Studies on the effect of drying on the chemical composition and antioxidant activities of the fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda)

By

Sukriti Nehra

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CERTIFICATE – I

This is to certify that this thesis entitled **"Studies on the effect of drying on the chemical composition and antioxidant activities of the fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda)"** submitted for the degree of Master of Science, in the subject of **Chemistry** of the Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Ms. Sukriti Nehra**, Admn. No. **2011BS83M** under my supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged.

(Dr. M. Khabiruddin)
Major Advisor
Professor of Chemistry
Department of Chemistry and Physics
CCS Haryana Agricultural University
Hisar – 125004,
Haryana

CERTIFICATE – II

This is to certify that this thesis entitled **"Studies on the effect of drying on the chemical composition and antioxidant activities of the fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda)"** submitted by **Ms. Sukriti Nehra**, Admn. No. **2011BS83M** to the Chaudhary Charan Singh Haryana Agricultural University, Hisar, in partial fulfilment of the requirements for the degree of Master of Science, in the subject of C**hemistry**, has been approved by the Student's Advisory Committee after an oral examination on the same.

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CHAPTER–I

INTRODUCTION

Recent years have witnessed a renewed interest in plants as pharmaceuticals. This interest has been focused on the adoption of crude extract of plants. With this context, considerable interest has arisen in the possibility that the impact of several diseases may be prevented by improving the intake of phytochemicals and natural nutrients with antioxidant properties. Among the various food plants, some endemic species are of particular interest because they may be used for the production of raw materials or preparations containing phytochemicals with significant antioxidant capacities and health benefits (Exarchou *et al*., 2002).

Crude extracts of fruit, herbs, vegetables, cereals and other plant materials rich in phenolics are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The preservative effect of many plant spices and herbs suggests the presence of antioxidative and antimicrobial constituents in their tissue (Hirasa and Takemasa, 1998). Many food plants contain large amounts of antioxidants other than vitamin C, vitamin E and carotenoids (Spanos and Wrolstad, 1990). The approach of phytochemicals in medicinal plants is mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Oxidative stress releases free oxygen radicals in the body which result in damage to membrane lipids, proteins, nucleic acids, and carbohydrates, which can result in cancer, neurological diseases, lung diseases, diabetes, vascular diseases, autoimmune diseases, premature aging, and eye diseases (Lachance *et al.,*2001).

Free radicals and reactive oxygen species (ROS) like superoxide, hydroxyl radical, peroxyl radical as well as non radical species such as hydrogen peroxide (H_2O_2) are highly reactive substances formed in the body's cells as a result of metabolic processes (Niki, 1992; Niki, 2001). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer, and AIDS (Pourmorad *et al*., 2006). Because of their highly reactive nature, these react rapidly with adjacent molecules via a variety of reactions including hydrogen abstraction (capturing), electron donation and electron sharing (Mc Cord, 2000). These may cause reversible or irreversible damages to biological molecules such as DNA, proteins or lipids (Goldberg, 2003).The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and

cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (Loliger, 1991).

Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al*., 1998). A number of factors are known to provide protection against this oxidative stress like free-radical traps (e.g. phenols) or anti oxidative enzyme like glutathione peroxidase (GP-X), and glutathione reductase (GR). Antioxidants are divided into two categories, preventive antioxidants and chain breaking antioxidants. The preventive antioxidants (e.g. glutathione peroxidase and catalase) deactivate the active species (e.g. hydrogen peroxide) without further generation of free radicals and thereby, reduce the rate of chain initiation. Chain breaking antioxidants have the ability to scavenge chain propagating oxygen radicals to produce stable, non- radical products and suppress lipid peroxidation (Niki, 1987).

The antioxidative effect is mainly due to phenolic components, such as flavonoids , phenolic acids and phenolic diterpenes (Pietta *et al*.,1998). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Nakatani *et al*.,1997). Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydro peroxides to non radical forms and functions as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different remedies, consuming antioxidants as free radical scavengers may be necessary (Halliwell, 1994 ; Kuhnan, 1976 ; Younes, 1981) . Currently available synthetic antioxidants like butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butylated hydroquinones and gallic acid esters have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990).

Momordica charantia commonly known as bitter gourd (Karela) is member of the Cucurbitaceae family. It is known as bitter melon, balsam pear, and pare. It grows in tropical areas of the Amazon, East Africa, Asia, India, South America, and the Caribbean and is used traditionally as both food and medicine. All parts of the plant, especially roots, leaves, fruits and seeds are widely used as traditional medicine throughout Asia, East Africa and South America. The roots are useful in treatment of coloptosis and Opthalmopathy. The leaves are useful in vitiated conditions of pita, helminthiasis, constipation, intermittent fever, burning sensation of the sole and nyctalopia. Leaves are also used in treatment of menstrual troubles, fever (malaria), colic, infections, worms and parasites, as an emmenogogue, measles and hepatitis (Kumar *et al*., 2010). In Guyana traditional medicine, leaf tea is used for diabetes, to expel intestinal gas, to promote menstruation, and as an antiviral for measles, hepatitis, and

feverish condition. It is used topically for sores, wound, infections and internally and externally for worms and parasites (Jagessar *et al*., 2008).

The fruits are useful in skin diseases leprosy, ulcers, wounds, burning sensations, constipation, anorexia, colic, helminthiasis, rheumatalgia, diabetes, asthama and dysmenorrhoea. Seeds are useful in treatment of ulcers, pharyngodynia and obstructions of the liver and spleen. The leaves and fruits are used for external application in lumbago, ulceration and bone fracture and internally in leprosy, haemorrhoids and Jaundice (Warrier *et al*., 1995). Fruits and seeds of bitter gourd possess medicinal properties such as anti- HIV, anti-ulcer, anti- inflammatory, anti- leukemic, anti-microbial, anti- tumor and antidiabetic property (Taylor, 2002), besides both are hypoglycemic, cytotoxic and anti- feedent (Hossain *et al*., 1992) and reduce the blood cholesterol level (Taylor, 2002).

Juice of the Karela leaves used to treat piles. Karela is used as a blood purifier due to its bitter tonic properties. It can heal boils and other blood related problems that show up on the skin. Juice of Karela is also beneficial in treating and preventing the liver damage (Agharkar, 1953; Garau *et al*., 2003). The pharmaceuticals studies of fruit extract of Momordica charantia have shown that the plant has antimicrobial (Mwambete, 2009), antioxidant (Kubola and Siriamornpun, 2008) and anti- inflammatory and immunomodulatory (Manabe *et al*., 2003), hypoglycemic and hypolipidiamic action (Abd El Baky, 2009), antiallergic (Gupta *et al*., 1993), anthelmintic (Grover and Yadav, 2004). antileshmania (Gupta *et al*., 2010).

Momordica dioica Roxb. Ex. Wild. (Cucurbitaceae) known as kankoda is a perennial, dioiceous climber with tuberous roots found throughout India from Himalayas to Ceylon, up to an altitude of 1,500 m. The plant is sometimes found growing wild and is common in hedges. *Momordica dioica* commonly known as Teasle Gourd, Kakrol, Kankro, Kartoli, Kantoli, Kantola, Kantroli, Ban karola or Small bitter-gourd is a relatively small oval to ovoid vegetable. It is also called as janglee karela (Harish, 2008). It is often cultivated for its fruits, which are used as vegetable (Sastri, 1962). The whole plant is used for treatment of eye diseases, poisoning and fever (Satyavati *et al*., 1987). Roots of the *Momordica dioica* are full of medicinal values. Juice of root is stimulant, astringent and antiseptic; tubers are used in cases of bleeding piles and similar affections. The roots of the plant are also recommended for scorpion sting (Kirtikar and Basu, 1999). The root ground into a paste and smeared over the whole body is believed to act as a sedative in high fever with delirium (Satyavati, 1987; Anjaria, 2002). Root is also used to stop bleeding from piles, as an expectorant and also in urinary and bowel complaints (Kirtikar *et al*., 1981). Powder of root is applied to skin to make it soft and to reduce perspiration.

Fruits are used as vegetables and also used in the treatment of inflammation caused by lizard excretion, mental and digestive disorders (Nadkarni, 1976). Fruit powder or

infusion of dried fruits produces a powerful errhine effect in nostrils and provokes a copious discharge from the nasal mucous membrane (Chatterjee, 1997; Kiritikar, 1987). The fruit is cooked in a small amount of oil and consumed for treating diabetes. Tender fruits are rubbed on skin for pimples and acne. Seeds are roasted and taken for eczema and other skin problems (Sharma, 2004). Leaves of the plant are antihelminthic, aphrodisiac. It is also used to cure tridosha, fever and alters pitta, jaundice, asthma, bronchitis, piles, hepatic damages, mental digestive disorders, bleeding piles bowel affection and urinary complaints. The juice of the leaves are mixed with coconut, pepper, red sandalwood etc. in order to form an ointment and applied to the head to relieve pain in the head. Leaf paste applied externally to skin and orally two or three times daily for skin disease (Nadkarni, 1976). Mishra *et al* (2006) studied *Momordica dioica* as an insecticide against mustard aphid, lipaphis erysimi kalt in farm of mustard crop . They concluded that seed oil of small bitter gourd was found to be satisfactory natural insecticide giving 100% mortality at 4% conc. in 24 hrs. Jain *et al.,* (2008) reported the antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of *Momordica dioica*. The antioxidant and free radical scavenging activities were positive for both ethanolic and aqueous extracts.

The fruits of Karela and Kankoda are used as vegetables but their availability is seasonal and limited to certain pockets of the country. Their highly perishable nature leads to 35 per cent loss during the post harvest stage. In order to make it available throughout the year in adequate quantity these are used in dried form. So the present study was aimed at the study of chemical composition and antioxidant activities of different extracts of fresh and dried fruits of Karela and Kankoda by using three testing methods: 2,2'-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$), β-carotene bleaching test (BCBT) and Ferric Thiocyanate (FTC) method, which may help to explore the possibility of using the most suitable route with regard to the preparation of extracts rich in anti-oxidative active components, as a functional ingredient in product formulations. The high content of polyphenolic compounds and flavonoids in various medicinal plants act as antioxidant against oxidative stress and scavenge free radicals.

Hence the objectives of study were

1. To estimate total phenols, flavonoids and ascorbic acid in the extracts of fresh and oven dried fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda).
2. To observe the contribution of these compounds to anti-oxidant and antiradical activity of these extracts.

CHAPTER–II

In the present study, the estimation of total phenols, flavonoids and ascorbic acid in different extracts of fresh and dried fruits of karela and kankoda will be done and contribution of these compounds to antioxidant activity will be studied. The literature pertaining to these aspects has been reviewed in this chapter under the following headings:-

2.1 Chemical constituents of Karela.
2.2 Chemical constituents of Kankoda.
2.3 Antioxidant activity of Karela.
2.4 Antioxidant activity of Kankoda.
2.5 Other activities of Karela.
2.6 Other activities of Kankoda.

2.1 Chemical constituents of Karela

2.1.1. Karela proteins

Polypeptide- p

Polypeptide- p, P- insulin or plant insulin is a protein isolated from fruit, seeds, and tissue cultures of *Momordica charantia* Linn. It has a molecular weight of about 11,000 Dalton and consists of 166 amino acids. Invariably accepted for this name the polypeptide has been proved to show hypoglycaemic effects in different animal models and patients with type I and Type II diabetes. Clinical trials suggest that subcutaneously injected peptide has hypoglycemic effects in gerbils, langurs and humans (Khanna *et al*., 1981), type -1 diabetics (Welihinda *et al*., 1982) and is more effective than orally administered dose because proteolytic digestion by the gut enzymes. Experimental data also suggest that therapy using this peptide is safe with no adverse effects (Raman and Lau, 1996). Recently Wang *et al.*, (2011) have cloned and expressed the 498 bp gene sequence coding for the *Momordica charantia* Polypeptide p gene and have also proved the hypoglycemic effect of the recombinant polypeptide in alloxan induced diabetic mice.

Bitter melon (and several of its isolated phytochemicals) also has been documented with *in vitro* antiviral activity against numerous viruses including Epstein-Barr, herpes and HIV viruses (Frame, 1998). In an *in vivo* study, a leaf extract demonstrated the ability to increase resistance to viral infections as well as to provide an immunostimulant effect in humans and animals (increasing interferon production and natural killer cell activity) (Huang, 1990). Two proteins known as alpha- and beta-momorcharin (which are present in the seeds, fruits, and leaves) have been reported to inhibit the HIV virus *in vitro* (Lee-Huang, 1990,

1995). In one study, HIV-infected cells treated with alpha- and beta-momorcharin showed a nearly complete loss of viral antigen while healthy cells were largely unaffected (Lee-Huang, 1990).

In 1996 the inventors of the chemical protein analog MAP-30 filed a U.S. patent, stating it was "useful for treating tumors and HIV infections. In treating HIV infections, the protein is administered alone or in conjunction with conventional AIDS therapies (Lifson, 1989). Another clinical study showed that MAP-30'antiviral activity was also relative to the herpes virus *in vitro* (Bourinbaiar, 1996). The fruit and fruit juice has antibacterial properties and, in another study, a fruit extract has demonstrated activity against the stomach ulcer-causing bacteria *Helicobacter pylori* (Yesilada, 1999).

2.1.2 Amino Acid in Karela

Freeze dried melon flesh was high in lysine. Flesh was relatively lower in glutamic acid and arginine. Essential amino acids, including threonine, valine, methionine, isoleucine, leucine, and phenylalanine are comparable in amount to soy proteins and other legume proteins. On the other hand, oven dried flesh had a much lower percentage of lysine and a lower percentage of arginine while other amino acids were almost similar. Seed protein was higher in glutamic acid and arginine but lower in lysine compared to flesh proteins. Glycine was also higher in bitter melon compared to soy proteins (Islam *et al.,* 2011)

2.1.3 Karela alkaloids

Seeds of bittergourd contain pyrimidine nucleoside vicine. Vicine has been found to induce hypoglycemia in rats, when administered intraperitoneally (Dutta *et al*., 1981 ; Barron *et al*., 1982).

Vicine

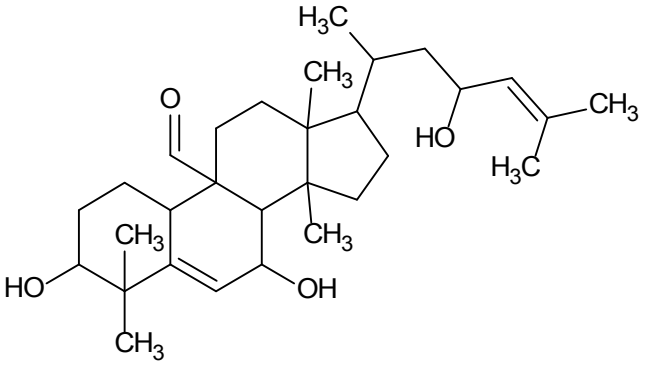
It is a glycol-alkaloid (pyrimidine nucleoside) isolated from the seeds of *M. charantia*. This pyrimidine nucleoside has been shown to induce hypoglycemia in non-diabetic fasting rats by intraperitoneal administration of dose, equivalent to 16 g seeds per kg body weight. Thus vicine may not account for all the activity of the seeds. Vicine found in faba bean has been shown to induce favism, an acute disease characterized by haemolytic anaemia (Raman and Lau, 1996), in individuals with a hereditary loss of the enzyme glucose-6-phosphate dehydrogenase (Basch *et al*., 2003). Although there have been no reports on favism induced by bitter melon, individuals susceptible to the disease should avoid eating the fruit. Further studies are suggested to ensure the safety and efficacy of using vicine to treat hyperglycemia (Dutta *et al*., 1981).

2.1.4 Triterpenoids

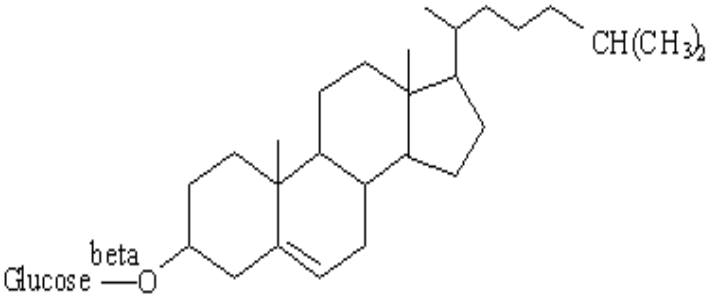
The terpenoids, referred to as isoprenoids, are a class of natural products and related compounds formally derived from five-carbon isoprene units. This class is subdivided according to the number of carbon atoms. The triterpenoids are terpenoids having a C30 skeleton. These C30 constituents are isolated and characterized from various sources in nature, particularly in resins and may occur as either esters or glycosides (Mahato *et al*., 1992; Connolly and Hill, 2008). The cucurbitacins are a typical group of cucurbitane-type triterpenoids found in plants belonging to the cucumber family (Cucurbitaceae). The natural cucurbitacins are well-known for their bitterness and toxicity (Hylands and Mansour, 1982; Chen *et al*., 2008).

Charantin

Charantin or momocharin, is a typical cucurbitane-type triterpenoid (steroidal glycoside) in *M. charantia* and is a potential substance with antidiabetic properties (Krawinkel and Keding, 2006) because of its insulin like chemical effects. It has been extracted from the seeds, leaves and fruits with alcohol. It has a molecular weight of 9.7 KD and is vastly accepted as a mixture of two steroidal saponin compounds sitosteryl glucoside and stigmasteryl glucoside. However some workers indicate the probability of presence of other specific components. Upon oral and intravenous administration, charantin has been found to reduce blood glucose levels significantly in both normal and diabetic rabbits (Raman and Lau, 1996). Studies have also reported that the compound is more effective than the oral hypoglycemic agent tolbutamide (Cousens, 2008). The major compound present in the methanolic fruit extract of *M. charantia* as identified by GC-MS was Gentisic acid with RT 16.544 and 8.406 % relative peak area. The molecular formula of gentisic acid is $C_7H_6O_4$, molar mass is 150.12 g/mol and biologically active as antioxidant. Recently, two other anti-diabetic constituents were isolated from *M. charantia* and both substances exerted hypoglycemic effects in mice. The cucurbitane triterpenoids were found to have the structures, 5β, 19-epoxycucurbita-6, 5β, 19-epoxy-19, 25-dimethoxycucurbita-6 ,23-(E)-dien-3β-ol and 3β,7β- 25-trihydroxy-cucurbita-5, 23 (E)-dien-19-al. These two compounds have more or less the same parent structure as α-β momocharin , and momordicin (Puspawati, 2008). More recently, momordicin was isolated from *M. charantia* and its chemical structure was characterized as momordicin1 3, 7, 23,-Trihydroxycucurbitan-5, 24-dien-19-al (Puspawati, 2008). This compound is more or less similar to the one identifies by Harinantenaina *et al., (*2006).



Momordicin



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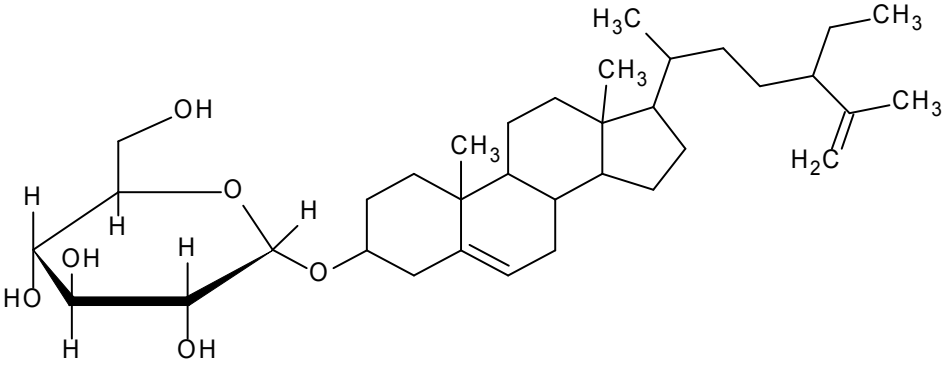
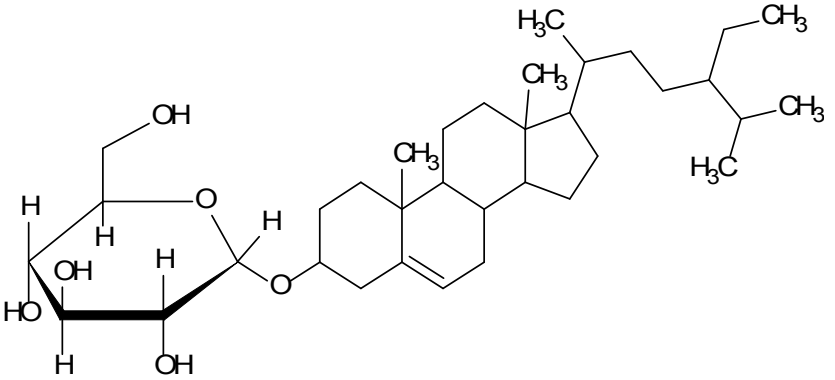
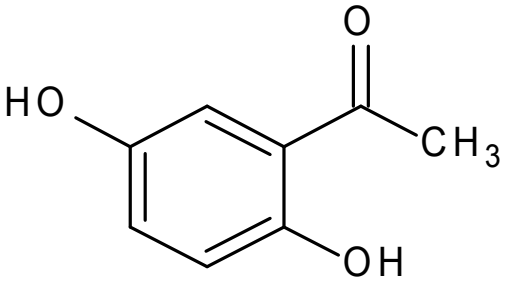


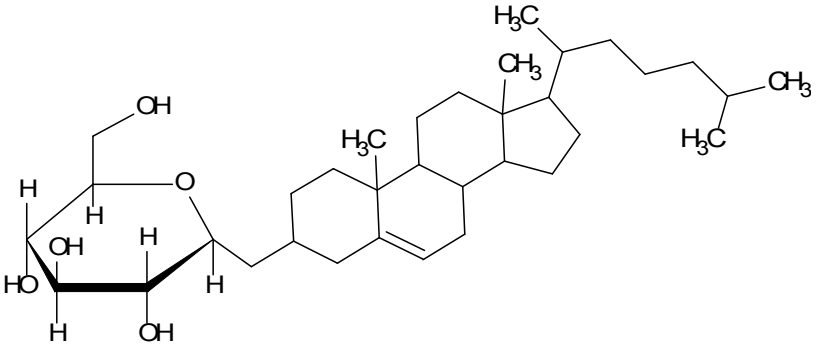
Sitosteryl glucoside



Stigmasteryl glucoside



Gentisic acid



Momorcharin

2.1.5 Karela phenolics

Hassan *et al*., (2011) reported that total phenolics in *M. charantia* were 126 ± 0.14 mg GAE/100 fresh weight. The main phenolic acids, which were present in bitter melon flesh, were gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin. Gallic acid, gentisic acid, catechin, chlorogenic acid, and epicatechin contents of the bitter melon ranged from 8.04 to 39.76, 16.99 to 32.39, 23.06 to 82.45, 4.55 to 15.83, and 16.14 to 44.28 mg respectively per 100 g dry material. Protocatechuic acid, vanillic acid, syringic acid, *p*-coumaric acid, and benzoic acid were present in small amount (less than 10 mg/g dry material) in the flesh of all varieties of the bitter melons. The amounts of these constituents ranged from 2.07 to 8.78, trace to 2.42, 1.77 to 3.67, 1.83 to 8.23, and ND to 5.35 mg/100 g dry material for protocatechuic acid, vanillic acid, syringic acid, and o-coumaric acid, respectively (Horax *et al*., 2005).

2.2 Chemical constituents of Kankoda

It contains Lectins, proteins, triterpenes and vitamins (Naik, 1951).The fruit contains a high amount of vitamin C (Bhuiya *et al*., 1977). The fruit is rich in ascorbic acid and contain iodine (Rao, 2007). The fruit also contain alkaloids, flavonoids, glycosides and amino acids (Kushwaha *et al*., 2005). *Momordica dioica* also contains a fragrant extractive matter and ash

3 to 4 percent. Ash contains a trace of manganese (National Plant Data Center, 2010). *Momordica dioica* as the average nutritional value per 100 g edible fruit was found to contain 84.1% moisture, 7.7 g carbohydrate, 3.1 g protein, 3.1 g fat, 3.0 g fiber and 1.1 g minerals. It also contained small quantities of essential vitamins like ascorbic acid, carotene, thiamin, riboflavin and niacin (Singh *et al*., 2006). It also content protein in the leaves and dry weight of aerial plant parts remained higher in male as compared to female defruited, and monoecious plants (Ghosh, 2005).

From *Momordica dioica* fruit isolated 8-methyl hentracont-3-ene along with the known sterol pleuchiol. Momodicaursenol, an unknown pentacylic triterpene isolated from the seeds, had been identified as urs-12, 18(19)-dien-3 beta-ol. Phytochemical investigations have revealed the presence of traces of alkaloids and ascorbic acid in fruits. Lectins, β-sitosterol, saponin glycosides, triterpenes of ursolic acid, hederagenin, oleanolic acid, spiranosterol, stearic acid, gypsogenin, two novel aliphatic constituents (Ali and srivastava, 1998; Sadyojatha and Vaidya, 1996; Ghosh *et al*., 1981; Luo *et al*., 1998).

From the dry root of *Momordica dioica* isolated three triterpenes and two steroidal compounds. These were alphaspinasterol octadecanonate(I), alphaspinasterol-3-O-beta-D-glucopyranoside(II), 3-O-beta-D-glucuronopyranosyl gypsogenin(III), 3-O-beta-D-glucopyranosyl gypsogenin(IV) and 3-O-beta-D-glucopyranosyl hederagenin(V). Constituent III was a new compound (Luo *et al*., 1998). Aberoumand and Deokule,(2010) reported that total phenolic amounts of *Momordica dioicia* is 3.69±2.1mg GAE/g of dry weight. Masao *et al*., (2011) reported that concentration of ascorbic acid in *M.dioica* is 25.1mg/g, α-tocopherol 121.5mg/g, β-carotene 10.5 mg/g, linoleic acid 43.3mg/g, oleic acid 9.7mg/g and palmitic acid 23.4 mg/g of dry weight.

2.3 Antioxidant activity of Karela

Different parts of this plant have been used in the Indian medicinal system for a number of ailments besides diabetes. Antioxidant activity of extracted phenolic compound from bitter melon has been reported by Horax *et al*., (2005). Antioxidant properties of *Momordica charantia* (Karela) Seeds on Streptozotocin induced-diabetic rats has been studied and results clearly suggest that seeds of *M.charantia* may effectively normalize the impaired antioxidant status in streptozotocin induced-diabetes (Sathishsekar and Subramanian, 2005). There was no significant difference in the antioxidant activities [% inhibition] of the methanolic extracts from bitter melons among varieties and drying methods [oven and freeze-dried]. The antioxidant activities of Indian green, Indian white, China green and China white ranged from 79-88, 79-87, 80-86, and 79-87% inhibition, respectively. The antioxidant activities of the oven-dried samples and the freeze-dried samples were 79-88 and 79-86% inhibition, respectively.

The antioxidant activities of the methanolic extracts of flesh and SCT were not significantly different, while they were significantly higher than that of seeds. Shu-Jing and Lean-Teik (2008) reported that bitter melon extracts possess potent antioxidant and free radical scavenging activities. These antioxidant activities could have contributed at least partly to the therapeutic benefits of certain traditional claims of wild bitter melons. The scavenging action of plant constituents has been found to relate to polyphenolic compounds (Hatono *et al*., 1988). Although the constituents of bitter melon, which show free radical scavenging action is still unclear, it is possible that the antioxidative properties of bitter melon are caused, at least in part, by the presence of polyphenols and other yet to be discovered antioxidant compounds. The result of scavenging activity this study were 37% to 64.48%, there were ranges of DPPH on wild of bitter melon were 36.6% to 75.8% (Wu and Ng, 2007). This result showed more ripened of bitter melon had a lower of DPPH. RS 4 was lowest and RS 3 was highest of DPPH value. However, all the samples showed no significant difference ($p>0.05$).Last reported, that radical scavenging activity for green fruit was 11.0% and ripe fruit 27.6% (Kubola and Siriamornpun, 2008).

2.4 Hepatoprotective, antioxidant and anti-inflammatory activities of Kankoda

Momordica dioica roots alcoholic extract significantly reduced CCl_4 induced hepatotoxicity in rats (Shreedhara and Vaidya, 2006). *Momordica dioica* Roxb. leaves ethanolic extract found more potent hepatoprotective activity against aqueous extracts was evaluated against carbon tetrachloride (CCl_4) induced hepatic damage in rats. Also in vivo antioxidant and free radical scavenging activities were also screened which were positive for both extracts due to the presence of flavonoids in the extracts (Jain *et al*., 2008). *Momordica dioica* fruits ethanolic extract shows hepatoprotective activity against carbon tetrachloride (CCl_4) induced hepatic damage. Fruit is reported for hepatoprotective activity (Kushwaha *et al*., 2005).

2.5 Other activities of Karela

2.5.1 Anti -cancer activity

There is absolutely no evidence that it can treat cancer. Bitter Melon and Bitter Melon Extracts inhibit cancer and tumor. A novel phytochemical in bitter melon has clinically demonstrated the ability to inhibit an enzyme named guanylate cyclase. This enzyme is thought to be linked to the pathogenesis and replication of not only psoriasis, but leukemia and cancer as well. One clinical trial found very limited evidence that bitter melon might improve immune cell function in people with cancer, but this needs to be verified and amplified in other research. Other phytochemicals that have been documented with cytotoxic activity are a group of ribosome-inactivating proteins named alpha- and beta-momorcharin,

momordin, and cucurbitacin B. A chemical analog of bitter melon proteins was developed and named MAP-30 and its inventors reported that it was able to inhibit prostate tumor growth.

The phytochemical momordin has clinically demonstrated cytotoxic activity against Hodgkin's lymphoma in vivo, and several other in vivo studies have demonstrated the cytostatic and antitumor activity of the entire plant of bitter melon. Further studies reportd that, a water extract blocked the growth of rat prostate carcinoma and a hot water extract of the entire plant inhibited the development of mammary tumors in mice. Numerous in vitro studies have also demonstrated the anti-cancerous and anti-leukemic activity of bitter melon against numerous cell lines including liver cancer, human leukemia, melanoma and solid sarcomas ("About Herbs: Bitter Melon" 2007 ; Cunnick *et al*.,1990). It has been shown that *M. charantia* fruit juice, peel, pulp, seed and whole fruit extract modulate detoxification pathways in diabetic rats, specifically altering P450 and GSH dependent metabolism (Raza *et al*., 1996; Singh *et al*., 1998).

Modulation of biotransformation system enzymes may be the cause of anticarcinogenic properties of *M. charantia* (Singh *et al*., 1998).The study by Kumara *et al*., (1998) determined the effects of *M. charantia* on the levels of phase I enzymes, which include cytochrome P450 (P450), aniline hydroxylase (ANH) and aminopyrine-N-demethylase (AMD) and to induce the phase II enzymes [i.e. glutathione S-transferase (GST)] in rat liver. It was demonstrated that bitter-gourd fruits contain phases I and II enzyme inducers and compounds capable of repressing some monooxygenases, especially those involved in the metabolic activation of chemical carcinogens. In another study (Ganguly *et al*., 2000) carcinogen-induced lipid peroxidation in liver and DNA damage in lymphocytes were reduced following treatment with *M. charantia*. The fruit extract was found to significantly activate the liver enzymes glutathione-S-transferase, glutathione peroxidase and catalase, which showed a depression following exposure to the carcinogen. The results suggest a preventive role of water-soluble constituents of *M. charantia* fruit during carcinogenesis, which is mediated possibly by their modulatory effect on enzymes of the biotransformation and detoxification system of the host. While the mechanism of the effects of this natural product remains to be clarified, there were no adverse effects of treatment with these agents as estimated from body weight, food and water intake and various plasma component levels as well as external appearance.

2.5.2 Antidiabetic Activity

Karela contains bitter chemicals like, charantin, vicine, glycosides and karavilosides along with polypeptide-p a plant insulin, which are hypoglycemic in action and improve blood sugar levels by increasing glucose uptake and glycogen synthesis in the liver, muscles and fat cells. Reports indicate that they also improve insulin release from pancreatic beta cells, and repair or promote new growth of insulin-secreting beta cells. P-Insulin, a

polypeptide from the fruits and seeds rapidly decreased and normalized the blood sugar level in rats. Bitter melon contains another bioactive compound i.e. lectin that has insulin like activity. The insulin-like bioactivity of lectin is due to its linking together 2 insulin receptors. This lectin lowers blood glucose concentrations by acting on peripheral tissues and, similar to insulin's effects in the brain, suppressing appetite. This lectin is a major contributor to the hypoglycemic effect that develops after eating Karela. Charantin extracted by alcohol, is a potent hypoglycemic agent composed of mixed steroids which is sometimes used in the treatment of diabetes to lower the blood sugar levels (Kumar *et al*., 2010; Indian Medicinal Plants 1995; Nadkarni 1993).

2.5.4 Antifertility Activity

Stepka *et al*., (1974) have demonstrated *in vivo* antifertility effect of fruit and leaf of bitter melon in female animals.

2.5.5 Anti-Malarial Activity

Karela is traditionally regarded by Asians, as well as Panamanians and Colombians, as useful plant for preventing and treating malaria. Laboratory studies have confirmed that various species of Karela have antimalarial activity. Leaves brewed in hot water to create a tea to treat malaria.

2.6 Other activities of Kankoda.

2.6.1 Antibacterial Activity

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al*., 1998). The antibacterial activity of the plants may be due to the presence of various active principles in the plant parts (Mukesh and Sharma, 2010). Many reports of the antibacterial sensitivity of plant extracts are observed using cup-plate agar diffusion method by measuring the diameter of zone of growth inhibition. Both the extracts have exhibited some degree of inhibition against all the tested organisms. The anti-microbial activity of extracts was shown in concentration dependent manner. The results illustrated that extracts of *Momordica dioica* were most active against *S. typhi* and S. *dysenteriae* in the 100 to 500 µg/ml concentrations. Steroids and flavonoids are known to be biologically active. These compounds are known to be toxic to microorganisms. The site and number of hydroxyl groups on the phenol group are thought to be related to their relative toxicity to the microorganisms. The probable mechanism responsible for phenolic toxicity to microorganisms include enzyme inhibition by oxidized compounds possibly through reaction with sulfhydryl groups or through more nonspecific interaction with proteins (Sharma and Arora, 2006). Similarly in our study, the phytochemical analysis of the extracts had shown the presence of bioactive compounds like steroids, fatty acids, saponin, glycosides and triterpenes. Thus, the antimicrobial activity might be due to the presence of these compounds.

2.6.2 Hypoglycemic and hypolipidemic activities

Momordica dioica Roxb fruit pulp extracts shows the hypoglycemic and hypolipidemic activities on alloxan-induced diabetic rats. *Momordica dioica* fruits show the anti-hyperglycemic activity in alloxan-induced diabetic rats. In this study the ethyl acetate and ethanol extract showed significant antidiabetic activity in comparision to chloroform extract (Reddy *et al*., 2006). This plant also possesses hypoglycaemic (Fernandopulle and Karunanyake, 2010)

2.6.3 Analgesic and anti-inflammatory activity

Momordica dioica fruits pulp hexane extract and ethyl acetate extract significantly exhibited analgesic and anti-inflammatory activities (Ilango *et al*., 2003). *Momordica dioica* root ethanolic extract exhibited significant analgesic activity. The aqueous extract was found paralysis of earth worms after 1hr. as 91.6 percent (Vaidya and Shreedhara, 2003).

2.6.4 Acute renal failure activity

Momordica dioica seeds ethanol extract possesses marked nephroprotective and curative activities without any toxicity due to its antioxidant activity and could offer a promising role in the treatment of acute renal injury caused by nephrotoxin-like gentamicin (Jain and Singhai, 2009).

2.6.5 Antiallergic activity

Momordica dioica roots shows antiallergic activity for alcoholic extract (Gupta *et al*., 1993)

2.6.6 Anticancer activity

The $CHCl_3$ extract of *Momordica dioica* roots and five isolated constituents showed anticancer activity in pharmacologic testing on cancer cell (Luo *et al*., 1998)

2.6.7 Antifeeedant activity

Momordica dioica fruit pulp hexane extract and ethyl acetate soluble fraction of methanolic extract exhibited moderate and concentration dependent antifeedant activity against *Spodoptera litura* (Narasimhan *et al*., 2005).

2.6.8 Antimalarial activity

Momordica dioica alcoholic extract screened in vivo & in vitro for antimalarial against NK 65strain of Plasmodium bergheli, Jurinea macrocephala, Aegle marmelos, were found to posses schizontocidal activity (Misra *et al*., 1991).

CHAPTER–III

MATERIALS AND METHODS

This chapter provides information regarding the experimental procedures employed during the course of investigation. Various chemicals used methods of extraction procedures and estimation of different parameters of extract are discussed in this chapter.

3.1 Research material

3.1.1 Fruit material

Fresh fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda) were procured from local market of Hisar during July-August, 2012. These were cleaned with water and external moisture wiped out with a dry cloth. The cleaned fruits were then chopped into thin slices, which were then extracted saparately with acetone, ethyl alcohol and distilled water. 500g chopped samples of both Karela and Kankoda in triplicate were shade dried for 4-days. Then they were placed in oven for further drying at 55 ºC for 2-days. These dried fruit slices were then crushed in a grinder into fine powder form. The powdered samples were then extracted separately with acetone, ethyl alcohol and distilled water. These extracts were then used for determination of total phenols, flavonoids, ascorbic acid and the antioxidant activity using various methods.

3.1.2 Chemicals

The commercially available chemicals from Sigma-Aldrich, Qualigens, Merk and Ranbaxy of high purity, were used for various experimental procedures.

3.2 Extraction of plant materials

500g fresh (40gm dried) fruits samples of each plant were extracted separately with acetone, ethyl alcohol and distilled water by refluxing for six hours and the process repeated three times. The solvent was removed to get extractives. These extracts were filtered and concentrated under reduced pressure and used for estimation of following parameters:

1. Total Phenols
2. Flavonoids
3. Ascorbic acid
4. Antioxidant activity by

a) 2, 2’-Diphenyl-1-picrylhydrazyl **(**DPPH) method

b) Ferric Thiocyanate (FTC) method

c) β-carotene bleaching method

3.2.1 Preparation of extracts

The water extracts of Karela and Kankoda fruits were prepared by boiling 100g of fresh fruit sample with 200ml of distilled water for 6 hours and 40 g of dried fruit sample with 100ml of distilled water, then leaving overnight at room temperature for further extraction. The mixtures were subsequently filtered and centrifuged at 5000-6000 rpm for 10-20 minutes and the filtrates were stored in deep freeze. The alcohol and acetone extracts of Karela and Kankoda fruits were prepared under reflux conditions using ethanol and acetone for a period of 6 hours respectively. The extracts were filtered and the residues were extracted again with fresh ethanol and acetone, respectively under the same conditions. The solvent of the combined filtrates was evaporated using a rotary evaporator.

3.3. Determination of total phenolics content

Reagent

Gallic acid

Methanol

Sodium carbonate (20% w/v)

Preparation of Folin-Ciocalteu reagent

Dissolved 100g of sodium tungstate ($Na_2WO_4.2H_2O$) and 25g of sodium molybdate ($Na_2MoO_4.2H_2O$) in about 700ml of water. Added 50ml of 85% phosphoric acid followed by 100ml conc. HCl in 1.5 litre flask. Fitted with a condenser and was refluxed gently for 10 hrs. Allowed to cool and then added 150g of lithium sulphate, 50ml of water and a few drops of bromine water. Boiled the mixture for 15 minutes without condenser to remove excess bromine. Cooled, diluted to 1 litre and filtered. The reagent should not have a greenish tint. The acid concentration of the reagent was determined by titration with 1N NaOH to phenolphthalein end point.

Method

The total phenolics were determined by the Folin-Ciocalteu reagent (Singleton and Rossi, 1965) method using gallic acid as standard for which a calibration curve was obtained with solutions of 0.1, 0.08, 0.06, 0.04, 0.02, and 0.01 mg/ml of gallic acid. A 1.0ml of diluted extract (all fraction were diluted with methanol to adjust the absorbance within the calibration limits), 1.0ml of 1mol/L Folin–ciocalteau reagent (diluted to 1:2 ratio) and 2.0ml of Na_2CO_3 (20% w/v) were mixed and the volume was made to 50ml. After 8 minutes, the mixture was centrifuged at 600 rpm for 10 minutes. Then the absorbance of supernatant solution was measured at 730nm using Spectronic 20 (Milton Roy Company) spectrophotometer and against a blank prepared similarly with the same solvent but omitting the extract. The concentration of phenolics thus obtained was multiplied by the dilution factor and the results were expressed as the equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

3.4 Determination of flavonoid content

Reagents

Catechin

5% $NaNO_2$

10% $AlCl_3$

1M NaOH

Method

The aluminium chloride colorimetric assay, as described by Zhishen *et al.,* (1999) was used. Briefly, 1ml of extracts or obtained solution of catechin (0.02, 0.04, 0.06, 0.08 and 0.01 mg/ml) was added to test tubes containing 4ml of double distilled water. To the mixture was added 0.3ml 5% $NaNO_2$. After 5 minute, 0.3ml 10% $AlCl_3$ was added. Immediately, 2ml 1M NaOH was added and the total volume was made upto 10ml with double distilled water. The solution was mixed thoroughly and the absorbance of both the samples, blank and standard was read at 510 nm using UV visible spectrophotometer Model Spectronic 20 (Milton Roy Company). Total flavonoid content was expressed as mg catechin equivalents per gram of the extract (mg CAE/g).

3.5 Determination of ascorbic acid content:

Ascorbic acid content was determined by titrating a known weight of sample with 2, 6 dichloro phenol-indophenol dye (AOAC, 1975). Ascorbic acid reduces the 2, 6 dichloro phenol-indophenol dye to a colourless leuco-base and itself gets oxidised to dehydroascorbic acid. Though the dye is a blue coloured compound, the end point is the appearance of pink colour. The dye is pink coloured in acidic medium. Oxalic acid was used as the titrating medium.

Reagents

Oxalic acid 4%

Sodium bicarbonate

2, 6 dichloro phenol-indophenol

Ascorbic acid

Method

Dye solution: weigh 42mg sodium bicarbonate in to a small volume of distilled water. Dissolve 52mg 2, 6 dichloro phenol-indophenol in it and make up to 200ml with distilled water. Standard stock solution: Dissolved 100mg ascorbic acid in 100ml 4% oxalic acid solution .Then different test sample are titrated against dye by adding 10 ml 4% oxalic acid solution.

Calculation

Amount of ascorbic acid mg/100g sample = (0.5mg/V_1 ml) × (V_2 /5ml) × (100ml/ Wt. of the sample) × 100

V_1 = vol. of dye used for standard solution

V_2 = vol. of dye used for sample.

3.6 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

Reagents

DPPH: 0.025gL^{-1} in 50% methanol

Method

The antioxidant activity of the extracts was evaluated by DPPH free radical scavenging method. The effect of extracts on DPPH radical was estimated according to the method of Hatano *et al*., (1988). 2, 2'-Diphenyl-1-picrylhydrazyl (DPPH) is a stable free radical that shows a maximum absorption at 517 nm in methanol. When DPPH encounters proton donating substances such as an antioxidant and a radical species, the absorbance at 517nm disappears because the DPPH radical is scavenged. On the basis of this principle, the radical scavenging effect of each fraction was measured. Briefly 0.3, 0.6, 0.9, 1.2, 1.5 mg of extract was added to 2.5ml of 2,2'-diphenyl-2-picrylhydrazyl radical (DPPH: 0.025gL^{-1} in methanol) final volume made to 10 ml with methanol and mixed by vortex for 5 minute. The absorbance of the sample was measured at 517nm every 10 minutes till a steady state is reached (2 hrs) using the spectrophotometer Spectronics 20 (Milton Roy Company). Similarly, a control sample was also prepared. For each sample, three separate determinations were carried out. The antioxidant activity was expressed as the percentage of decline of the absorbance after 2 hrs, relative to the control, corresponding to the percentage of DPPH that was scavenged.

Calculation

The percentage of DPPH, which was scavenged (%DPPH*$_{sc}$) was calculated using:

$$\%DPPH^*_{sc} = \{(A_{cont}-A_{samp})/A_{cont}\} \times 100$$

Where A_{cont} is the absorbance of control and A_{samp} is the absorbance of sample.

3.7 Ferric Thiocyanate (FTC) method

Reagents

2.51% (w/v) linoleic acid in ethanol

30% (w/v) ammonium thiocyanate

0.02 mol/L ferrous chloride in 3.5% (v/v) hydrochloric acid

75% ethanol

0.05 mol/L of phosphate buffer (pH 7.0)**:** 0.2M solution of monobasic sodium phosphate was prepared by dissolving 31.2g of $NaH_2PO_4.2H_2O$ in 1000 ml of water and 0.2M solution of dibasic sodium phosphate was prepared by dissolving 71.7g of $Na_2HPO_4.12H_2O$ in 1000 ml of water. 39.0 ml of 0.2M solution of monobasic sodium phosphate and 61.0 ml 0.2M solution of dibasic sodium phosphate were mixed diluted to a total of 200 ml.

Method

The FTC method of Kikuzaki and Nakatani, (1993) was used to evaluate the antioxidant activity of the extract. Linoleic acid emulsion was prepared by mixing linoleic acid (0.28g), Tween 20 (0.28g) and phosphate buffer (50ml, 0.2M, pH 7.0). Test samples were prepared in ethanol-water (6: 4 v/v). Different test samples of conc. 0.3, 0.6, 0.9, 1.2,1.5 mg were mixed with 5ml of Linoleic acid emulsion and final volume made to 10ml with phosphate buffer (0.2M, pH 7.0) and incubated at 37ºC for 96 hours (4 days). The mixture prepared as above without the test sample served as control. Aliquots (0.1ml) were drawn from the incubation mixture at intervals of 24 hour and mixed with 0.1ml of 30% ammonium thiocyanate, 0.1 ml of 20mM ferrous chloride in 3.5% HCl and final volume made to 10 ml with 75% ethanol and allowed to stand at room temperature for 3 minutes. The colour developed was measured at 500 nm in a spectrophotometer. This method depends on peroxide formation in the aqueous emulsion of linoleic acid. In this method, the higher the absorbance increase is, the higher the concentration of peroxide formed and hence, the lower the antioxidant activity of the sample tested.

Calculation

Antioxidant activity was expressed as

Antioxidant activity (%) = (100 - increase in abs. of sample/ increase in abs. of control) x 100

3.8 β-carotene bleaching method

Reagents

β-carotene

Linoleic acid

Tween 20

Chloroform

Method

The β-carotene bleaching method of Hidalgo *et al.,* (1994) was used to evaluate the antioxidant activity of the extract. β-carotene (0.2mg), linoleic acid (20mg) and tween 20 (200mg) were mixed in 0.5ml of chloroform. The solvent was subsequently removed at 40ºC in a vacuum evaporator and the mixture was diluted with 50ml of triply distilled water. Aliquots (4ml) of this emulsion were transferred into test tubes, to which were then added 0.3, 0.6, 0.9, 1.2, 1.5 mg of aliquots of test samples in ethanol. A control containing 0.2ml of ethanol and 4ml of emulsion was also used. The test tubes were covered with aluminium foil and placed in a water bath at 50°C. The absorbance at 470nm was recorded with a Spectronic 20 (Milton Roy Company) spectrophotometer at intervals of 30 minute, until the colour of β-carotene has disappeared from the control tubes. The above mixture without β-carotene served as blank. All determinations were carried out in triplicate.

Calculation

The antioxidant activity was expressed as percentage inhibition relative to the control using the equation

$$AA\ (\%) = 100\ [1-\{(A_0-A_t)/\ (A_0^{\ 0}-A_t^{\ 0})\}]$$

Where A_0 and $A_0^{\ 0}$ are the absorbance values measured at zero time of incubation for the test sample and control respectively and A_t and $A_t^{\ 0}$ are the corresponding values at the end of the reaction time.

CHAPTER–IV

RESULTS

Table 1. Yield percentage of different extracts of fresh and dried fruits of Karela and Kankoda

Fruit samples	Acetone extract (%)	Ethanol extract (%)	Water extract (%)
Fresh Karela	1.96±0.21	2.25±0.15	2.37±0.22
Dried Karela	6.75±0.27	12.25±0.38	27.75±0.39
Fresh Kankoda	3.34±0.08	3.63±0.14	1.76±0.11
Dried Kankoda	4.06±0.12	16.41±0.65	16.47±0.72

Values are mean of three replicates ± standard error

Table 2. Moisture content in fruits of Karela and Kankoda

Fruits sample	Moisture (%)
Karela	90.57±0.97
Kankoda	84.18±0.88

Values are mean of three replicates ± standard error

Table 3. Chemical composition of different extracts of fresh fruits of Karela

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	12.08±0.23	10.21±0.36	9.18±0.29
Flavonoids (mg CAE/g)	6.86±0.31	7.61±0.21	1.28±0.24
Ascorbic acid (mg/g)	1.01±0.02	0.78±0.01	0.54±0.01

Values are mean of three replicates ± standard error
mg GAE/g- milligrams gallic acid equivalent/g of the extract
mg CAE/g- milligrams catechin equivalent/g of the extract

Table 4. Chemical composition of different extracts of dried fruits of Karela

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	6.83±0.12	3.18±0.08	0.57±0.06
Flavonoids (mg CAE/g)	4.78±0.14	2.64±0.10	0.27±0.04
Ascorbic acid (mg/g)	0.27±0.01	0.18±0.01	0.05±0.01

Values are mean of three replicates ± standard error
mg GAE/g- milligrams gallic acid equivalent/g of the extract
mg CAE/g- milligrams catechin equivalent/g of the extract

Table 5. Chemical composition of different extracts of fresh fruits of Kankoda

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	4.67±0.27	4.10±0.25	5.45±0.26
Flavonoids (mg CAE/g)	3.81±0.20	1.25±0.19	0.87±0.09
Ascorbic acid (mg/g)	0.63±0.01	0.43±0.01	0.47±0.01

Values are mean of three replicates ± standard error
mg GAE/g- milligrams gallic acid equivalent/g of the extract
mg CAE/g- milligrams catechin equivalent/g of the extract

Table 6. Chemical composition of different extracts of dried fruits of Kankoda

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	3.58±0.09	1.11±0.32	0.67±0.09
Flavonoids (mg CAE/g)	4.22±0.13	0.73±0.06	0.33±0.03
Ascorbic acid (mg/g)	0.35±0.01	0.14±0.01	0.08±0.01

Values are mean of three replicates ± standard error
mg GAE/g- milligrams gallic acid equivalent/g of the extract
mg CAE/g- milligrams catechin equivalent/g of the extract

Table 7. Ascorbic acid content in 3% metaphosphoric acid extract of fresh and dried fruits of Karela and Kankoda

Sample	Ascorbic acid (mg/100g)
Karela fresh	114.61±5.51
Karela dry	81.21±5.01
Kankoda fresh	83.03±7.01
Kankoda dry	63.11±9.53

Values are mean of three replicates ± standard error

Table 8. Antioxidant activities (%) of different extracts of fresh fruits of Karela by different methods

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	86.11±0.55	62.06±0.8	67.16±0.96
Ethanol extract (1.0 mg/ml)	86.51±0.60	50.43±0.83	56.41±0.78
Water extract (1.0 mg/ml)	91.21±0.65	51.11±1.31	47.11±0.75
BHA (Standard) (1.0 mg/ml)	86.11±0.40	65.41±1.15	72.46±1.15
BHT (Standard) (1.0 mg/ml)	87.21±0.35	76.46±1.17	82.03±0.85

Values are mean of three replicates ± standard error

Table 9. Antioxidant activities (%) of different extracts of dried fruits of Karela by different methods

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	86.41±1.11	80.53±1.25	85.51±0.96
Ethanol extract (1.0 mg/ml)	86.21±1.11	65.21±1.22	72.11±0.91
Water extract (1.0 mg/ml)	61.21±1.77	77.43±1.11	84.21±0.63
BHA (Standard) (1.0 mg/ml)	86.11±0.40	65.41±1.15	72.46±1.15
BHT (Standard) (1.0 mg/ml)	87.21±0.35	76.46±1.17	82.03±0.85

Values are mean of three replicates ± standard error

Table 10. Antioxidant activities (%) of different extracts of fresh fruits of Kankoda by different methods

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	76.11±0.02	65.36±1.28	70.43±0.75
Ethanol extract (1.0 mg/ml)	87.02±0.04	46.41±0.95	54.31±0.79
Water extract (1.0 mg/ml)	58.01±0.05	52.21±1.12	56.31±1.09
BHA (Standard) (1.0 mg/ml)	86.11±0.40	65.41±1.15	72.46±1.15
BHT (Standard) (1.0 mg/ml)	87.21±0.35	76.46±1.17	82.03±0.85

Values are mean of three replicates ± standard error

Table 11. Antioxidant activity (%) of different extracts of dried fruits of Kankoda by different methods

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	55.51±0.70	70.46±0.98	75.21±0.97
Ethanol extract (1.0 mg/ml)	85.61±0.95	39.51±1.22	43.51±1.19
Water extract (1.0 mg/ml)	89.03±0.75	66.36±1.28	72.26±1.12
BHA (Standard) (1.0 mg/ml)	86.11±0.40	65.41±1.15	72.46±1.15
BHT (Standard) (1.0 mg/ml)	87.21±0.35	76.46±1.17	82.03±0.85

Values are mean of three replicates ± standard error

Table 12. Percent increase (+) / decrease (-) in concentration of various chemical constituents of fruits of Karela on drying

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	- 43.41±2.01	- 68.61±1.11	- 93.53±0.86
Flavonoids (mg CAE/g)	- 30.36±0.91	- 65.56±1.12	- 78.31±1.49
Ascorbic acid (mg/g)	- 72.61±1.15	- 76.11±1.75	- 92.61±1.65

Values are mean of three replicates ± standard error

Table 13. Percent increase (+) / decrease (-) in concentration of various chemical constituents of fruits of Kankoda on drying

Constituents	Acetone extract	Ethanol extract	Water extract
Total Phenols (mg GAE/g)	- 23.03±1.32	- 73.33±0.87	- 87.41±0.98
Flavonoids (mg CAE/g)	+ 11.38±0.89	- 41.44±1.33	- 62.25±1.06
Ascorbic acid (mg/g)	- 44.66±0.97	- 68.26±1.12	- 83.41±1.2

Values are mean of three replicates ± standard error

Table 14. Percent increase (+) / decrease (-) in antioxidant activity of fruits of Karela on drying

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	+0.30±0.05	+18.47±0.88	+18.48±0.81
Ethanol extract (1.0 mg/ml)	- 0.49±0.07	+14.64±0.88	+15.61±1.01
Water extract (1.0 mg/ml)	- 30.34±0.94	+26.59±1.12	+37.3±1.11

Values are mean of three replicates ± standard error

Table 15. Percent increase (+) / decrease (-) in antioxidant activity of fruits of Kankoda on drying

Extract	DPPH	FTC	β-carotene
Acetone extract (1.0 mg/ml)	- 20.61±1.05	+5.42±0.93	+4.62±0.76
Ethanol extract (1.0 mg/ml)	- 1.44±0.10	- 6.75±0.90	- 10.61±0.90
Water extract (1.0 mg/ml)	+31.07±1.41	+14.55±0.90	+15.72±1.11

Values are mean of three replicates ± standard error

CHAPTER–V

DISCUSSION

5.1 Yield percentage

The yield of extractable compounds relative to the weight of fresh and dried fruit material ranged from 1.96 ± 0.21% (acetone extract) to 2.37 ± 0.22% (water extract) in fresh fruits of Karela, 6.75 ± 0.27% (acetone extract) to 27.75 ± 0.39% (water extract) in dried fruits of Karela, 1.76 ± 0.11% (water extract) to 3.63 ± 0.14% (ethanol extract) in fresh fruits of Kankoda and 4.06 ± 0.12% (acetone extract) to 16.47 ± 0.72%.in dried fruits of Kankoda (Table1).

5.2 Moisture percentage

The moisture content in fruits material ranged from 84.18 ± 0.88% (Kankoda) to 90.57 ± 0.97% (Karela) Table 2.

5.3 Total phenolic content

Phenolics are aromatic secondary plant metabolites are high-level antioxidants because of their ability to scavenge free radicals and active oxygen species such as singlet, superoxide free radicals and hydroxyl radicals. Natural polyphenols have chain-breaking antioxidant activities. It is well known that phenolic substances contribute directly to the antioxidant activity of plant materials. In fact, phenolic compounds exhibit considerable free radical-scavenging activities (through their reactivity as hydrogen-donating or electron-donating agents) and metal ion-chelating properties (Rice-Evans *et al.,* 1996). Therefore, the amounts of total phenols in the extracts were determined (Table 3, 4, 5 and 6). Our results showed that the content of total phenols varied from 9.18 ± 0.29 mg GAE/g (water extract) to 12.08 ± 0.23 mg GAE/g (acetone extract) in fresh fruits of Karela, 0.57 ± 0.06 mg GAE/g (water extract) to 6.83 ± 0.12 mg GAE/g (acetone extract) in dried fruits of Karela, 4.10 ± 0.25 mg GAE/g (ethanol extract) to 5.45±0.26 mg GAE/g (water extract) in fresh fruits of Kankoda and 0.67 ± 0.09 mg GAE/g (water extract) to 3.58 ± 0.09 mg GAE/g (acetone extract) in dried fruits of kankoda. There is much more decrease in the total phenol content in the water extract of dried fruits of Kankoda. This is explained as during drying there occurs enzymatic hydrolysis of polyglycosylated phenols and aglycones gets free from sugar moiety and ester linkage. The free aglycone becomes more soluble in acetone hence its concentration increases in the acetone extract and the order of phenolic content becomes acetone extract > ethanol extract > water extract.

The antioxidant activity of fractions may not only be due to the presence of phenolic compounds but also related to the presence of some individual active components in the extracts. The unclear relationship between the antioxidant activity and total phenolic content may be explained by the fact that the total phenolic content does not incorporate all the

antioxidants. In addition, the synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration but also on the structure and interaction between the antioxidants. Phenolic groups play an important role in antioxidant activity (Huang and Frankel, 1997; Baratta *et al.,* 1998). It is well known that the antioxidant activity of phenols is affected by their chemical structure and can be decreased or increased depending upon the group attached to a basic aglycon.

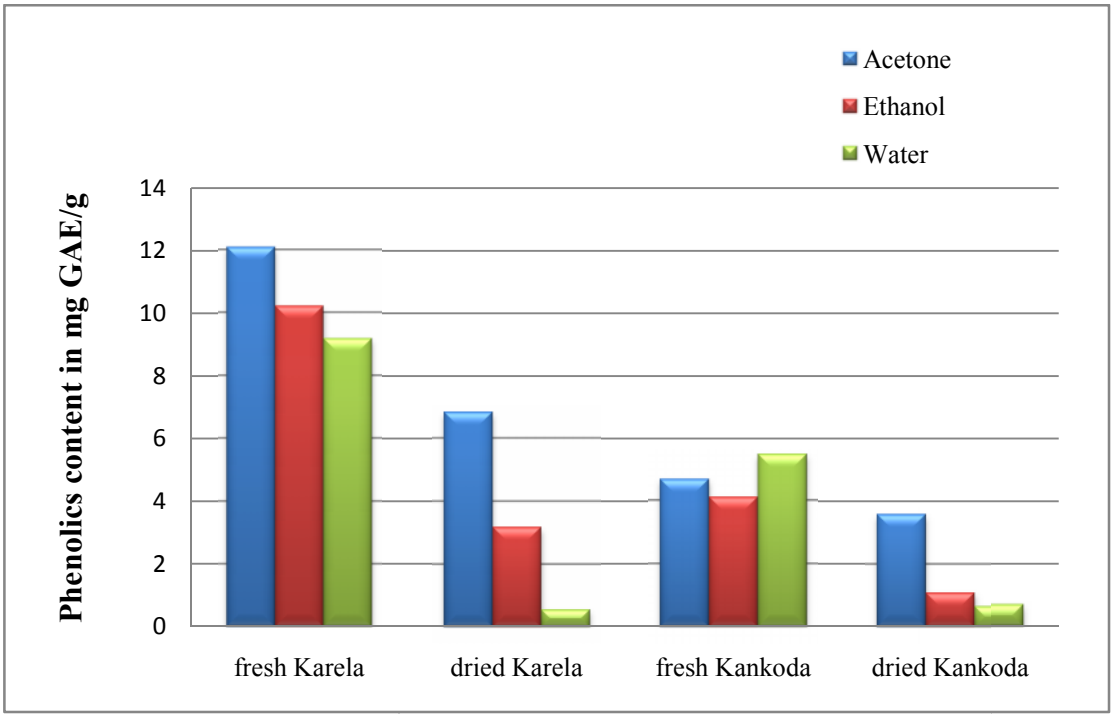


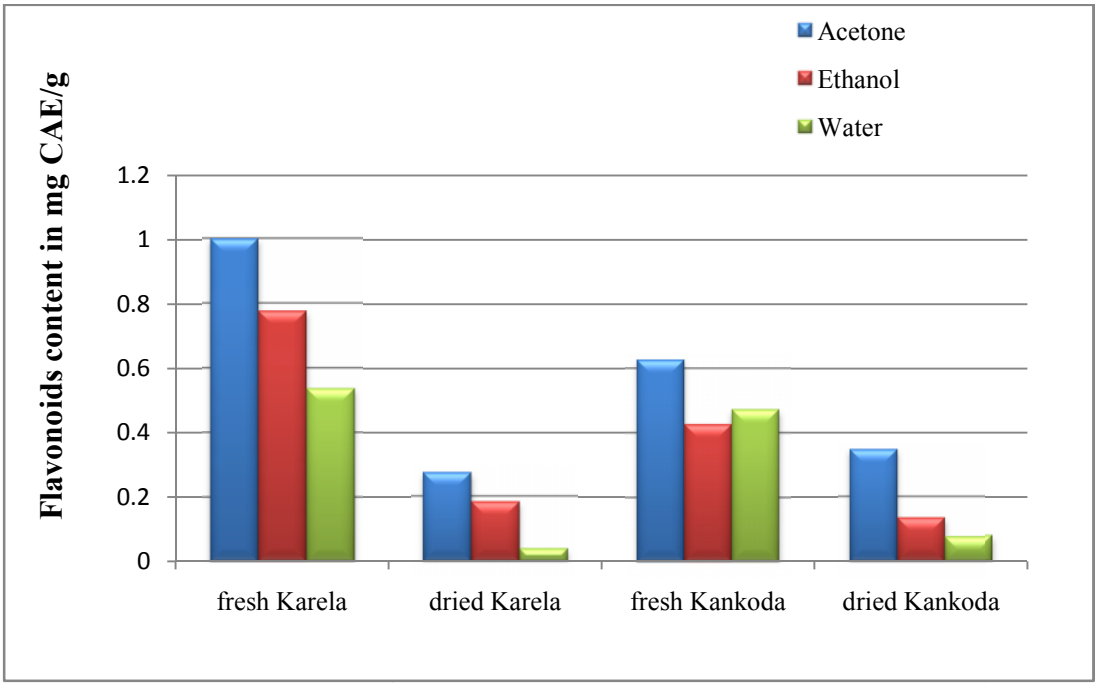
Figure 1. Total phenolics content of different extracts of fresh and dried fruits of Karela and Kankoda

5.3 Flavonoid content

Flavones and flavonols are the subgroups of flavonoids. Flavonols are known to act as antioxidant, both as radical scavengers (Bors and Saran, 1987) and as metal chelators (Afanaslejev *et al.,* 1989). The aglycones of these flavonols were reported to be more active than their glycosides (Hopia and Heinonen, 1999). Flavonoids have the ability to scavenge active oxygen radical, superoxide and hydroperoxide by single electron transfer. Superoxide is a biologically important substance which can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Korycka-Dahl and Richardson, 1978). The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids and proteins (Grootveld and Jain, 1989)

In the present study the flavonoid content in fresh fruits of Karela varied from 1.28 ± 0.24 mg CAE/g (water extract) to 6.86 ± 0.31 mg CAE/g (acetone extract). In dried fruits of Karela, it varied from 0.27 ± 0.04 mg CAE/g (water extract) to 4.78 ± 0.14 mg CAE/g (acetone extract). While in fresh fruits of Kankoda it varied from 0.87±0.09 mg CAE/g (water

extract) to 3.81±0.20 mg CAE/g (acetone extract). In dried fruits of Kankoda it was lowest (0.33 ± 0.03 mg CAE/g) in water extract and highest (4.22 ± 0.13 mg CAE/g) in acetone extract (Table 3, 4, 5 and 6). It was reported by Heim *et al.,* (2002) that the presence of glycosides attached to flavonoid aglycons decreases the antioxidant activity of flavonoid. The reason for this is the glycoside moiety, which interferes with the coplanarity of the flavonoid molecule, decreases the ability to delocalise electrons which results in decreases in antioxidant activity of flavonoid.



Figurse 2. Flavonoids content of different extracts of fresh and dried fruits of Karela and Kankoda

5.4 Ascorbic acid content

Ascorbic acid is a well-known antioxidant compound. Vegetables are poor resources of ascorbic acid as compared to fruits but abundance of vegetables in local diets contributes to a significant portion of ascorbic acid requirement of human body. In the present study the ascorbic acid content in Fresh fruits of Karela varied from 0.54 ± 0.01 mg/g (water extract) to 1.01 ± 0.02 mg/g (acetone extract). In dried fruits of Karela, it varied from 0.05 ± 0.01 mg/g (water extract) to 0.27 ± 0.01 mg/g (acetone extract).While in fresh fruits of Kankoda it varied from 0.43 ± 0.01 mg/g (ethanol extract) to 0.63 ± 0.01 mg/g (acetone extract). In dried fruits of Kankoda it was lowest (0.08 ± 0.01 mg/g) in water extract and highest (0.35 ± 0.01 mg/g) in acetone extract (Table 3, 4, 5 and 6).

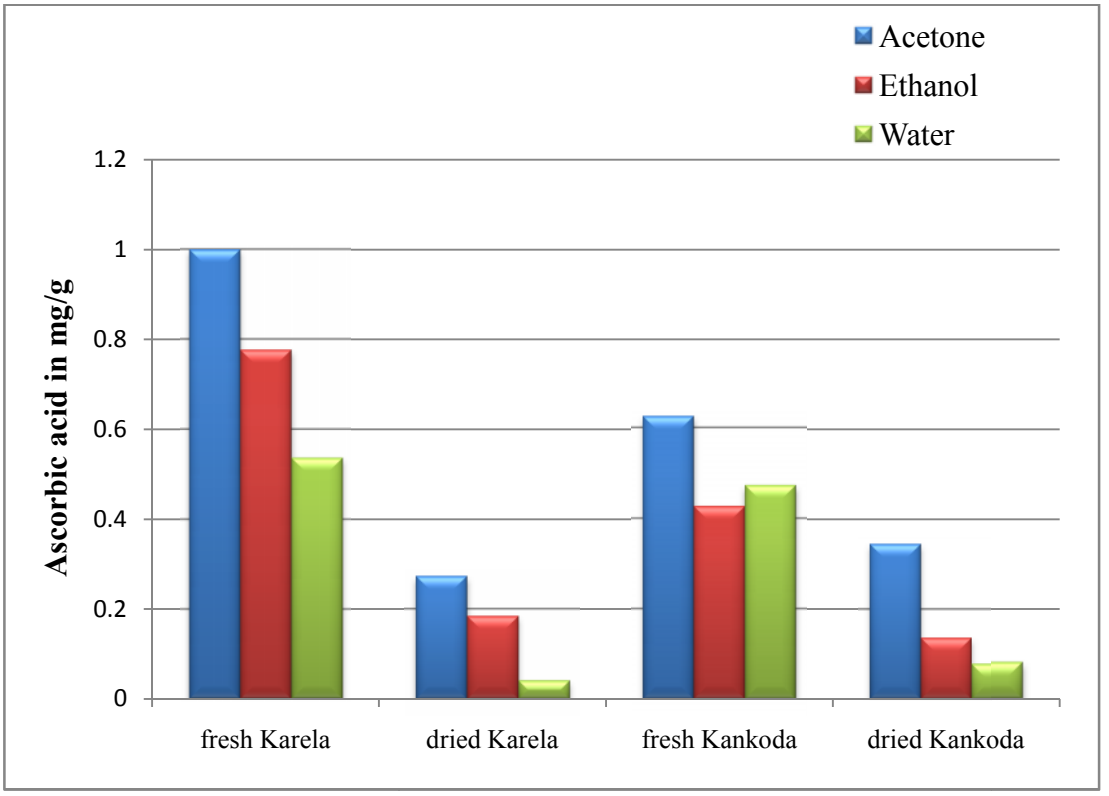


Figure 3. Ascorbic acid content of different extracts of fresh and dried fruits of Karela and Kankoda

5.5 Antioxidant activity

Antioxidants affect the process of lipid oxidation at different stages due to differences in their mode of action (Larson, 1997). Oxidation of lipids is a very complex process resulting in a great variety of oxidation products. Many factors particularly temperature, light and the presence of initiators (metal enzymes), influence the oxidation process and resulting products. For this reason different methods are needed for monitoring oxidation processes to assess primary or secondary oxidation changes and the efficiency of antioxidants. The results obtained by different methods can also differ distinctly, as they involve different conditions, reaction phases and reaction systems. Therefore, to obtain more comprehensive information on antioxidants, the present study is aimed at the evaluation of the antioxidant activity of different extracts of fresh and dried fruits of Karela and Kankoda by using three testing methods.

I. 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) method
II. Ferric thiocyanate method
III. β-carotene method

5.5.1 DPPH method

Various radicals formed during lipid oxidation are among the main causes for oxidative damage to human health (Williams,1993) Antioxidants can exercise their protective function by scavenging free radicals, which are the main propagators of lipid oxidation.

2,2'-diphenyl-1-picrylhydrazyl radical is one of the few stable and commercially available organic nitrogen radical ($DPPH^{\bullet}$), often used in the evaluation of radical scavenging activity of antioxidants-natural and synthetic pure compounds (Brand-Williams *et al.,* 1995; Yen and Duh, 1994). Alcoholic solutions of $DPPH^{\bullet}$ have a characteristic absorption maximum at 517 nm. When an electron or hydrogen atom donating antioxidant (AH) is added to $DPPH^{\bullet}$ a decrease in absorbance at 517 nm takes place due to the formation of the non-radical form DPPH-H, which does not absorb at 517 nm. Originally, it was monitored by ESR spectroscopy and relied on the signal intensity of $DPPH^{\bullet}$ being inversely related to the antioxidant concentration and the reaction time. More recently, this reaction has been measured by the de-coloration assay where the decrease in absorbance at 517 nm produced by the addition of the antioxidant to the $DPPH^{\bullet}$ in methanol or ethanol is measured.

$$DPPH^{\bullet} + AH \longrightarrow DPPH\text{-}H + A^{\bullet}$$

All the above described extracts were screened for radical scavenging activity against $DPPH^{\bullet}$. The antioxidant activity exhibited by acetone, ethanol and water extracts of fresh fruits of Karela were 86.11 ± 0.55%, 86.51 ± 0.60%, and 91.21 ± 0.65% respectively at the concentration of 1.0 mg/ml of the extract. The corresponding values of dried fruits of Karela at the same concentration were 86.41 ± 1.11%, 86.21 ± 1.11% and 61.21 ± 1.77% respectively. The values for fresh fruits of Kankoda were as 76.11 ± 0.02%, 87.02 ± 0.04% and 58.01 ± 0.05% at the concentration of 1.0 mg/ml of acetone, ethanol and water extracts respectively. The corresponding values for dried fruits of Kankoda at the same concentration were as 55.51 ± 0.71%, 85.61 ± 0.95% and 89.03 ± 0.75% respectively. The most active extract was water extract of fresh fruits of Karela. The opposite trend in the phenolic content and antioxidant activity of extracts of fresh fruits of Karela can be explained by the fact that the Folin – Ciocalteu method measures other constituents than phenolics. The Folin – Ciocalteu reagent detect all phenolic groups found in the extracts (Shahidi and Naczk, 1995). This indicates also that factors other than total phenolics may play a role in the antioxidant activity of water extracts. Moreover, all the phenolics do not have the same antioxidant activity, some are powerful, others are weak and they develop antagonistic or synergistic effects with themselves or with the other constituents of the extracts (Rice-Evans *et al*., 1996; Moran *et al*., 1997; Lien *et al*., 1999). This fact could mean that either their components do not possess, good hydrogen donating properties or that some kinetic factors influenced their reaction with the radical, or that their components interfere with the radical scavenging process. Bondet *et al.,* (1997) have found that the radical scavenging activity of a particular antioxidant depends on structure as well as on the type of reaction kinetics.

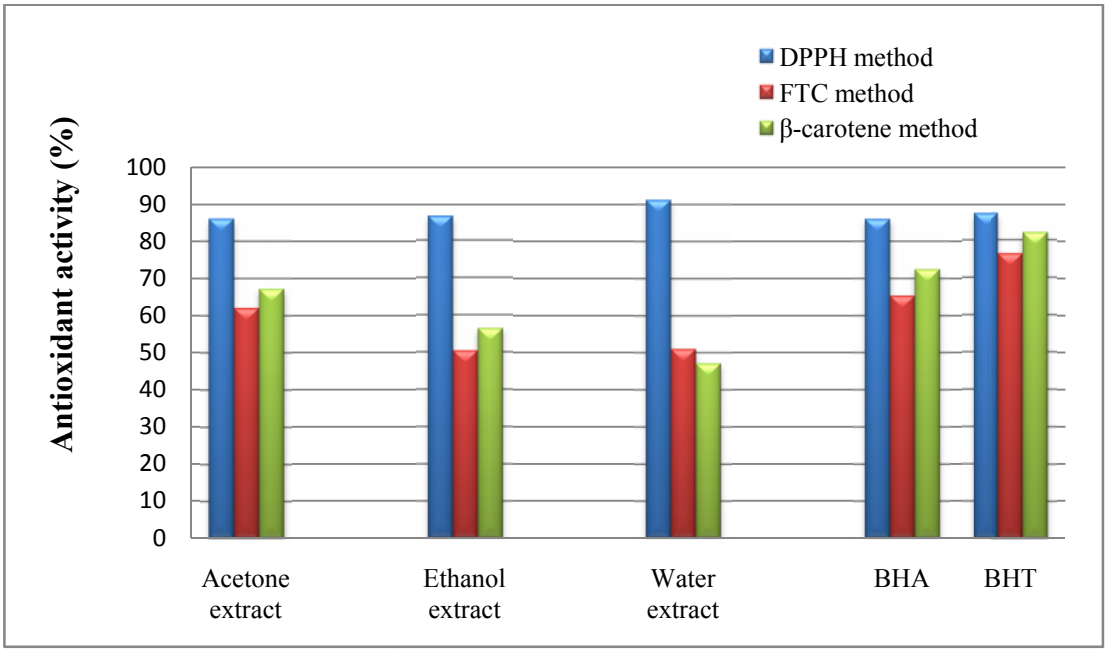


Figure 4. Antioxidant activity of different extracts of fresh fruits of Karela at a concentration of 1.0 mg/ml by DPPH, FTC and β-carotene methods

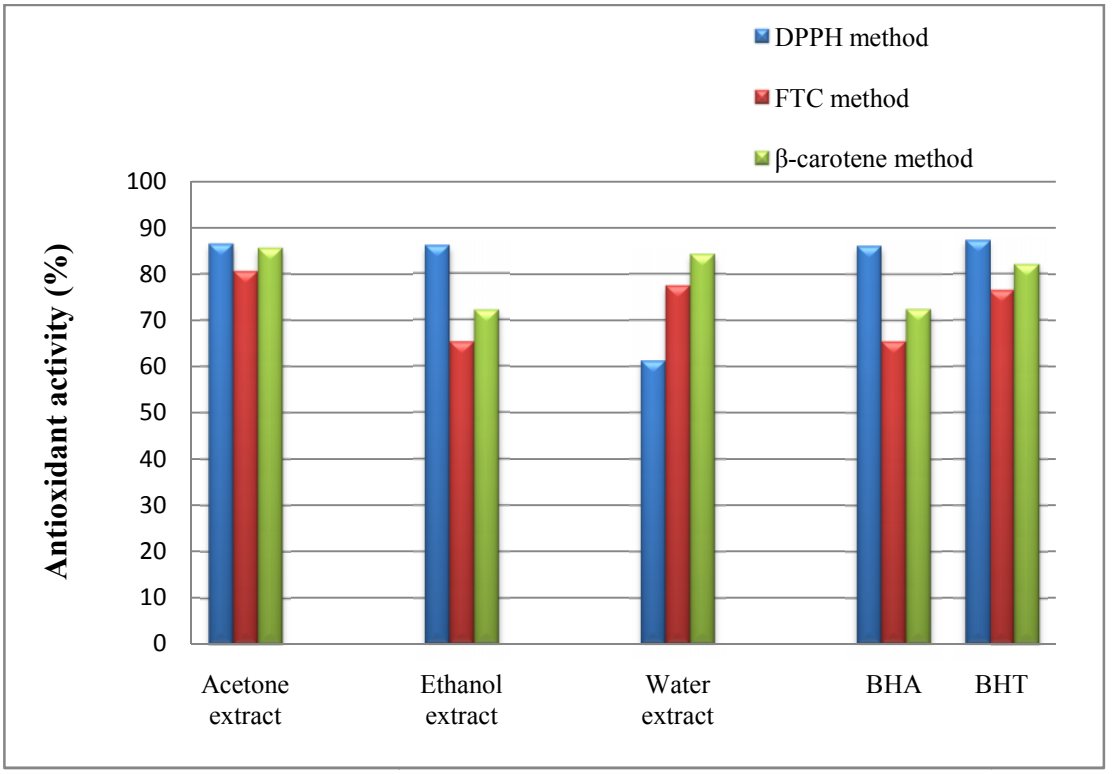


Figure 5. Antioxidant activity of different extracts of dried fruits of Karela at a concentration of 1.0 mg/ml by DPPH, FTC and β-carotene methods

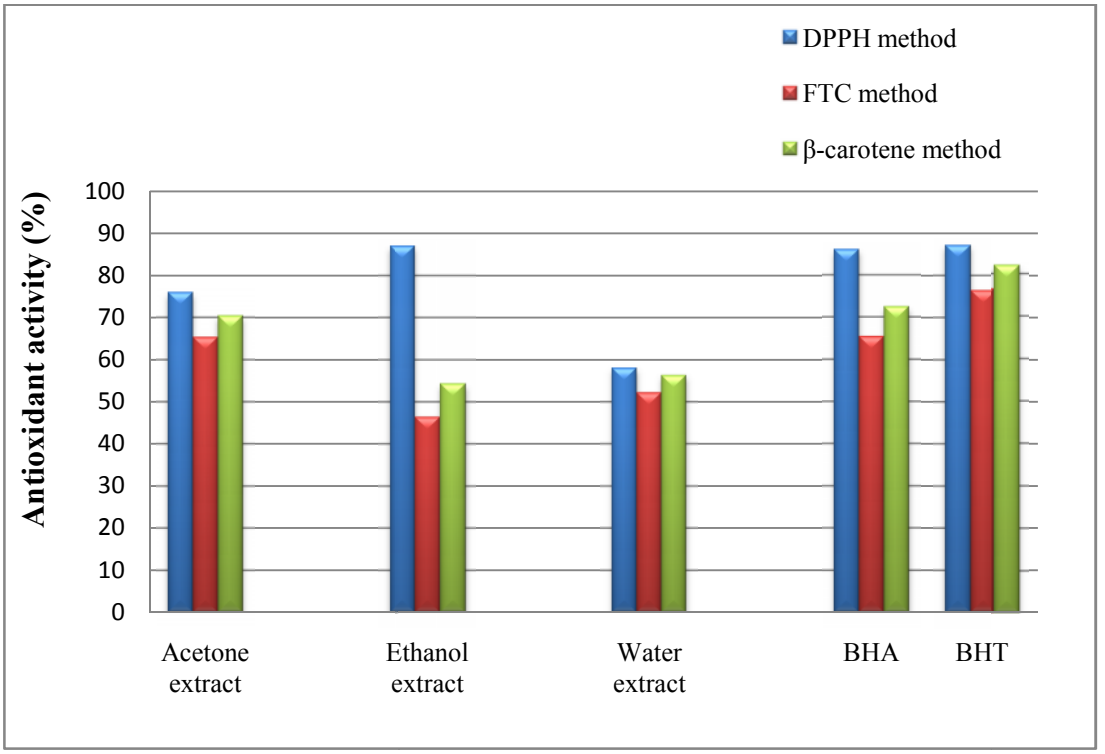


Figure 6. Antioxidant activity of different extracts of fresh fruits of Kankoda at a concentration of 1.0 mg/ml by DPPH, FTC and β-carotene methods

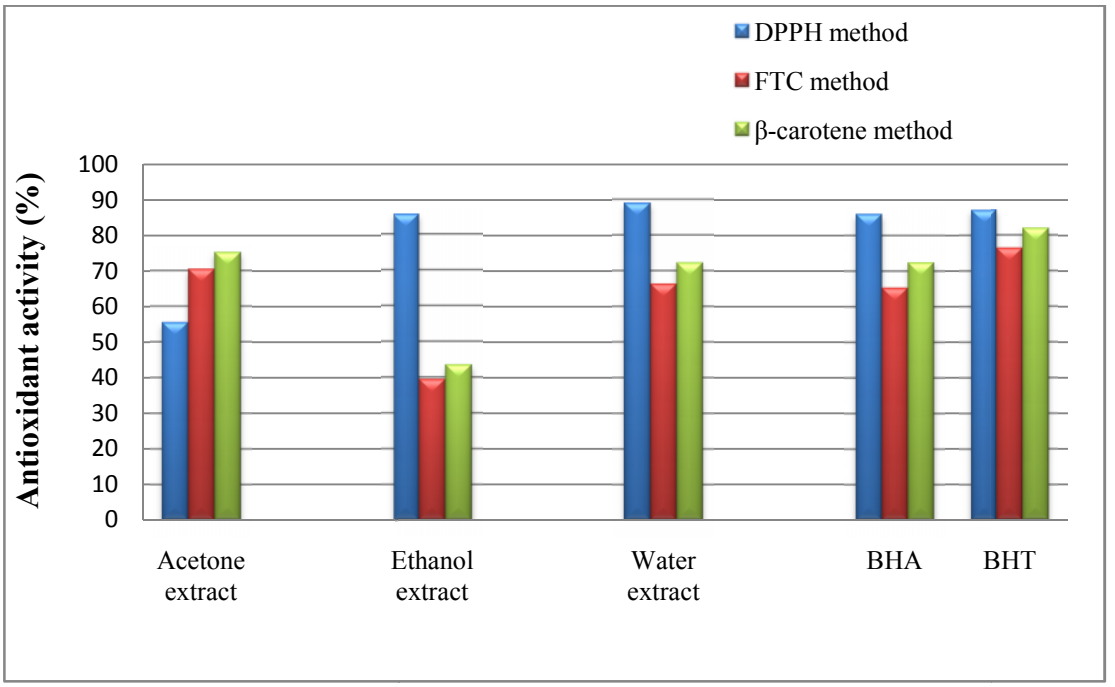


Figure 7. Antioxidant activity of different extracts of dried fruits of Kankoda at a concentration of 1.0 mg/ml by DPPH, FTC and β-carotene method

5.6 Antioxidant activity by Ferric Thiocyanate Method

Real food systems generally consist of multiple phases in which lipid and water coexists with some emulsifier. Hence an antioxidant assay using a heterogeneous system such as an oil-in-water emulsion is required. Autoxidation of linoleic acid in ethanol buffer is one of the model systems for such evaluation, satisfying the above conditions (Osawa and Namiki, 1981). The linoleic acid emulsion system/thiocyanate method has been used here for evaluation under the above conditions. The results are shown in table 8, 9, 10 and 11. During peroxidation of linoleic acid at 37°C in an incubator, the absorbance values increased owing to the oxidation products, which react to form ferric thiocyanate, the colour of red blood (Jayaprakasha *et al.,* 2001). Antioxidants can hinder the oxidation and, consequently, the increase in absorbance will be less. The antioxidant activity exhibited by acetone, ethanol and water extracts of fresh fruits of Karela were 62.06±0.81%, 50.43 ± 0.83%, and 51.11 ±1.3% respectively at the concentration of 1.0 mg/ml of the extract. The corresponding values of dried fruits of Karela at the same concentration were 80.53 ± 1.25%, 65.21 ± 1.22% and 77.43 ± 1.11% respectively. The values for fresh fruits of Kankoda were as 65.36 ± 1.28%, 46.41± 0.95% and 52.21 ± 1.12% at the concentration of 1.0 mg/ml of acetone, ethanol and water extracts respectively. The corresponding values of dried fruits of Kankoda at the same concentration were 70.46 ± 0.98%, 39.51 ± 1.22% and 66.36 ± 1.28% respectively. The antioxidant activity of the extracts by thiocyanate method supports the results of the β-carotene bleaching method.

5.7 Antioxidant activity by β-carotene method

In the β-carotene bleaching method, the antioxidant activity of carotenoids is based on the radical adducts of carotenoid with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene molecules. The presence of different antioxidants can hinder the extent of β-carotene bleaching by neutralizing the linoleate free radical and other free radicals formed in the system (Jayaprakasha *et al.,* 2001). In this way the antioxidant activity of various added substances can be monitored. Our results showed that the antioxidant activity of fresh fruits of Karela varied from 47.11 ± 0.75% (water extract) to 67.16 ± 0.96% (acetone extract) and from 72.11 ± 0.91% (ethanol extract) to 85.51 ± 0.96% (acetone extract) in dried fruits of Karela at the concentration of 1.0 mg/ml of the extract. The antioxidant activity of fresh fruits of Kankoda varied from 54.31 ± 0.79% (ethanol extract) to 70.43 ± 0.75% (acetone extract) and from 43.51 ± 1.19% (ethanol extract) to 75.21 ± 0.97% (acetone extract) in dried fruits of Kankoda at the concentration of 1.0 mg/ml of the extract. It seems that antioxidant activity of all the extracts showed different value from the extracts of the different polar solvents. It has been reported that most natural anti-oxidative compounds often work synergistically with each other to produce a broad spectrum of anti-oxidative activities that create an effective defence system against free-

radical attack (Lu and Foo, 1995). The composition of the extract is very complex; it consists of various classes of organic compounds which may exert opposite effects on the process of lipid oxidation. Based on the results obtained, it is highly possible that some constituents of non-phenolic components may contribute to the anti-oxidative activity of the extract.

CHAPTER–VI

SUMMARY AND CONCLUSION

Recent years have witnessed a renewed interest in plants as pharmaceuticals. The approach of phytochemicals in medicinal plants is mainly concentrated on their role in preventing diseases caused as a result of oxidative stress. Oxidative stress releases free oxygen radicals in the body which result in various diseases. Hence reactive oxygen species need to be scavenged to maintain the normal cellular functions and to avoid damages. A number of factors are known to provide protection against this oxidative stress like free-radical traps (e.g. phenols) or anti-oxidative enzyme like glutathione peroxidase (GP-X), and glutathione reductase (GR).

Momordica charantia commonly known as bitter gourd (Karela) is a member of the Cucurbitaceae family. It is known as bitter melon, balsam pear, and pare. It grows in tropical areas of the Amazon, East Africa, Asia, South America, and the Caribbean and is used traditionally as both food and medicine.

Momordica dioica Roxb. Ex. Wild. (Cucurbitaceae) known as Kankoda is a perennial, dioiceous climber with tuberous roots found throughout India from Himalayas to Ceylon, up to an altitude of 1,500 m. The plant is sometimes found growing wild and is common in hedges. *Momordica dioica* climber plant commonly known as Janglee Karela or small bitter-gourd is a relatively small oval to ovoid in shape. It is often cultivated for its fruits, which are used as vegetable. The whole plant is used for the treatment of eye diseases and fever.The fruits of Karela and Kankoda are used as vegetables but their availability is seasonal and limited to certain pockets of the country. In order to make it available throughout the year in adequate quantity these are used in dried form. So the present study was aimed at the study of chemical composition and antioxidant activity of different extracts of fresh and dried fruits of Karela and Kankoda by using three testing methods: 2,2'-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), β-carotene bleaching test (BCBT) and Ferric Thiocyanate (FTC) method, which may help to explore the possibility of using the most suitable route with regard to the preparation of extracts rich in anti-oxidative active components, as a functional ingredient in product formulations.

Hence the objectives of study were

1. To estimate total phenols, flavonoids and ascorbic acid in the extracts of fresh and oven dried fruits of *Momordica charantia* (karela) and *Momordica dioica* (kankoda).
2. To observe the contribution of these compounds to anti-oxidant and antiradical activity.

6.1 Yield percentage of different extracts of fresh and dried fruits of Karela and Kankoda was found as follows:

1. It was found that the yields of acetone, ethanol and water extract of fresh Karela were 1.96 ± 0.21%, 2.25 ± 0.15% and 2.37 ± 0.22% respectively.
2. The yields of acetone, ethanol and water extract of dried Karela were 6.75 ± 0.27%, 12.25 ± 0.38% and 27.75 ± 0.39% respectively.
3. The yields of acetone, ethanol and water extract of fresh Kankoda were 3.34 ± 0.08%, 3.63 ± 0.14% and 1.76 ± 0.11% respectively.
4. The yields of acetone, ethanol and water extract of dried Kankoda were 4.06± 0.12%, 16.41± 0.65% and 16.47 ± 0.72% respectively.

6.2 Moisture percentage of fresh fruits of Karela and Kankoda was as follows:

1. It was found that the moisture content in Karela was 90.57±0.97%.
2. The moisture content in Kankoda was found to be 84.18±0.88%.

6.3 Chemical composition of different extracts of fresh fruits of Karela was found as follows:

1. It was found that acetone extract contains 12.08 ± 0.23 mg GAE/g total phenols, 6.86 ± 0.31 mg CAE/g flavonoids, and 1.01 ± 0.02 mg/g ascorbic acid.
2. Ethanol extract was found to contain 10.21± 0.36 mg GAE/g total phenols, 7.61 ± 0.21 mg CAE/g flavonoids, and 0.78 ± 0.01 mg/g ascorbic acid.
3. Water extract contained 9.18 ± 0.29 mg GAE/g total phenols, 1.28 ± 0.24 mg CAE/g flavonoids, and 0.54 ± 0.01 mg/g ascorbic acid.

6.4 Antioxidant activity of different extracts of fresh fruits of Karela was found as follows:

1. According to DPPH method, antioxidant activity of acetone extract was found to be 86.11 ± 0.55% while for ethanol extract it was found to be 86.51 ± 0.60% and it was maximum for water extract and found to be 91.21 ± 0.65% at a concentration of 1.0 mg/ml of the extract.
2. According to FTC method, antioxidant activity of acetone extract was found to be maximum that is 62.06 ± 0.81% while for ethanol extract it came out to be 50.43 ± 0.83% and for water extract it was 51.11 ± 1.31% at a concentration of 1.0 mg/ml of the extract.
3. According to β-carotene method, antioxidant activity of acetone extract was found to be maximum that is 67.16 ± 0.96% while for ethanol extract it came out to be 56.41 ± 0.78% and for water extract it was 47.11 ± 0.75% at a concentration of 1.0 mg/ml of the extract.

6.5 Chemical composition of different extracts of dried fruits of Karela was found as follows:

1. It was found that acetone extract contains 6.83 ± 0.12 mg GAE/g total phenols, 4.78 ± 0.14 mg CAE/g flavonoids, 0.27 ± 0.01mg/g ascorbic acid.
2. Ethanol extract was found to contain 3.18 ± 0.08 mg GAE/g total phenols, 2.64 ± 0.10 mg CAE/g flavonoids, 0.18± 0.01 mg/g ascorbic acid.
3. Water extract was found to contain 0.57 ± 0.06 mg GAE/g total phenols, 0.27 ± 0.04 mg CAE/g flavonoids, 0.05± 0.01 mg/g ascorbic acid.

6.6 Antioxidant activity of different extracts of dried fruits of Karela was found as follows:

1. According to DPPH method, antioxidant activity of acetone extract was found to be maximum that is 86.41 ± 1.11% while for ethanol extract it came out to be 86.21 ± 1.11% and for water extract it was 61.21 ± 1.77 % at a concentration of 1.0 mg/ml of the extract.
2. According to FTC method, antioxidant activity of acetone extract was found to be maximum that is 80.53 ± 1.25% while for ethanol extract it came out to be 65.21 ± 1.22% and for water extract it was 77.43 ± 1.11% at a concentration of 1.0 mg/ml of the extract.
3. According to β-carotene method, antioxidant activity of acetone extract was found to be maximum that is 85.51 ± 0.96% while for ethanol extract it came out to be 72.11 ± 0.91% and for water extract it was 84.21 ± 0.63% at a concentration of 1.0 mg/ml of the extract.

6.7 Chemical composition of different extracts of fresh fruits of Kankoda was found as follows:

1. It was found that acetone extract contains 4.67 ± 0.27 mg GAE/g total phenols, 3.81 ± 0.20 mg CAE/g flavonoids and 0.63± 0.01 mg/g ascorbic acid.
2. Ethanol extract contains 4.10 ± 0.25 mg GAE/g total phenols, 1.25 ± 0.19 mg CAE/g flavonoids and 0.43 ± 0.01 mg/g ascorbic acid.
3. Water extract contained 5.45 ± 0.26 mg GAE/g total phenols, 0.87 ± 0.09 mg CAE/g flavonoids and 0.47 ± 0.01 mg/g ascorbic acid.

6.8 Antioxidant activity of different extracts of fresh fruits of Kankoda was found as follows:

1. According to DPPH method, antioxidant activity of ethanol extract was found to be maximum that is 87.02 ± 0.04% while for acetone extract it came out to be 76.11 ± 0.02% and for water extract it was 58.01 ± 0.05% at a concentration of 1.0 mg/ml of the extract.

2. According to FTC method, antioxidant activity of acetone extract was found to be maximum that is 65.36 ± 1.28% while for water extract it came out to be 46.41 ± 0.95% and for ethanol extract it was 52.21 ± 1.12% at a concentration of 1.0 mg/ml of the extract.
3. According to β-carotene method, antioxidant activity of acetone extract was found to be maximum that is 70.43± 0.75% while for ethanol extract it came out to be 54.31 ± 0.79% and for water extract it was 56.31 ± 1.09% at a concentration of 1.0 mg/ml of the extract.

6.9 Chemical composition of different extracts of dried fruits of Kankoda was found as follows:

1. It was found that acetone extract contains 3.58 ± 0.09 mg GAE/g total phenols, 4.22 ± 0.13 mg CAE/g flavonoids and 0.35 ±0.01 mg/g ascorbic acid.
2. Ethanol extract contains 1.11 ± 0.32 mg GAE/g total phenols, 0.73 ± 0.06 mg CAE/g flavonoids and 0.14 ± 0.01 mg/g ascorbic acid.
3. Water extract contained 0.67 ± 0.09 mg GAE/g total phenols, 0.33 ± 0.03 mg CAE/g flavonoids and 0.08 ± 0.01 mg/g ascorbic acid.

7.0 Antioxidant activity of different extracts of dried fruits of Kankoda was found as follows:

1. According to DPPH method, antioxidant activity of water extract was found to be maximum that is 89.03 ± 0.75% while for ethanol extract it came out to be 85.61 ± 0.95% and for acetone extract it was 55.51 ± 0.70% at a concentration of 1.0 mg/ml of the extract.
2. According to FTC method, antioxidant activity of acetone extract was found to be maximum that is 70.46 ± 0.98% while for ethanol extract it came out to be 39.51 ± 1.22% and for water extract it was 66.36 ± 1.28% at a concentration of 1.0 mg/ml of the extract.
3. According to β-carotene method, antioxidant activity of acetone extract was found to be maximum that is 75.21 ± 0.97% while for ethanol extract it came out to be 43.51 ± 1.19% and for water extract it was 72.26 ± 1.12% at a concentration of 1.0 mg/ml of the extract.

Therefore, the following conclusion can be drawn from this research:

1. The contents of total phenols, flavonoids and ascorbic acid decreased in the acetone, ethanol and water extracts of the dried fruits of Karela.
2. On drying the fruits of Kankoda, there is increase in the concentration of flavonoids in the acetone extract. The increase is due to enzymatic hydrolysis of polyglycosylated flavonoids during drying and the aglycones gets free from sugar moiety and ester linkages. The free aglycone becomes more soluble in acetone and hence its concentration increases in the acetone extract. The antioxidant activity of the acetone extract of the

dried fruits of Karela increases in all the test methods. But the ethanol and water extracts shows decrease in antioxidant activity by DPPH method and increase in antioxidant activity by FTC and β- carotene bleaching method.

3. The water extracts of the dried fruits of Kankoda showed increase in antioxidant activity in all the three test methods while acetone extract show increase in the antioxidant activity in FTC and β- carotene bleaching methods.

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ABSTRACT

a)	Title of Thesis	:	**Studies on the effect of drying on the chemical composition and antioxidant activities of the fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda)**
b)	Full name of the degree holder	:	**Ms. Sukriti Nehra**
c)	Admn. No.	:	2011BS83M
d)	Title of degree	:	Master of Sciences
e)	Name and address of Major Advisor	:	**Dr. M. Khabiruddin** Professor of Chemistry Department of Chemistry and Physics CCS Haryana Agricultural University Hisar -125004 ,India
f)	Degree awarding University/ Institute	:	CCS Haryana Agricultural University Hisar -125004 (Haryana),India
g)	Year of award of degree	:	2013
h)	Major subject	:	Chemistry
i)	Total number of pages in the thesis	:	38 + vii
j)	Number of words in the abstract	:	Approximately 300

Key words: *Momordica charantia*, *Momordica dioica*, phenols, flavonoids, ascorbic acid, antioxidant activity

Antioxidant compounds in food play an important role as a health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms that lead to degenerative diseases. The fruits of *Momordica charantia* (Karela) and *Momordica dioica* (Kankoda) are used traditionally as both food and medicine. The availability of these vegetables is seasonal and limited to certain pockets of the country. In order to make it available throughout the year in adequate quantity, these are used in dried form. So the aim was to determine the content of total phenols, flavonoids and ascorbic acid in the acetone, ethanol and water extracts of fresh and dried fruits of Karela and Kankoda and the antioxidant activity of these extracts by: 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) method, Ferric Thiocyanate (FTC) method and β-carotene bleaching test (BCBT). It was found that the contents of total phenols, flavonoids and ascorbic acid decreased in the acetone, ethanol and water extracts of the dried fruits of Karela. On drying the fruits of Kankoda, the concentration of flavonoids increased in the acetone extract. The antioxidant activity of the acetone extract of the dried fruits of Karela increased in all the three test methods. But the ethanol and water extracts showed decrease in antioxidant activity by DPPH method and increase in FTC and BCBT methods.The water extracts of the dried fruits of Kankoda showed increase in antioxidant activity in all the three test methods while acetone extract showed increase in the antioxidant activity in FTC and BCBT methods.

SIGNATURE OF THE STUDENT **MAJOR ADVISOR**

HEAD OF DEPARTMENT

CURRICULUM VITAE

Name	:	Sukriti Nehra
Date of birth	:	1 January, 1991
Place of birth	:	Hisar
Mother's name	:	Mrs. Sulochana Devi
Father's name	:	Mr. Krishan kumar
Permanent address	:	Mr. Krishan Nehra Vill&PO – Rawalwas khurd, Distt & Teh – Hisar , PIN- 125001
Mobile	:	9729525032
E-mail	:	Sukritinehrasn@gmail.com

Academic qualifications

Degree	**University/Board**	**Year of passing**	**Percentage of marks**	**Subjects**
Matriculation	CBSE Board	2006	84	English, Science, Math, Social Science, Hindi
10+2	CBSE Board	2008	76.2	English, Physics, Chemistry, Biology, Hindi
B.Sc.	K.U.K.	2011	80.68	Chemistry, English, E.Vs. Zoology, Botany,Sanskrit

Co-curricular activities : Reading books and novels and listening music.

Medals/ Honours received : District topper graduation, During M.Sc. Promotion Of Science and Education (POSE) Scholarship from DST, Haryana

SIGNATUR OF THE STUDENT

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