 Leaf area per plant (cm^2)

Leaf area was recorded with leaf area meter (CI-203, CID, Inc.) and their mean values were used for statistical analysis.

3.4.5 Fresh weight (gm)

Fresh weight of leaves was recorded during different time intervals and their mean values were used for statistical analysis.

3.5. Biochemical Parameters

Total chlorophyll content ($\mu g/g$) .

3.5.1 Total Chlorophyll Content (Hiscox and Israelstan, 1979)

From each sample 100 mg of fresh leaf portion was finely chopped and was dipped in a test tube containing 5ml of D.M.S.O. The test tube was then placed in an oven at 60°C for about 4 hrs. After requisite period and on attaining room temperature absorbance was read at 645 nm and 665 nm on spectrophotometer (Genesys 10 Vis) and the chlorophyll content was calculated using the following equation-

Total Chlorophyll Content ($\mu g/g$) = $20.2 \times A_{645} + 8.02 \times A_{665}$

Where, A = Absorbance

3.5.2 Determination of Ascorbic Acid (AOAC, 1990)

Weighed 5g of fresh leaves, brought to the laboratory just after plucking, and ground with 5ml of 2% oxalic acid as extraction medium in order to grt slurry. The total weight of slurry was recorded. 1g of this slurry was taken in a beaker and its volume was made upto 5 ml with 1% oxalic acid. The content of the beaker was filtered properly through Whatman filter paper No.1 and 0.25 ml of this filterate was pipetted out and titrated against a dye solution (2,6-dichlorophenol indophenols) prepared by taking 52 mg of dye in 200 ml volumetric flask adding 100 ml of hot distilled water. The volume was then made upto 200 ml with distilled water. After cooling 42 mg of $NaHco_3$ was added and mixed properly.

Photograph of *Aloe vera* plant

T_1- Sand

T_2- Soil

T_3 - Soil+Sand

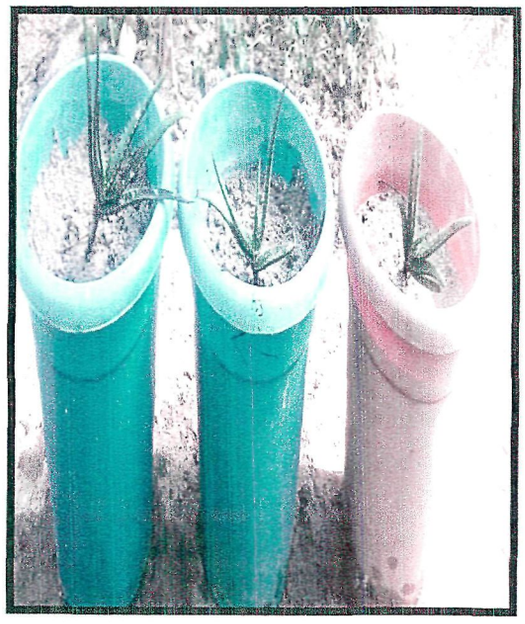
T_4-Soil+FYM

T_5-Soil+Sand+FYM

T_6-Soil+Sand+Vermicompost

Photographs of *Aloe perryi*

T_1-Sand



T_2-Soil

T_3-Soil+Sand

T_4- Soil+FYM

T_5-Soil+Sand+FYM

T_6-Soil+Sand+Vermicompost

3.5.2 i Preparation of Standard for Ascorbic Acid

At the same time 100 mg of ascorbic acid was dissolved in 500 ml of 1% oxalic acid, solution was used as standard (always prepared fresh). The amount of ascorbic acid in sample expressed as mg/100g as:-

$$\text{Ascorbic acid} = \frac{\text{Standard conc. of Ascorbic acid x A x volume made up x B}}{\text{Titre value of standard x Wt. of slurry x S x Volume used for titre}} \times 100$$

Where A= Titre value of sample

B= Total weight of slurry

S= weight of sample

3.5.3 Antioxidant activity (Kordali *et. al* (2005) and Sharma & Bhat (2009)

Free radical scavenging activity of aqueous solution of the samples were evaluated by the methods of Kordali *et. al* (2005) and Sharma & Bhat (2009).

REAGENTS :-

i) **DPPH Solution (200 *u*m)** : 0.002 g of 2,2 DPPH (Sigma USA) was dissolved in 25 ml of methanol(AR)

ii) **Sample solution:** Crushed 10 mg of sample in 10 ml of methanol.

PROCEDURE :-

To a known volume of (20, 40, 60, 80, 100 *u*l) 1mm of ascorbic acid (taken in two different sets of test-tubes and each set comprising of five test-tubes) was added methanol to make the final volume of 3 ml. 1 ml of 200 *u*m DPPH solution prepared in methanol was added to the test tube containing solutions of ascorbic acid . The contents were mixed thoroughly with the help of vortex and allowed to incubate at 30°C for 30 minutes in the dark. Absorbance was measured at 517 nm with the help of Spectrophotometer.

3.5.4 Mineral Content:-

The mineral contents were determined by using flame photometer and atomic adsorption spectrophotometer. Sample was wet digested for studying the minerals.

3.6 Statistical Analysis

Statistical analysis of data was carried out using analysis of variance method (ANOVA) and comparisons between the different parameters were tested at 5 per cent level of significance as suggested by Panse and Sukhatme (1985)

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

The present investigation entitled, "**Morphological and Antioxidant activities of *Aloe* species of Palampur and its Surrounding Area**" was undertaken to gather information regarding the species variability in different Morphological and Biochemical constituents of *Aloe* species.

The results on various parameters of the present study are presented as follows:

4.1. Variability in Morphological Parameters

4.1.1. Shape of the leaves

Shape of the leaves of the species of *Aloe*, is presented in **Table:- 4.1**. The shape of the leaves of *Aloe vera* and *Aloe perryi* was lanceolate, edges were serrated with numerous little spines. Leaves were crowded and tips were pointed. *Aloe vera* was greyish green in color whereas *Aloe perryi* was dark green in color. Leaves were thick in both species but in *Aloe perryi* the width was narrower whereas in *Aloe vera* it was broader. Leaves were mottled in both the species . Leaf surface was covered with wax.

Table-4.1 Variation in Shape of the leaves of *Aloe* species

Species	Shape of leaves
Aloe vera	Fleshy and thick, lanceolate (lance shaped), greyish green, serrated edges that are pinkish with numerous little spines, crowded, parallel venation, mottled surface, pointed tip, leaf surface covered with wax.
Aloe perryi	Lanceolate, dark green, spiny margins, fleshy and thick serrated edges, narrow width, mottled, dark green, waxy surface.

4.1.2. Plant Height

Plant height increased with time in both species . Analysis of varaiance indicated that application of different treatments significantly influenced the plant height.

A perusal of **Table:-4.2** indicated that the plant height after 150 days of plantation varied between 32.00 cm to 44.87 cm. The maximum plant height of *Aloe* species was recorded in T_6 (Soil+Sand+Vermicompost) (44.87cm) followed by T_5 (Soil+Sand+FYM) (43.63 cm) and T_4 (Soil+FYM) (43.30 cm). T_3 (Soil+Sand) (37.07 cm) also results in attaining a good increase in plant height. Least plant height in *Aloe vera* was found in control pot T_1 (Sand) (32.00 cm) followed by T_2 (Soil) (33.63 cm).

These results are supported by Hasanuzzaman *et al.* (2008) in which higher growth rate was observed in case of *Aloe vera* leaf treated with the application of 50% to 10% cow dung . The growth rate was highest where more cowdung was applied. The dose of cow dung improved the *Aloe vera* plant growth by providing the essential nutrient which influenced the leaf growth. Pichgram (1987) also observed similar results . In *Aloe vera* length decreased with the decrease in cowdung percentage so organic manure was found to be effective in growth trend.

In *Aloe perryi* after 150 days the plant height varied between 19.73 cm to 35.97 cm.**(Table:-4.3).** Plant height of *Aloe perryi* was highest with the application of T_6 (Soil+Sand+Vermicompost) (35.97 cm) followed by T_5 (Soil+Sand+FYM) (33.73 cm), T_4 (Soil+FYM) (32.40 cm), T_3(Soil+Sand) (26.57 cm) and T_2(Soil) (23.50 cm). The growth in control treatment T_1(Sand) (19.73 cm) was lower, where no organic manures and other mixtures were added. T_6 (Soil+Sand+Vermicompost) and T_5 (Soil+Sand+FYM) were found to be statistically at par with each other in both the species.

The growth of control treatment was lower but consistent. These findings are also in agreement with the results of Chatterjee *et al.* (1979) and Van Schaik *et al.* (1997) in that case organic manures has significant effect over control where no organic manure was applied. T_6 (Soil+Sand+Vermicompost) proved to be a better treatment for obtaining maximum increase in plant height.

Table:- 4.2 Plant Height of vegetatively propagated *Aloe vera* at different time intervals:-

	Plant height (cm) of *Aloe vera*									
Treatments	**15 D**	**30 D**	**45 D**	**60 D**	**75 D**	**90 D**	**105 D**	**120 D**	**135 D**	**150 D**
T_1 (Sand)	26.10	27.00	27.97	28.67	29.43	30.27	30.83	31.33	31.77	32.00
T_2 (Soil)	27.77	28.50	29.07	29.93	30.83	31.57	32.27	33.00	33.43	33.63
T_3 (S+S)	27.33	28.43	30.37	32.30	34.30	35.23	35.93	36.57	36.97	37.07
T_4 (S+F)	28.60	30.93	33.70	36.50	38.40	39.77	40.80	42.27	43.23	43.30
T_5 (S+S+F)	29.43	31.50	34.40	37.50	39.40	41.57	42.47	42.97	43.30	43.63
T_6 (S+S+V)	29.13	31.97	35.67	39.13	40.77	42.80	43.60	44.20	44.60	44.87
CD (5%)	1.64	2.09	1.80	2.18	2.23	2.49	2.50	2.46	2.86	2.84

Table :- 4.3 Plant height of vegetatively propagated *Aloe perryi* at different time intervals:-

	Plant height (cm) of *Aloe perryi*									
Treatments	**15 D**	**30 D**	**45 D**	**60 D**	**75 D**	**90 D**	**105 D**	**120 D**	**135 D**	**150 D**
T_1 (Sand)	16.03	16.67	17.07	17.63	18.03	18.57	18.97	19.37	19.53	19.73
T_2 (Soil)	16.67	17.67	18.93	20.07	20.90	21.83	22.30	22.70	23.23	23.50
T_3 (S+S)	17.80	19.17	20.03	22.27	23.33	24.70	25.23	25.93	26.33	26.57
T_4 (S+F)	17.33	19.97	24.10	26.60	28.47	30.17	30.70	31.57	32.10	32.40
T_5 (S+S+F)	17.93	22.23	25.73	28.93	30.53	31.97	32.33	33.03	33.43	33.73
T_6 (S+S+V)	18.97	22.17	26.47	29.40	32.30	33.90	34.50	35.00	35.53	35.97
CD (5%)	1.06	2.25	1.58	2.10	2.20	3.36	3.24	3.12	3.24	3.28

4.1.3. Number of leaves /plant

Analysis of variance showed that T_6 (Soil+Sand+Vermicompost) had a significant effect on number of leaves/plant of *Aloe vera* and *Aloe perryi*. This trend was followed by pots treated with T_5 (Soil+Sand+FYM). The lowest number of leaf per plant was observed in case of control . The difference in number of leaves became significant after 40 days of plantation. Mean number of leaves after 150 days ranged from 9.33 to 16.33 in *Aloe vera* and 7.00 to 14.33 in *Aloe perryi*. Maximum number of leaves after 150 days were found to be in with the application of T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM).**(Table:-4.4).**These results are in agreement with those of Hasanuzzaman *et al.* (2008) in which increased application of organic manure significantly increased the number of leaves. Hazrati *et al.* (2012) also reported number of leaves as (20.62± .47 to 22.17± .33) in *Aloe vera.*

In both the *Aloe* species T_4 (Soil+FYM) and T_5 (Soil+Sand+FYM) were found to be statistically at par with each other. Mean no. of leaves increased with time in both the species. The plants on an average bear 9-16 leaves in number in *Aloe vera* and 7-14 leaves in *Aloe perryi*. Values reported for these are almost close to these with some variations reported by some authors (Bently 2000; Anon and Bhattacharjee 2004; Lele 2007 ; Rodriguez *et al.* 2008).

Table :- 4.4 Number of leaves of vegetatively propagated *Aloe vera* and *Aloe* perryi at different time intervals:-

	Number of leaves							
	Aloe vera				*Aloe perryi*			
Treatments	**40D**	**80 D**	**120 D**	**150 D**	**40 D**	**80 D**	**120 D**	**150 D**
T_1 (Sand)	7.00	7.33	8.33	9.33	4.33	5.33	6.00	7.00
T_2 (Soil)	7.67	9.33	11.33	12.33	6.33	8.00	9.67	10.33
T_3 (S+S)	7.00	8.67	10.33	11.67	6.67	8.33	10.00	10.67
T_4 (S+F)	7.67	10.33	12.67	14.00	7.33	10.33	12.33	13.67
T_5 (S+S+F)	8.00	11.00	13.33	14.67	7.67	10.67	12.67	14.00
T_6 (S+S+V)	9.33	12.33	15.33	16.33	7.33	10.67	13.00	14.33
CD (5%)	0.74	1.41	1.27	1.41	1.04	1.20	1.47	1.70

4.1.4 Leaf area:-

Leaf area increased with time in both the species. **(Table:-4.5)**. Analysis of variance showed significant increase for mean leaf area of vegatatively propagated plants of *Aloe vera* and *Aloe perryi* after 40 days of plantation. The application of T_6 (Soil+Sand+Vermicompost) had a significant effect on leaf area of *Aloe vera* and *Aloe perryi* followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+FYM). At the interval of 150 days after plantation (DAP) in *Aloe vera*, leaf area varied from 78.67 to 201.33 cm^2 and 9.67 to 49.00 cm^2 in case of *Aloe perryi*. Least leaf area was found in the control T_1 (Sand) in *Aloe vera* and *Aloe perryi* so all other treatments were found to be significant over control T_1. Leaf area of *Aloe vera* leaves was higher than *Aloe perryi* in all the treatments. Leaf area of *Aloe vera* and *Aloe perryi* in treatments T_6 (Soil+Sand+Vermicompost) and T_5 (Soil+Sand+FYM) were found to be statistically at par with each other at the interval of 120 days in *Aloe vera* and at the interval of 40 and 80 days in case of *Aloe perryi*. The results of increase in leaf area with the application of organic manures are supported by Hasanuzzaman *et al.* (2008) in which they also reported that with the increase in application of organic manure or cow dung , leaf area increased significantly.

Table:- 4.5 Leaf area of vegetatively propagated *Aloe vera* and *Aloe perryi* at different time intervals:-

	Leaf Area (cm^2)							
	Aloe vera				*Aloe perryi*			
Treatments	**40D**	**80 D**	**120 D**	**150 D**	**40 D**	**80 D**	**120 D**	**150 D**
T_1 (Sand)	68.67	71.67	74.67	78.67	7.33	8.33	9.00	9.67
T_2 (Soil)	77.67	90.67	104.67	119.67	12.33	25.33	32.33	35.33
T_3 (S+S)	90.33	106.00	118.00	130.00	15.33	24.00	29.00	31.33
T_4 (S+F)	115.67	136.67	154.67	172.00	14.67	26.33	34.00	38.33
T_5 (S+S+F)	131.67	160.33	174.00	194.00	23.33	30.33	39.67	44.33
T_6 (S+S+V)	140.67	170.33	180.67	201.33	25.33	34.67	44.67	49.00
CD (5%)	5.19	7.33	7.21	7.04	4.61	6.09	3.67	4.20

4.1.5 Fresh Weight:-

Table 4.6 depicted that fresh weight of *Aloe vera* and *Aloe perryi* increased with time. Luxuriant increase in fresh weight of *Aloe vera* and *Aloe perryi* occur in the T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+ FYM). T_3 (Soil+Sand) and T_2 (Soil) also yielded a good amount of fresh weight. Least fresh weight was observed in control pot (100 % Sand). All the treatments were found to be significant over control. Overall the mean fresh weight of *Aloe vera* was much more than that of *Aloe perryi* and at all the time intervals organic manures showed significant effect over control and other treatments. It was due to the beneficial effect of organic matter in soil properties and plant growth (Dexter 1988; Tisdall and Oades 1982; Uyanoz *et al.* 2002).

Table 4.6 Fresh weight of vegetatively propagated *Aloe vera* and *Aloe perryi* at different time intervals:-

	Fresh Weight (gm)							
	Aloe vera				*Aloe perryi*			
Treatments	**40D**	**80 D**	**120 D**	**150 D**	**40 D**	**80 D**	**120 D**	**150 D**
T_1 (Sand)	42.43	45.39	50.08	59.01	13.27	15.26	18.37	18.96
T_2 (Soil)	52.93	70.67	97.40	107.89	18.51	22.94	25.93	26.07
T_3 (S+S)	64.89	84.37	104.64	121.60	25.27	28.44	29.95	30.08
T_4 (S+F)	84.63	115.78	130.34	142.93	24.49	30.93	35.50	36.40
T_5 (S+S+F)	96.97	133.80	148.74	156.82	32.70	37.26	39.23	39.83
T_6 (S+S+V)	106.56	145.87	158.25	177.34	33.73	42.01	46.93	47.60
CD (5%)	7.65	8.47	9.58	9.95	4.01	5.09	3.78	3.85

Fresh weight ranged from 59.01 to 177.34 gm and 18.96 to 47.60 gm in *Aloe vera* and *Aloe perryi* respectively at the interval of 150 DAP. T_2(Soil) and T_3 (Soil+Sand) were found to be statistically at par with each other at the interval of 120 days after

plantation (DAP) whereas T_6 (Soil+Sand+Vermicompost) and T_5 (Soil+Sand+FYM) were found to be statistically at par with each other at the intervals of 40 and 80 DAP in *Aloe perryi*. Mirza *et al.* 2008 also observed the same trend in increasing fresh weight of *Aloe vera* when treated with organic manure. Using 50% organic manure was effective in increasing leaf fresh weight per plant. The effect of cowdung was superior to the control in the experiment supported by Saha *et al.* (2005) and Nobel *et al.* (1991).

Hazrati *et al.* 2012 studied effect of application of chemical fertilizer (Nitrogen) on leaf fresh weight of *Aloe vera* plants and found that application of four levels of Nitrogen significantly increased the leaf fresh weight of *Aloe vera* plant (i.e) 262.36±24.85 to 317.52±14.24 gm. The difference in the values could be due to the environmental conditions and due to different fertilizers and their doses.

Variations in the values of physical parameters of *Aloe* species reported in this study and those in the literature earlier can be due to the variations in the species and agro climatic conditions under which the *Aloe* leaves were grown and collected by the author to initiate the study.

4.2 Biochemical Parameters

4.2.1 Chlorophyll Content

Chlorophyll content increased with time in both the species but at the intervals of 150 days the rate of increase of chlorophyll content was ceased . Analysis of variance showed significant variations in chlorophyll content of leaves in vegetatively propagated plants of *Aloe species* after 40 days, 80 days and 120 and 150 days. Both *Aloe* species with the application of T_2 (Soil) and T_3 (Soil+Sand) were found to be statistically at par each other in *Aloe vera.* Maximum amount of chlorophyll content was recorded in T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM) in both the species. In case of *Aloe vera* the overall variation ranged from 8.18 to 17.77 *ug*/g and in case of *Aloe perryi* it ranged from 11.82 to 31.78 *ug*/g so *Aloe perryi* showed far more content of chlorophyll than the other *Aloe vera* (i.e) Data related to this attribute is depicted in **Table-4.7.** Data obtained from the table indicates that all treatments significantly increased chlorophyll content at different time intervals over control pot.

Table: -4.7 Chlorophyll content of vegatatively propagated *Aloe vera* and *Aloe perryi* at different time intervals:-

	Chlorophyll content (*ug*/g fresh weight)							
	Aloe vera				*Aloe perryi*			
Treatments	**40D**	**80 D**	**120 D**	**150 D**	**40 D**	**80 D**	**120 D**	**150 D**
T_1 (Sand)	4.53	7.20	8.06	8.18	8.71	11.02	11.55	11.82
T_2 (Soil)	6.89	9.11	10.49	10.71	14.78	17.00	18.81	19.18
T_3 (S+S)	6.21	8.16	9.26	9.42	13.01	14.82	16.37	16.89
T_4 (S+F)	6.91	10.17	12.89	13.44	16.52	18.86	21.38	21.99
T_5 (S+S+F)	9.41	13.64	16.55	17.56	18.75	22.71	25.41	26.45
T_6 (S+S+V)	10.27	15.48	17.44	17.77	20.27	25.23	31.13	31.78
CD (5%)	1.12	1.87	1.99	2.33	1.38	1.61	1.85	2.24

4.2.2 Determination of Antioxidant Activity

DPPH Radical Scavenging Activity

DPPH has been widely used to evaluate the free radical scavenging effectiveness of various antioxidant substances. The method has been used extensively to predict antioxidant activities because of the relatively short time required for analysis. The method is based on the reduction of alcoholic DPPH solution in the prescence of a hydrogen donating antioxidant due to the formation of the non radical form DPPH-H by the reaction (Glucin, 2006). Higher the IC_{50} value, lower the free radical scavenging activity.

Acccording to the DPPH assay **(Table:-4.8)** all the extracts exhibited a noticeable concentration-dependent antiradical effect but differed in their inhibiting activities. The scavenging activity was found more in *Aloe vera* with the application of T_6 (Soil+Sand+Vermicompost) [IC_{50}=156.4063μg/ml] followed by T_5 (Soil+Sand+FYM)

[IC_{50}=163.3870 *u*g/ml] and T_4 (Soil+FYM) [IC_{50}= 185.1891 *u*g/ml], T_3 (Soil+Sand) [IC_{50} = 215.6043 *u*g/ml] and T_2 (Soil) [IC_{50}= 276.8881 *u*g/ml]. Least scavenging activity was observed from control pot T_1 (Sand) [IC_{50} = 326.7672 *u*g/ml] and similarly extracts of *Aloe perryi* showed highest scavenging activity from T_6 (Soil+Sand+Vermicompost) [IC_{50}= 270.8545 *µ*g/ml] followed by T_5 (Soil+Sand+FYM) [IC_{50}=286.1340 *u*g/ml] ,T_4 (Soil+FYM) [IC_{50}= 299.1803 *u*g/ml] ,T_3 (Soil+Sand) [IC_{50} = 303.5762 *u*g/ml] and T_2 (Soil) [IC_{50}= 423.5714 *u*g/ml]. Least scavenging activity was observed from control pot T_1 (Sand) [IC_{50}=456.8217 *u*g/ml]. Data obtained from the table help in concluding the result that antioxidant activity of *Aloe vera* was far more than the *Aloe perryi* and with the addition of organic manures antioxidant activity of *Aloe* species was enhanced significantly.

Table 4.8 :- IC_{50} values of DPPH˙ scavenging activities of the studied *Aloe* species extract :-

Treatment	*Aloe vera*	*Aloe perryi*
T_1 (Sand)	326.7672	456.8217
T_2 (Soil)	276.8881	423.5714
T_3 (S+S)	215.6043	303.5762
T_4 (S+F)	185.1891	299.1803
T_5 (S+S+F)	163.3870	286.1340
T_6 (S+S+V)	156.4063	270.8545

The results are supported by Kumar *et al.* (2011). 10 different drugs were extracted and subjected to screening for their possible antioxidant activity and study clearly indicated that extracts of *Aloe vera* and 9 other plants possess antioxidants.

4.2.3 Ascorbic Acid :-

Table 4.9 depicts the ascorbic acid content of *Aloe vera* and *Aloe perryi* on fresh weight basis. The data revealed that *Aloe vera* and *Aloe perryi* contains very less amount of ascorbic acid (1.28mg/100g) and (0.95 mg/100g) respectively. The data on the prescence of this constituent is scanty; however, some studies have been reported which

are supportive to the data (Paul 2003; Gautam and Awasthi 2007; Chauhan *et al.* 2007).The maximum content of ascorbic acid was observed from the pots given treatment T_6 (Soil+Sand+Vermicompost) in both *Aloe species* followed by T_5 (Soil+Sand+FYM), T_4 (Soil+FYM), T_3 (Soil+Sand),T_2 (Soil) and T_1 (Sand). In *Aloe* vera pots treated with T_4 (Soil+FYM) and T_5 (Soil+Sand+FYM) ascorbic acid content was found to be statistically at par with each other.

Table:- 4.9 Ascorbic Acid Content of vegetatively propagated *Aloe* plants

	Ascorbic acid (mg/100g fresh weight)	
Treatments	***Aloe vera***	***Aloe perryi***
T_1 (Sand)	0.80	0.70
T_2 (Soil)	0.86	0.76
T_3 (S+S)	0.94	0.80
T_4 (S+F)	1.01	0.84
T_5 (S+S+F)	1.08	0.92
T_6 (S+S+V)	1.28	0.95
CD (5%)	0.08	0.03

4.2.4 Mineral Content

The data regarding mineral content of *Aloe vera* and *Aloe perryi* used in the present investigation is presented in **Table 4.10**.

Table depicts that in *Aloe vera* on dry weight basis minerals Cu, Ca and Zn were present as 0.89 mg/100g, 31.49 mg/100g and 1.87 mg/100g respectively in T_6 (Soil+Sand+Vermicompost), and in *Aloe perryi* as 0.85 mg/100g, 27.42 mg/100g and 1.82 mg/100g in T_6 (Soil+Sand+Vermicompost). Iron in *Aloe vera* was reported to be 78.89 mg/100g and in *Aloe perryi* 73.92 mg/100g. The results of the mineral contents of iron and copper are in agreement to some extent with those reported by Gautam and Awasthi (2007).

Table 4.10 Mineral Content of *Aloe vera* and *Aloe perryi*

	Mineral Content (mg/100g dry weight)							
	Aloe vera				*Aloe perryi*			
Treatments	**Fe**	**Cu**	**Zn**	**Ca**	**Fe**	**Cu**	**Zn**	**Ca**
Sand	58.19	0.66	1.27	18.38	44.64	0.52	1.17	13.50
Soil	61.51	0.74	1.87	21.29	54.06	0.67	1.43	16.91
S+S	65.84	0.74	1.30	22.59	58.07	0.66	1.56	17.58
S+F	71.22	0.85	1.73	26.67	63.21	0.80	1.68	22.63
S+S+F	73.72	0.88	1.85	28.78	68.37	0.83	1.73	24.96
S+S+V	78.89	0.89	1.87	31.49	73.92	0.85	1.82	27.42
CD (5%)	2.32	0.02	0.14	1.97	2.08	0.04	0.04	0.80

Among various mineral elements analyzed in *Aloe* species (i.e) *Aloe vera* and *Aloe perryi*, they were found rich in iron and poor in copper.

SUMMARY AND CONCLUSIONS

5. SUMMARY AND CONCLUSIONS

Aloe is an important plant and has a long history of use in Ayurvedic medicine. It is a member of Family Liliaceae and comprises of 400 species. A number of species have been introduced in India which grow in varied climates and different types of soils. It is a succulent, xerophytic and perennial plant. The leaves are of spreading nature rather than ascending type, tapering to a blunt point. Leaf surfaces are quite smooth and shining, dark green or pale green coloured on both surfaces. The plant produces a single, unbranched flowering stalk that is topped by a cluster of bright yellow, red purple or pale stripped flowers. The plant is reported to grow to a huge tree in Africa, its native home, but normally it grows 1-2 feet high and 2-3 feet wide. The active constituent of *Aloe* is a mixture of glycosides called aloin which vary in different species of *Aloe*. The chief constituent of aloin is barbaloin which is a pale, crystalline, glycoside soluble in water. The minerals including Zn, Cu, Ca and Fe are also found in considerable amounts. The plant has numerous uses in cosmetics also. The plant is used in many facial treatments, in minimizing frostbite damage and in relieving digestion problems etc . Besides this it is also rich in polyphenolic compounds and is well known for its antioxidant properties.

The salient findings of the study based on field observations and laboratory investigations are summarized as follows:-

- Plant height and number of leaves were increased with time in both the species. Maximum number of leaves and plant height were recorded in T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+FYM).

- Leaf area , fresh weight of leaves was recorded at four different time intervals. Number of leaves and leaf area increased in both the species but significant variation was found in the treatments. At the interval of 40, 80,120 and 150 days after plantation maximum number of leaves were recorded in T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM). Least number of leaves and fresh weight were recorded in T_1 (Sand).

Among *Aloe vera* and *Aloe perryi* maximum amount of fresh weight and number of leaves were recorded in *Aloe vera* .

- Chlorophyll content increased significantly upto 120 days and after that at the interval of 150 days rate of increase in chlorophyll content was ceased in both the species. Maximum chlorophyll content was found in T_6 (Soil+Sand+Vermicompost) followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+Sand+FYM). T_1 (Sand) showed least amount of chlorophyll content in both the species at all the time intervals.

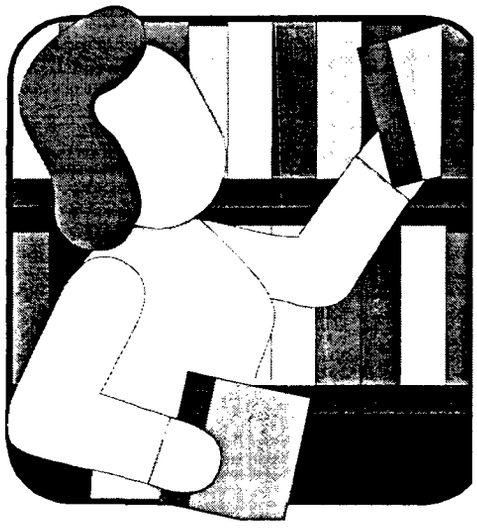
- Maximum ascorbic acid was evaluated in *Aloe vera* followed by *Aloe perryi.* In both the species pots treated with T_6 (Soil+Sand+Vermicompost) showed higher content of ascorbic acid followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+FYM) over control T_1 (Sand)

- Among *Aloe vera* and *Aloe perryi* , mineral content was higher in *Aloe vera* than *Aloe perryi. Aloe* species were found to be rich in iron whereas poor in copper. T_6 (Soil+Sand+Vermicompost) proved to be a better treatment for obtaining maximum amount of mineral content followed by T_5 (Soil+Sand+FYM) and T_4 (Soil+FYM).

- According to DPPH$^{\cdot}$ assay, maximum free radical scavenging activity and lowest IC_{50} was found in the sequence as T_6 (Soil+Sand+vermicompost) followed by T_5 (Soil+Sand+FYM), T_4 (Soil+FYM), T_3 (Soil+Sand), T_2 (Soil) and T_1(Sand) in both the species so minimum free radical scavenging activity and highest IC_{50} values were analysed in T_1 (Sand) . Antioxidant activity in *Aloe vera* was found more than that of *Aloe perryi.*

Free radicals are chemical entities that can exist separately with one or more unpaired electrons. The generation of free radicals can bring about thousands of reactions and thus cause extensive tissue damage. Lipids , proteins and DNA are all susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging the free radicals.

The results of this study showed that the application of Vermicompost and FYM enhance the growth and yields of *Aloe* species significantly because *Aloe vera* and *Aloe perryi* are succulent plants and more responsive to nutrients in comparison to the other plants. Fertilizers are sources of plant nutrients that can be added to the soil to supply its natural productivity. There is usually a dramatic improvement in both quality and quantity of plant growth when appropriate fertilizers are added and *Aloe* are favorable free radical scavengers as well as primary antioxidants that may react with free radicals and limit ROS (i.e.) reactive oxygen species attack on biological and food systems.



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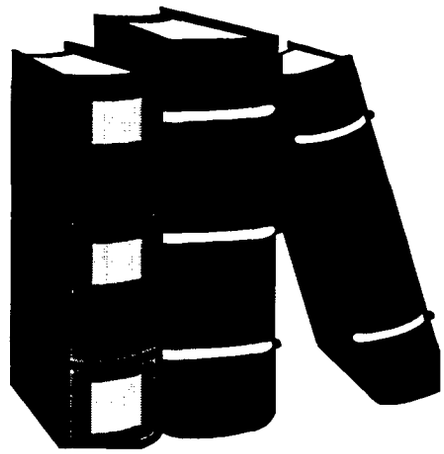

APPENDICES

APPENDIX(CES)

Mean weekly weather data for the year 2011-12 at CSK HPKV, Palampur

Standard week	Temperature ^{0}C		Relative humidity	Rainfall (mm)
	Max	Min	%	
40 (9th Oct. 2011)	25.5	15.8	86.5	32.2
41	27.1	14.4	72.5	0.0
42	25.9	12.5	64.5	0.0
43	24.2	11.7	63.5	2.6
44	23.4	12.6	74.0	1.8
45	23.6	11.5	79.5	0.0
46	23.0	10.1	79.0	0.0
47	22.8	9.7	81.0	0.0
48	20.7	7.1	76.5	0.0
49	21.9	8.7	88.0	6.0
50	18.5	6.6	85.5	0.0
51	17.9	3.6	88.0	0.0
52	18.1	3.8	67.5	0.0
1 Jan. (2012)	17.1	4.6	66.8	1.7
2	11.6	1.7	65.7	10.4
3	10.1	2.8	84.4	15.7
4	15.0	3.5	63.2	0.0
5	16.3	3.0	59.1	0.1
6	14.3	3.2	66.6	4.6
7	13.9	4.8	71.7	5.1
8	18.2	7.6	69.1	1.5
9	19.8	8.0	47.6	0.0
10	18.9	6.7	53.0	2.9
11	20.8	8.4	53.1	1.7
12	25.5	11.0	53.6	0.0
13	25.9	13.5	45.2	0.0
14	28.3	14.8	39.6	0.5
15	25.0	12.2	61.4	2.6
16	25.5	13.8	63.0	1.0
17	26.2	12.9	54.2	3.8
18	26.9	14.4	45.2	0.0
19	31.1	16.7	21.0	4.8
20	30.5	18.0	35.0	0.0
21	32.8	19.5	29.0	0.1
22	35.5	21.6	28.2	0.0

Brief Biodata of the Student

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Mother's Name : Smt. Maya Devi

Date of Birth : 25-06-90

Permanent Address : V.P.O Ghar and Teh. Palampur Distt. Kangra(H.P) Pin-176103.

Academic Qualifications:

Class	**Month, Year**	**School**	**Board/University**	**Marks (%)**	**Division**
10th	March 2005	Govt. High School Ghar	H.P.Board Dharamshala	79.85%	1st
12th	March 2007	Crescent Public Sr. Sec. School Banuri	H.P.Board Dharamshala	66.4%	1st
B.Sc. (Medical)	March 2010	G.G.D.S.D College Rajpur	H.P.University Shimla	72.10%	1st
M.Sc. (Biology)	July 2012	CSK HP Krishi Vishvavidyalaya, Palampur	CSK HPKV, Palampur	70.90%	1st