Studies on chemical composition of some Indian medicinal plants and their antioxidant activity

By

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

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

α	:	Alpha
β	:	Beta
g	:	Gram
µg	:	Micro gram
kg	:	Kilogram
mg	:	Milligram
No.	:	Number
m.p.	:	Melting point
GAE	:	Gallic Acid Equivalent
CE	:	Catechin Equivalent
ppm	:	Parts Per Million
ml	:	Millilitre
L	:	Litre
M	:	Molar
N	:	Normal
nm	:	Nanometer

Chapter-I

INTRODUCTION

Early research on the role of antioxidants in biology is focused on their use in preventing the oxidation of unsaturated fats, which is the cause of rancidity. Antioxidants are important not only for food protection but also for the defense of living cells against oxidative damage. Free radicals, produced by metabolic reactions, promote oxidative stress and even atherosclerosis and cancer. Increased oxidative stress damage cells, including their proteins, lipids, DNA and contribute to aging. Although, food industries have used effective synthetic antioxidants but recently consumers prefer natural antioxidants on the basis of the assumption that natural compounds are safe. Several plants have been studied to evaluate their phenolic contents and antioxidant activity. The importance of this study lies in the fact that all kinds of food containing phenolic compounds usually have high antioxidant activity, which means they may have positive effect on preserving the quality of food and human health when sufficient quantity is present in the diet.

Some studies have demonstrated that phenolic compounds having antioxidant activity may retard aging, as well as prevent degenerative diseases, such as cancer, cardiovascular diseases and cerebral dysfunctions (Ames *et al.,* 1993). Due to difficulty in evaluating the anti-oxidant activity that food can present in the human physiology and its consequences on human health, it is necessary to identify methods which correlate to this biological function. Quantifying their phenolic composition and determining their free radical scavenging activity, and the solvent effect on the efficiency of the antioxidant are some indicators of this property (Becker *et al.,* 2004). The following plants having medicinal/ spices properties have been selected for present study.

Terminalia arjuna (Arjuna) is found throughout the subcontinent of India, from the foothills of the Himalayas southwards to Sri Lanka (Warrier *et al.,* 1995; Kirtikar and Basu, 1935). It is a member of family Combretaceae. Arjuna tree is known for its ethnomedicinal significance. Its bark is used to maintain the cholesterol level and for the treatment of coronary artery disease (Dwivedi and Jauhari, 1997). It is beneficial in heart failure (Dwivedi and Jauhari, 1997), edema, asthma and angina. Its bark possesses diuretic, prostaglandin enhancing and coronary risk factor modulating properties. Bark of some species of the genus *Terminalia* like *T. macroptera, T. superba* and *T. vorensi* have also been reported for their use as antidiarrheal, antidysentric (Alawa *et al.,* 2002) and trypanocidal (Adewunmi *et al*., 2001). Decoction prepared from the bark of *T. arjuna* is used as an anthelmintic, both in man and animals.

Kigelia pinnata (Balam Kheera) is grown throughout Africa and many places of India. It belongs to the family Bignoniaceae and commonly called the Sausage tree because of its huge fruits. The sausage tree has a long history of use by rural African communities especially for its medicinal properties (Asekun *et al.*, 2007). The fruits are believed to be a cure for a wide range of ailments including rheumatism, snakebites, evil spirits and veneral diseases like syphilis. The fruits are a popular source of traditional medicine throughout Africa (Asekun *et al*., 2007; Kolodziej, 1997). *Kigelia africana* fruit pulp and extracts have traditionally been used as folklore, dietary/herbal supplement, cosmeceutical, nutraceutical and pharma-ceutical purposes. It has strong anti-oxidative effects against hepatotoxicity induced by paracetamol (Olaleye and Rocha, 2008). It is speculated that the antioxidant activity is attributed to the caffeic acid derivative (Jung *et al*., 2006 and Gulcin, 2006) and compounds unique to *Kigelia* (Olthof *et al*., 2001). Other notable bioactivities include its antimicrobial action against sexually transmitted diseases (Grace *et al*., 2002), anti-protozoal activity against *Plasmodium falciparium, Tripanosoma cruzi, Tripanosoma brucei* and *Leishmania major* (Moiden *et al*., 1999 and Weiss *et al*., 2000), antiamoebic activity against *E. histolytica* (Bharti *et al*., 2006), anti-diarrhoeal activity (Akah, 1998), anti-inflammatory/ analgesic activity (Owolabi and Omogbai, 2007) and anticancer activity (Houghton *et al*., 1994 and Picerno *et al.,* 2005). The Bignoniaceae family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes, tannins, steroids, saponins and caffeic acid in the fruits, stem, leaves and roots. The anti-oxidant actions of Kigelia africana have been attributed to the abundance of flavonoids. Its bark and fruit extracts are used for treating diseases caused by microorganisms and as a remedy for skin cancer. The fruit extract shows antibacterial activity which inhibit the growth of yeast and are also active against several protozoal species associated with diseases.

Zingiber officinale (Ginger) is grown in India, Southeast Asia and West Africa (McGee and Harold, 2004). It is a member of family Zingiberaceae. Fresh ginger is one of the main medicinal plant and spices used as a stimulant, carminative and for treating dyspepsia and colic. Chen *et al.,* 2007 studied that ginger also decreases joint pain from arthritis, have blood thinning and cholesterol lowering properties that make it useful for treating heart disease. Rhizome extract is also taken as a carminative for relieving flatulence (Kikuzaki and Nakatani, 1993). In Malaysia, only ginger rhizomes are consumed as food flavoring and the leaves are thrown away. In Thailand, leaves of ginger are eaten as salad.

Foeniculum vulgare (Fennel) is found growing in many parts of the world. It belongs to family Apiaceae (Umbelliferae). It is an aromatic, anise-flavoured spice (Wright *et al.,* 2007). Fennel is chiefly used medicinally with purgatives to allay their side effects. Also used as a diuretic and a potential drug for treatment of hypertension (El Bardai *et al.,* 2001). Fennel juice is given for chronic coughs and for cattle condiments.

Terminalia arjuna (Arjuna)

Kigelia pinnata (Balam Kheera)

Zinziber officinale (Ginger)

Foeniculum vulgare (Fennel)

Cuminum cyminum (Cumin or Zira)

Trigonella foenum (Fenugreek)

Cuminum cyminum (Cumin or Zira) is native of Egypt and has been cultivated in the Middle East, India, China and Mediterranean countries; it belongs to family Apiaceae (Umbelliferae). It is a small annual plant. The cumin seeds are widely used in cooking. Traditional uses of cumin include anti-inflammatory, diuretic, carminative and antispasmodic effect (Evans *et al.,* 1989). It has also been used as an aid for dyspepsia, jaundice, diarrhea, flatulence and indigestion. The presence of sesquiterpenes in the plant material is known to possess a potent antiulcer activity (Yesilada *et al.,* 2004). It is also known to possess anti-carcinogenic (Nalini *et al.,* 2006), hepatoprotective (Kode *et al.*, 2005), antidiabetic (Dhandapani, 2002), antibacterial (Iacobellis and Cantore, 2005), antiepileptic (Janahmadi *et al*., 2006) and antioxidant (Satyanarayana *et al.,* 2004) activities.

Trigonella foenum (Fenugreek) is cultivated worldwide as a semi-arid crop and is a plant of the family Fabaceae. It is used both as an herb (the leaves) and as a spice (the seed). Fenugreek is rich in phenolic phytochemicals that are thought to account for many of its therapeutic effects. Fenugreek seeds lower serum cholesterol, triglyceride and low-density lipoprotein. It shows antidiabetic effects by reducing serum glucose and improving glucose tolerance (Daniel and Maria, 2000). Fenugreek is (*Trigonella foenum-graecum*) found in nature and is cultivated in India and Pakistan. It contains lecithin and choline that helps to dissolve cholesterol and fatty substances, minerals, B. complex, iron, phosphates, PABA (Para Amino Benzoic Acid) and vitamins A and D. It also contains neurin, biotin, trimethylamine which tend to stimulate the appetite by their action on the nervous system (Michael and Kumawat, 2003).

The present study is aimed at the evaluation of antioxidant activity of different extracts from the above six plants grown in Haryana, by using three testing methods: β-carotene bleaching method (BCBT), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric thiocyanate (FTC) method. As the extracts studied have been produced by the extraction routes, the results obtained show the most suitable route with regard to the preparation of extracts rich in antioxidative active components, as well as the most promising plants.

Meagre information is available on this aspect in the literature; therefore, the present study has been undertaken with the following objectives:

1. To estimate total phenols, flavonoids and minerals in the extracts of some Indian medicinal/ spice plants.
2. To observe the contribution of these compounds to anti-oxidant and antiradical activity.

Chapter-II

REVIEW OF LITERATURE

In the present studies, quantitative estimation of total phenols, flavonoids and minerals in the extract of bark of *Terminalia arjuna,* fruits of *Kigelia pinnata, Foeniculum vulgare* and *Cuminum cyminum;* rhizome of *Zinziber officinale* and seeds of *Trigonella foenum* was performed. The contribution of these compounds to anti-oxidant and antiradical activity of the extracts was studied. The literature pertaining to these aspects has been reviewed in this chapter under the following headings:

2.1 Constituents

2.2 Biological activity

2.1.1 Constituents of *Terminalia arjuna* (Arjuna)

Terminalia arjuna (Family: Combretaceae) is a large tree distributed throughout India. It is a commonly occurring medicinal plant growing as a 20-30 m high tree. It is also well recognized in Ayurveda for its various therapeutic values (Kalola and Rajani, 2006; Nadkarni, 2000). Chemical constituents of different classes such as hydrolysable tannins (Kandil and Nassar, 1998), triterpenoid acids and their glycosides (Tripathi *et al.,* 1992), flavonoids (Sharma *et al.,* 1982), phenolics, phytosterol (Row *et al.,* 1970), were reported from stem bark portion of *Terminalia arjuna* species. Additionally, arjunglucoside I-III, arjunic acid, arjunetin, arjunolic acid and terminoic acid also form group of important constituents of the bark. It has been demonstrated that bark extract from *T. arjuna* contains following compounds: acids such as arjundic acid, terminic acid, glycosides argentine arjunosides I-IV, strong antioxidants such as flavones, tannins, oligomeric proanthocyanodins and minerals (Kalola and Rajani, 2006). *Terminalia arjuna* bark is rich in many natural ingredients useful for heart problems. Current scientific research has proved that Arjuna contains specific medically active constituents namely triterpine glycosides like arjunetosides I, II, III, IV, arjunine and arjunetin.

i) It contains a lot of flavones (natural antioxidants). Recently arjunone has been isolated from fruits alongs with cerasidin, β-sitosterol, friedlin, methyl oleanolate, gallic, ellagic and arjunic acids. These are all useful components to take care of the heart naturally.

ii) The bark is rich in saponnins, natural anti-oxidants (flavonoids-arjunone, arjunolone, luteilin), gallic acid, ellagic acid, oligomeric proanthocyanidins, phytosterols, rich in minerals like calcium, magnesium, zinc and copper.

2.2.1 Biological activity of *Terminalia arjuna*

A number of previously published papers reported the therapeutic properties for *Terminalia arjuna* (Bharani *et al.,* 1995). The bark of *T. Arjuna* is used in India as cardio-protective agent in hypertension and ischaemic heart diseases. It is rich in various chemicals such as polyphenols (about 60-70%), phenyl propanoids, tannins (20-24%), flavones, and flavonols and thus it finds useful applications in the treatment of diseases like ulcers, blood diseases, anemia and asthma. It also finds potential applications in curing hepatic, congenital, venereal and viral diseases. The bark powder is reported to show hypocholesterolaemic and antioxidant effect (Gupta *et al.,* 2001). Various pharmacological studies deciphered usefulness of bark against angina pectoris, congestive heart failure, artheriosclerosis and myocardial necrosis. Besides, the medicinal use of bark as fungicidal, antimicrobial, antibacterial, antifertility and anti-human immune deficiency virus induced diseases has also been evaluated.

A number of terpenoid saponins (arjunic acid, arjunolic acid, arjunetin, arjun glycosides), flavonoids (arjunone, arjunolone, luteolin), gallic acid, ellagic acid and phytosterol have been isolated from the bark (Chopra and Ghosh, 1929). It is believed that the fracture bones can be regenerated in a faster rate if the bark paste is used and plastered with the bark itself. In practice, the decoction of the bark is used therapeutically to relieve the pain and inflammation. In this context, the isolation and evaluation of organic compounds present in the plant species that are responsible for observed therapeutic effects is worth studied. The bark of the arjuna tree containing calcium salts, magnesium salts, and glucosides have been used in traditional Ayurvedic herbalism. Juice of its leaves is used to cure dysentry and earache. It strengths the heart muscles and maintains the heart functioning properly. It also improves functioning of cardiac muscle. Arjuna is used for the treatment of coronary artery disease, heart failure, edema, angina and hypercholesterolemia. Its bark power possesses diuretic, prostaglandin enhancing and coronary risk factor modulating properties. It is also considered as beneficial in the treatment of asthma.

Ellagic acid was isolated from *T. arjuna* and its antimutagenic potential was evaluated in TA98 and TA100 strains of *Salmonella typhimurium* against direct- and indirect-acting mutagens. It was found to be quite effective against promutagen 2-aminofluorene (Kandil and Nassar, 1998). Gallic acid, ethyl gallate, and luteolin were reported to be active cancer-cell growth inhibitory constituents from *T. arjuna* by Pettit and co-workers using bioassay-guided separation methods (Pettit *et al.,* 1996). In addition, it also had a significant hypocholesterolemic effect (Dwivedi and Agarwal, 1994). No metabolic, renal, and hepatic toxicity has been reported even when patients were administered *T. arjuna* for more than 24 months (Bharani *et al.,* 2002). In a double-blind, placebo-controlled crossover study (Bharani *et al.,* 1995) comparing *T. arjuna* in chronic stable angina patients, 58 male patients with

chronic stable angina with evidence of provocable ischemia on the treadmill exercise test received *T. arjuna* (500 mg), isosorbide mononitrate (40 mg/day) or a matching placebo for 1 week each, separated by a wash-out period of at least 3 days.

The patients underwent clinical, biochemical, and treadmill exercise evaluation at the end of each therapy; the scores were compared during the three therapy periods. *T. arjuna* therapy was associated with a significant decrease in the frequency of angina and need for isosorbide dinitrate. The treadmill exercise test parameters improved significantly during therapy with *T. arjuna* compared with those with placebo. The total duration of exercise increased, maximal ST depression during the longest equivalent stages of submaximal exercise decreased, time to recovery decreased, and higher double products were achieved during the *T. arjuna* therapy. *T. arjuna* was also reported to provide marked improvement in a case of Stokes-Adam's attack following chest pain after 3 months of therapy (Srivastava *et al.,* 1992). Methanol extract of *T. arjuna* was found to inhibit the growth of human normal fibroblasts (WI-38) *in vitro* without any effect on normal cells. A cylin-dependent kinase inhibitor, was induced in the transformed cell by *T.arjuna.* It is likely that *T. arjuna* has components that can inhibit of transformed cell by p53-dependent and independent pathways (Seth *et al.,* 1998).

2.1.2 Constituents of *Kigelia pinnata* (Balam Kheera)

Kigelia pinnata (Family: Bignoniaceae), colloquially called the Sausage Tree, or Worsboom, on account of its large fruits, has a variety of medicinal uses throughout Africa where it grows as an endemic species in many areas. Chemical examinations have resulted in the isolation of iridoids and naphtoquinoids as important secondary metabolites but flavonoids and lignans have also been isolated. The roots, the wood and the leaves have been investigated chemically. They contain naphthoquinones, dihydroisocoumarines, flavonoids and aldehydic iridoids. Among the naphthoquinones kigelinole, isokigelinole, pinnatal and isopinnatal were isolated (Akunyili *et al.,* 1991) from the root and its bark the usual plant substances stigmasterol, ß-sitosterol, ferulic acid, the naphthoquinones lapachol, 6-methoxymellein and two new phenolic compounds could be isolated. Kigelin is the main component of the plant (m.pt. 144^0 C, molecular formula $C_{12}H_{14}$). A minor one, (m.pt. 76-77^0C, molecular formula $C_{11}H_{12}O_4$) was commonly referred as 6-methoxymellein. In a special investigation, the attributes of two cyclopenta-c-pyran aldehydes were determined: 1) Sonovoburtinal, a yellow compound, subliming at ambient temperature, molecular formula $C_9H_6O_2$. 2) Pinnatal, a phenolic substance with aromatic protons. The molecular formula is $C_{20}H_{18}O_5$. Biogenetically pinnatal is probably formed by cyclisation of geranylquinone. The root bark and stem bark from plants collected in Zimbabwe were successively extracted in a soxhlet apparatus with different solvents. The isolation with thick layer chromatography (Kieselgel PF254, 0.75 mm) afforded four naphthoquinones: Kigelinol (1), Isokigelinol (2),

Isopinnatal (3) and 2-(1-hydroxyethyl)-naphtol (2, 3-b) furan-4, 9-quinone (4). They all were assessed for their biological activity (Moiden *et al.,* 1999). In the polar (methanolic) extract of the fruit from *K. africana* verminoside ($C_{24}H_{28}O_{13}$), an iridoid as a major constituent and among a series of polyphenols verbascoside could be isolated (Picerno *et al.,* 2005).

2.2.2 Biological activity of *Kigelia pinnata*

Investigation into the biological activity of *Kigelia pinnata* has focussed on its antibacterial activity and its cytotoxic effects against cancer cell lines. These are related to the traditional uses of bark and fruit extracts for treating diseases caused by micro-organisms and as a remedy for skin cancer. The iridoids and naphtoquinones have been shown to display antibacterial activity and also the ability to inhibit the growth of yeast. Although little ethnopharmacological evidence exists, the naphtoquinones are active against several protozoal species associated with disease. The compounds also show cytotoxicity against mammalian cell lines. Investigation into the biological activity of *Kigelia pinnata* has focussed on its antibacterial activity and its cytotoxic effects against cancer cell lines. Of particular interest is the use of the fruit to treat cancer and especially from Southern Africa where it has a considerable reputation for being effective against solar keratosis which may develop into skin cancer (Hutchings *et al*., 1996). In African herbal medicine, the fruit is believed to be a cure for a wide range of ailments, from rheumatism, snakebites, evil spirits, syphilis and even tornadoes. Chemical examinations have resulted in the isolation of iridoids and naphtoquinoids as important secondary metabolites but flavonoids and lignans have also been isolated.

Most of the studies on the biological activity of *Kigelia pinnata* extracts and constituents have been connected in some way to its traditional uses. In many parts of Africa the extracts of *Kigelia pinnata* bark has been used as a treatment for Sexually Transmitted Diseases (STDs). An unpublished ethnobotanical survey amongst traditional healers of the Ibos in south eastern Nigeria conducted by Dr. Akunyili *et al*., 1991 from University of Nigeria revealed that they used an aqueous or dilute alcohol extract of *Kigelia pinnata* roots as a treatment for STDs. Extracts of the roots equivalent to those obtained using the traditional methods were found to contain the iridoids, specioside and minecoside as major constituents. The extracts, as well as two of the isolated iridoids, were tested and also their 1/10 and 1/100 dilutions, against four representative species of bacteria viz. *Staphylococcus aureus, Bacillus subtilis, Escherrichia coli and Pseudomonas aeruginosa,* and the yeast *Candida albicans* in the absence and presence of the enzyme emulsin. This enzyme converts catalpol-type iridoids to their more antimicrobially active non-sugar containing aglycones. The growth of the organism in culture broth was assessed by measuring the turbidity of the solution. The results showed that the aqueous extract had strong activity, even in the absence of emulsion, against all the bacteria tested but especially against the yeast *C. albicans*.

2.1.3 Constituents of *Zingiber officinale* (Ginger)

It is known to contain volatile oils including borneol, camphene, citral, eucalyptol, linalool, phenllandrene, zingiberine and zingiberol phenols (gingerol, zingerone and shogaol) and resin. The famous American herbalist Michael Tierra describes ginger as 'spicy', warm and mainly affecting the stomach and lungs. Some of ginger's medicinal properties are contained in the chemicals responsible for the taste, the most noteworthy being gingerol and shogaol. The fragrance of ginger is due to the volatile oil, which is composed of about 200 chemical substances and accounts for approximately 1-2.5% of the rhizome. Nutrients include carbohydrates, lipids, proteins, minerals and vitamins. Among these may be found phosphorus, potassium, riboflavin and vitamin C. Finally, synergists include zingibain, a protein digesting enzyme that is known to act in a similar manner to bromelain in pineapple and capsaicin, limonene and curcumin. The later is the main active constituent in turmeric, which is closely related to ginger. It is clear that ginger contains a vast and complex array of chemicals that, in combination, provide a powerful aid to healing. For example, the enzyme zingibain is believed to improve digestion as well as kill parasites and their eggs. Furthermore, zingibain enhances antibacterial and anti-inflammatory actions and it is thought to assist other antibacterials, such as antibiotics, by up to 50%. Ginger's ability to reduce inflammation is due to its neutralising action upon free radicals, which are known to contribute to the problem. Finally, ginger contains over 12 antioxidant constituents, the combined actions of which have been regarded as being more powerful than vitamin C.

2.2.3 Biological activity of *Zingiber officinale*

α-shogaol, one of the active constituents of adraka showed triphasic effect on blood pressure in rats, an initial fall followed by marked pressure response, bradycardia and apnea. It induced contractile response in isolated guinea pig trachea at 100.0 μM and showed positive inotropic and chronotropic activities on isolated atria in rats at 3.6 M (Suekawa Mamoru, 1986) gingerols I and II from rhizomes potentiated contractions induced by prostanoids.

Inhibitory effects of several edible plant extracts against 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase, a rate-limiting enzyme in the biosynthesis of cholesterol, were screened. Inhibition rates of 10-15% were observed with hot water extracts of *Allium fistulosum, Allium sativum* and *Cucurbita maxima.* Methanol extracts of *Aster scaber, Allium sativum, Zingiber officinale, Oenanthe javanic* and *Angelica keiskei* effectively reduced the enzyme activity with inhibition rates of 29-51%. The methanol extract of *Angelica keiskei* was fractionated sequentially with chloroform, ethyl acetate and n-butanol. Of the fractions, the ethyl acetate fraction showed the highest inhibition against the enzyme. Luteolin-7-O-β-D-glucoside and hyperoside isolated from the ethyl acetate fraction

of *Angelica keiskei* inhibited the enzyme activity by 65.5% and 14.8%, respectively, at the concentration of 30 mM (Park, Jeong-Ro *et al.,* 1997).

In order to investigate the possible use of spices as natural preservatives, antimicrobial activities of garlic and ginger were examined. Distilled components of garlic and ginger were also analyzed. Garlic extracts suppressed growth of Gram-negative bacteria more effectively than they inhibited Gram-positive bacteria. Extracts of garlic suppressed growth of yeasts. The ether extract of garlic was especially active. The ether extract of ginger was also effective for growth inhibition of bacteria. Distilled components of garlic and ginger were extracted by using a simultaneous steam distillation and extraction apparatus. The concentrations were analyzed with GC/MSD and Kovat's retention index and 13 and 21 components, respectively, were identified.α-zingiberene, β-phellandrene, a-sesquiphell-andrene and camphene were major compounds in ginger (Park, Jeong-Ro *et.al*., 1997).

2.1.4 Constituents of *Foeniculum vulgare* (Fennel)

The chemical constituents of fennel can vary depending upon the species or subspecies. The primary constituent of interest is fennel's essential oil, which is upwards of 2-4% of the total constituents, comprised of the sweet-tasting transanethole (60-80%) and estragole (5-10%), as well as fenchone (5-7.5%), limonene, phelandrene, camphene and pinene. Other constituents include tannins, a fixed oil, stigmasterol and coumarins (Mills and Bone, 2000). Fruits contain 1.5–8.6% (usually 2–6%) volatile oil; 9–28% (usually 17–20%) fixed oil composed primarily of petroselinic acid (60–75%), oleic acid, and linoleic acid with a relatively high concentration of tocopherols (mostly γ-tocotrienol); flavonoids (mainly quercetin-3-glucuronide, rutin, isoquercitrin, and quercetin-3-arabinoside, with minor amounts of kaempferol-3-arabinoside and kaempferol-3-glucuronide); umbelliferone (7-hydroxycoumarin); hydroxyl cinnamic acid derivatives; stigmasterol; protein (16–20%); sugars; vitamins; minerals (relatively high in calcium and potassium); and others. Low concentrations of polyacetylenes were recently detected in the root. An antimicrobial phenyl propanoid (dillapional) was also isolated from the stem.

The volatile oil contains mostly *trans*-anethole (72–74%), with lesser amounts of fenchone (11–16%), estragole (methyl chavicol, 3–5%), limonene, camphene, and α-pinene. Other compounds present include more monoterpene hydrocarbons (β-pinene; α-thujene, α-fenchene, 3-carene, sabinene, α-phellandrene, myrcene, α- and β-terpinene, *cis*- and *trans*-ocimenes, terpinolene, and *p*-cymene), fenchyl alcohol, anisaldehyde, panisic acid, *trans*-l,8-terpin, myristicin, and apiole, the last two reportedly only present in the cultivated sweet variety.

2.2.4 Biological activity of *Foeniculum vulgare*

Essential oil and extracts of fennel have demonstrated antispasmodic activities in experimental models using isolated smooth muscle. This action appears to be related to an

effect upon calcium metabolism. Anethole is stated to be chemically similar to the catecholamines adrenaline, noradrenaline and dopamine, and as a result fennel can exert a sympathetic activity, such as bronchodilation, or as its Greek name suggests, weight loss. The activity of fennel upon pentobarbitone-induced digestive immotility in rabbits was reported to be active within 30 minutes, via cholinergic mechanisms rather than a direct stimulation of smooth muscle. In human clinical trials polyherbal formulations that include fennel as primary ingredient have been shown to exhibit a superior antispasmodic activity when compared to the drug metoclorapramide, improving symptoms of pain, nausea and heartburn. Similar studies on infantile colic have demonstrated that traditional herbal digestive aids that include fennel are superior to placebo (57% vs. 27%), and are well tolerated (Mills and Bone, 2000).

Fennel oil administered by inhalation has been shown to exhibit a mild antitussive effect on cough stimulated by mechanical methods in experimental animal models. Anethole and fenchone were shown to enhance the expulsion of respiratory tract fluid and displayed a mucolytic activity (Mills and Bone, 2000). Fennel oil exhibits an *in vitro* antibacterial activity comparable to many common antibiotics, such as penicillin and tetracycline, against a range of potential pathogens, including *Escherischia coli, Strepococcus pyogens, Staphylococcus aureus, Mycobacterium avium, Bacillis subtilis* and *Proteus vulgaris* (Mills and Bone, 2000). It is reported to exhibit a positive effect upon lactation, improving the volume of milk produced and its fat content in goats and mice. An injection of fennel oil in sexually immature and ovariectomized mice was shown to trigger the mating response, and has been observed to stimulate the growth of mammary glands and female reproductive tissues, and in general, enhanced the chance of conception. In male mice however, fennel oil was shown to exhibit an antiandrogenic activity, with an increase in the size of the seminal vesicles and prostate. The chemical similarity of anethole with dopamine may explain why fennel enhances lactation, competing for receptor sites and generally down-regulating the inhibitory activity of dopamine upon prolactin secretion (Mills and Bone, 2000).

2.1.5 Constituents of *Cuminum cyminum* (Cumin)

This small annual plant is native of the Mediterranean region where it is cultivated extensively. The cumin seeds are widely used in cooking. The dried seeds resemble those of caraway, but are straighter in form and have a coarser taste and odor (Evans *et al*., 1989). Major cumin seed producers include Egypt, Iran, India, and Morocco (Leung, 1980). The United States is among the largest producers of cumin oil. This spice should not be confused with sweet cumin, which is a common name for anise (*Pimpinella anisum*). Black cumin (*Bunium persicum*) has smaller and sweeter seeds than *C. cyminum* but is not commercially important. Another black cumin (*Nigella sativa*) is not related to cumin (Simon *et al.,* 1971-80).

Cumin seeds contain up to 5% of a volatile oil. In addition, the seeds yield about 22% fats, numerous free amino acids, and a variety of flavonoid glycosides, including derivatives of apigenin and luteolin (Leung, 1980; Ishikawa *et al.,* 2002; Takayanagi *et al*., 2003 and Kitajima *et al.,* 2003).The volatile oil is composed primarily of aldehydes (up to 60%). The cuminaldehyde content varies considerably, depending on the source of the oil (fresh vs ground seeds). Fine grinding of the seed can result in the loss of up to 50% of the volatile oil, (Leung, 1980) with the greatest loss occurring within 1 h of milling. Another major component of the oil is monoterpene hydrocarbons. Sesquiterpenes constitute minor constituents (Gagandeep *et al.,* 2003), (Takayanagi *et al.,* 2003).

2.2.5 Biological activity of *Cuminum cyminum*

Traditional uses of cumin include anti-inflammatory, diuretic, carminative, and antispasmodic. It has also been used as an aid for dyspepsia, jaundice, diarrhea, flatulence, and indigestion. Cumin powder has been used as a poultice and suppository, and has been smoked in a pipe and taken orally (Dhandapani *et al*., 2002; Ishikawa *et al.,* 2002; Gagandeep *et al.,* 2003; Platel and Srinivasan, 2004; Platel and Srinivasan, 2000; Srivastava, 1989). Cumin is a major component of curry and chili powders and is used to flavor a variety of commercial food products (Leung, 1980). The oil, derived by steam distillation, (Simon *et al.,* 1971-80) is used to flavor alcoholic beverages, desserts, and condiments. It has been used as a fragrant component of creams, lotions, and perfumes. In studies conducted on rats with induced diabetes, cumin reduced blood glucose levels (Roman-Ramos *et al.,* 1995), (Talpur *et al.,* 2005). One mechanism for this reduction suggests the inhibition of aldose reductase and alpha-glucosidase (Lee, 2005). In addition, reductions in plasma and tissue cholesterol, phospholipids, free fatty acids, and triglycerides (secondary to diabetes) were demonstrated in another animal study (Dhandapani *et al.,* 2002).

Cumin, given at a level 5 times higher than the usual human intake, did not reduce serum or liver cholesterol levels in rats fed a hypercholesterolemic diet (Sambaiah and Srinivasan, 1991). In mice, the spice appears to have an anticancer effect as demonstrated by the ability of cumin seeds to inhibit the induction of gastric squamous cell carcinomas (Gagandeep *et al*., 2003; Aruna and Sivaramakrishnan, 1992). In rats fed cumin, a protective effect against induced colonic cancer was demonstrated. Decreased beta-glucuronidase and mucinase activity was evident, and the rats had fewer papillae, no infiltration into the submucosa, and fewer morphological changes (Nalini *et al.,* 1998).Cumin seeds were not carcinogenic when tested by the reverse mutation *Salmonella typhimurium* (TA100) test but demonstrated very weak oxidative mutagenicity with strain TA102 (Al-Bataina *et al*., 2003; Sivaswamy *et al.,* 1991). Cumin contains the mutagen safrole, which is degraded by cooking (Al-Bataina *et al*., 2003).

Stimulation of bile acid secretion and pancreatic enzymes has been demonstrated in rats given a continuous intake of dietary cumin. Variable results were obtained with a single dose of cumin (Platel and Srinivasan, 2004; Platel and Srinivasan, 2000; Ramakrishna *et al*., 2003). Cumin (extract in ether) inhibited arachidonate-induced platelet aggregation in human platelets in a dose-dependent manner (Srivastava, 1989). Cumin oil and cuminaldehyde have been reported to exhibit strong larvicidal and antibacterial activity. At *in vitro* concentrations of 300 or 600 ppm, cumin oil inhibited the growth of *Lactobacillus plantarum* (Kivanc *et al.,* 1991).

Cumin essential oil demonstrated activity (reported to be comparable with standard antibiotics) against common human pathogens in vitro experiments (Singh *et al.,* 2002) and against gram-negative and gram-positive plant pathogens (Iacobellis and Cantore, 2005). An aqueous extract of cumin inhibited rat jejunal ATPase in an *in vitro* experiment (Kreydiyyeh *et al.,* 2000).

2.1.6 Constituents of *Trigonella foenum* (Fenugreek)

It contains simple alkaloids consisting mainly of trigonelline (up to 0.13%), choline (0.05%), gentianine and carpaine; much of the trigonelline is degraded during roasting to nicotinic acid and other pyridines and pyrroles, which probably account for much of the flavor of roasted fenugreek. Other constituents include saponins that yield on hydrolysis 0.6–1.7% steroid sapogenins consisting mainly of diosgenin and its isomer yamogenin usually in a 3:2 ratio, with tigogenin and neotigogenin also present; treatment of the seeds with enzymes before acid hydrolysis has increased the yield of diosgenin and yamogenin by 10–90%; yamogenin tetrosides B and C have been reported to be two of the glycosides (saponins) present. Three minor steroidal sapogenins also have been found in the seeds: smilagenin, sarsapogenin, and yuccagenin (Gupta *et al.,* 1986).Flavonoids, including vitexin, vitexin-7-glucoside, orientin arabinoside, homoorientin, saponaretin (isovit-exin), vicenin-1, vicenin-2, quercetin, luteolin, and vitexin cinnamate. Fixed oils (5–8%), which on extraction with fat solvents yield an extract with a strong odour; varying from fishy to nutty, depending on age of the extract. Fenugreek gel consists chiefly of galactomannans characterized by their high water-holding capacity. These galactomannans have a unique structure and may be responsible for some of the characteristic therapeutic properties attributed to fenugreek (Madar and Stark, 2002).

Considerable amount of mucilage, which appears to be mostly a galactomannan and is probably responsible for swelling of the seed in water (Gupta *et al.,* 1986). Protein (23–25%), which is low in *S*-amino acids but high in lysine and tryptophan; it has been suggested as a supplement of cereal proteins. Free amino acids, including (2*S*, 3*R*, 4*R*)-4-hydroxyisoleucine, histidine, lysine, and arginine, with the first one isolated at 0.09% yield as the major component (Adamska and Lutomski, 1971). Volatile components (more than 50), which include *n*-alkanes, sesquiterpenes, and oxygenated compounds (undecane to

hexadecane, elemenes, muurolenes, γ-nonalactone, 5-methyl-δ-caprolactone, etc.); and others. The leaves contain at least 7 saponins, known as graecunins. These compounds are glycosides of diosgenin. Seeds contain 0.1% to 0.9% diosgenin and are extracted on a commercial basis (Sauvaire and Baccou, 1978; Elujoba and Hardman, 1987). Plant tissue cultures from seeds grown under optimal conditions have been found to produce as much as 2% diosgenin with smaller amounts of gitongenin and trigogenin.

2.2.6 Biological activity of *Trigonella foenum*

Faecal bile acid and cholesterol excretion are increased by fenugreek administration. This may be secondary to a reaction between the bile acids and fenugreek-derived saponins causing the formation of micelles too large for the digestive tract to absorb. Another hypothesis attributes the cholesterol-lowering activities to the fiber-rich gum portion of the seed that reduces the rate of hepatic synthesis of cholesterol. It is likely that both mechanisms contribute to the overall effect. Studies have clearly demonstrated the cholesterol-lowering activity of fenugreek in animals. (Valette *et al.,* 1984; Singhal *et al.,* 1982; Stark and Madar, 1993; Sauvaire *et al.,* 1991). In a typical study, fractions of fenugreek seeds were added to the diets of diabetic hypercholesterolemic and normal dogs. The defatted fraction, which contains about 54% fiber and about 5% steroidal saponins, lowered plasma cholesterol, blood glucose, and plasma glucagon levels from pretreatment values in both groups of dogs (Valette *et al.,* 1984).The hypocholesterolemic effect has been reproduced in rats (Singhal *et al.,* 1982 and Yadav *et al.,* 2004). Administration of the fiber-rich fraction of fenugreek to diabetic rats lowered total cholesterol, triglycerides, and low density lipoprotein (LDL) (Hannan *et al.,* 2003).

The level of high density lipoprotein (HDL) was increased. Serum triglycerides were reduced from baseline in patients with newly-diagnosed, mild, type-2 diabetes mellitus who received a hydroalcoholic extract of fenugreek seeds 1 g/day (Gupta *et al.,* 2001). Total cholesterol and proportions of LDL and HDL fractions were not altered by treatment. A systematic review identified 5 other randomized clinical trials (N = 140) investigating the cholesterol-lowering effects of fenugreek seeds (Thompson Coon and Ernst, 2003). Reductions (15 to 33%) of serum cholesterol from baseline were reported in all the trials identified. One small study using an aqueous extract of fenugreek leaves in healthy volunteers showed cholesterol reductions compared with control subjects after a single dose. Dose-dependent hypocholesterolemic effects of germinated fenugreek seeds also have been demonstrated (Sowmya and Rajyalkshmi, 1999).

Total serum cholesterol and LDL cholesterol were reduced; while HDL cholesterol remained unchanged. The galactomannan-rich soluble fiber fraction of fenugreek may be responsible for the antidiabetic activity of the seeds. Insulinotrophic and antidiabetic properties also have been associated with the amino acid 4-hydroxyisoleucine that occurs in

fenugreek at a concentration of about 0.55%. In vitro studies have indicated that this amino acid causes direct pancreatic β-cell stimulation. Delayed gastric emptying and inhibition of glucose transport also have been postulated as possible mechanisms. Multiple studies have been undertaken to demonstrate the glucose-lowering effects of fenugreek (Khosla *et al.,* 1995; Sharma *et al.,* 1990; Madar *et al.,* 1988; Ajabnoor and Tilmisany, 1988). A typical study evaluated the hypoglycemic effects of the seeds in dogs. The defatted fraction of the seeds lowered blood glucose levels, plasma glucagons, and somatostatin levels; carbohydrate-induced hyperglycemia also was reduced (Ribes *et al.,* 1984).

Glycemic control was improved in a small study of patients with mild type-2 diabetes mellitus. A reduction in glycosylated hemoglobin (HbA) levels and increased insulin sensitivity were observed in fenugreek recipients. The preparation was well tolerated, with no patients withdrawing from the study because of adverse effects. Patients receiving the fenugreek preparation also were allowed to receive adjuvant antidiabetic preparations if required; caution is advised in the interpretation of these results. Rats treated with a single dose of fenugreek extract 100 or 200 mg/kg showed a dose-related response when treated with carragenin. Inhibition of inflammatory swelling was 45% and 62% in the lower and higher dose groups, respectively, compared with 100% in untreated animals (Sur *et al.,* 2001).

A French patent has been granted to a product purported to have antitumor activity, especially against fibromas. The product contains extracts of several herbal products, including fenugreek. Pretreatment with a fenugreek extract was found to enhance macrophage cell counts in rats. When these rats were subsequently inoculated with tumor cells, tumor cell growth was inhibited. Simultaneous administration of an aqueous extract of fenugreek seeds with ethanol prevented the harmful effects of alcohol on lipid peroxidation and enzyme markers of hepatotoxicity (Thirunavukkarasu *et al.,* 2003). Histopathological examination of liver and brain confirmed these findings, indicating that fenugreek could offer some protection against ethanol toxicity. The seeds are rich in protein, and the plant is grown as animal forage. Diosgenin, a precursor used in commercial steroid synthesis, is extracted from the seeds. The remaining residue is rich in nitrogen and potassium and is used as an agricultural fertilizer. Because the seeds contain up to 50% of mucilaginous fiber, they have been used in the preparation of topical poultices and emollients; internally this ability to swell in volume has been utilized to relieve constipation and diarrhea.

Reduction in cataract incidence has been demonstrated in diabetic rats receiving an extract of fenugreek seeds and leaves (Vats *et al.,* 2004). After 115 days of treatment, cataracts were diagnosed in 25% of fenugreek recipients compared with 100% of diabetic controls. Oral administration of fenugreek seed fractions resulted in dose-dependent gastric protection against the effects of ethanol (a necrotizing agent) (Pandian *et al.,* 2002).The seeds were as effective as omeprazole, a clinically-recognized antiulcer agent. Ulcer scores

indicated that the soluble gel fraction was more effective than the aqueous extract or omeprazole.

Chapter-III

MATERIALS AND METHODS

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

3.1 Research material

3.1.1 Plant materials

The bark of *T. arjuna* and fruit of *K. pinnata* were procured from the university campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar. While fruit of *F. vulgare, C. cyminum;* rhizome of *Z. officinale* and seed of *T. foenum* were purchased from the local market of Hisar.

The plant materials were extracted with chloroform, methanol and water, separately. The plant materials were refluxed with the solvents for six hours each in round bottom flask and the process was repeated thrice to ensure complete extraction. These extracts were filtered and concentrated under reduced pressure. The total phenols, flavonoids, mineral contents and antioxidant activity of these extracts were then determined using various methods.

3.1.2 Chemicals

The commercially available chemicals of high purity were procured from Sigma-Aldrich, Qualigens, Merck and Ranbaxy, (LR grade) and used for various experimental procedures.

3.2 Extraction of plant materials

- Samples (100 g) of each plant material (bark of arjuna, fruit of balam kheera, rhizome of ginger and seeds of fennel, cumin and fenugreek) have been extracted separately with chloroform, methanol and distilled water.
- The plant materials were refluxed with the solvents for six hours each in round bottom flask.
- The process was repeated thrice to ensure complete extraction.
- These extracts were filtered, concentrated under reduced pressure and used for estimation of following parameters:
    1. Total phenols
    2. Flavonoids
    3. Minerals
    4. Antioxidant activity and antiradical activity by

a) 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

b) Ferric thiocyanate (FTC) method

c) β-carotene bleaching method (BCBT)

3.3 Determination of various parameters

3.3.1 Determination of total phenols

Reagent

Gallic acid

Methanol

Sodium carbonate (20% w/v)

Preparation of Folin-Ciocalteu reagent

A mixture containing 100 gm of sodium tungstate (Na_2WO_4. $2H_2O$), 25 gm of sodium molybedate ($Na_2MoO_4.2H_2O$). 700 ml of water, 50 ml of 85% phosphoric acid and 100 ml concentrated HCl in 1.5 L flask was refluxed gently for 10 h. Then 150 g of lithium sulphate, 50 ml of water and a few drops of bromine water were added. The mixture was boiled for 15 minutes without condenser in order to remove excess of bromine, cooled, diluted to 1 L by distilled water and filtered. The acid concentration of the reagent was determined by titration with 1N NaOH using phenolphthalein indicator.

Method

The total phenols 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 0.01, 0.02, 0.04, 0.06 and 0.08 mgL^{-1} of gallic acid. A 1.0 ml of diluted extract (all fraction were diluted with methanol to adjust the absorbance within the calibration limits), 1.0 ml of 1M Folin–ciocalteau reagent and 2.0 ml of Na_2CO_3 (20% w/v) were mixed and the volume was made to 10 ml. After 8 minutes, the mixture was centrifuged at 6000 rpm for 10 minutes. Then the absorbance of supernatant solution was measured at 730 nm using Spectronic 20 spectrophotometer against a blank prepared similarly but containing distilled water instead of extract. The concentration of phenols thus obtained was multiplied by the dilution factor and the results were expressed as equivalent to milligrams of gallic acid per gram of extract (mg GAE/g).

3.3.2 Determination of flavonoids

Reagents

Catechin

5% $NaNO_2$

10% $AlCl_3$

1M NaOH

Method

The aluminium chloride colorimetric assay, as described by Marinova *et al*., 2005 was used for the determination of flavonoid content. Briefly, 1 ml of each extract and standard solution of catechin (0.20, 0.40, 0.60, 0.80 and 1.0 mgL^{-1}) was added to test tubes containing 4 ml of double distilled water. To the mixture was added 0.3 ml 5% $NaNO_2$. After 5 minute, 0.3 ml 10% $AlCl_3$ was added. Immediately, 2 ml, 1 M NaOH was added and the total volume was made upto 10 ml 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. Total flavonoid content was expressed as mg catechin equivalents.

3.3.3 Determination of minerals

Reagent

Perchloric acid

Nitric acid

Method

The sample was digested by wet oxidation. Each extract of 0.5 g was transferred to a conical flask, containing a mixture of 1 ml of perchloric acid and 5 ml nitric acid, mixed well and kept overnight at room temperature followed by digestion first on low temperature 70-80ºC and then at higher temperature until the volume of the solution was reduced to about 1ml. After the digestion, the volume of solution was made to 10 ml with distilled water and analyzed by using atomic absorption spectrometer (AAS).

3.3.4 Antioxidant Activity

3.3.4.1 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

Reagents

DPPH; 0.025gL^{-1} in 50% ethanol

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 517 nm disappears because the DPPH radical is scavenged. On the basis of this principle, the radical scavenging effect of each fraction was measured. Briefly, 0.1 ml of each extract (diluted with distilled water and centrifuged) was added to 2.46 ml of 2, 2'-diphenyl-1-picrylhydrazyl radical (DPPH; 0.025gL^{-1} in 50% ethanol) and mixed thoroughly on vortex mixture for 5 minutes. The absorbance of the sample was measured at 517 nm at every 1 minute for 5 minutes using the spectrophotometer. For each sample, three separate determinations were

carried out. The antioxidant activity was expressed as the percentage of decline of the absorbance after 1 minute, 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:

$$\%DPPH_{sc} = (A_{cont} - A_{samp}) \times 100/A_{cont}$$

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

3.3.4.2 Ferric thiocyanate (FTC) method

Reagents

2.51% (w/v) Linoleic acid in ethanol

30% (w/v) Ammonium thiocyanate

0.02 M Ferrous chloride in 3.5% (v/v) hydrochloric acid

75% Ethanol

Solution of 0.05 M of phosphate buffer (pH 7.0): 0.2 M solution of monobasic sodium phosphate was prepared by dissolving 31.2 g of $NaH_2PO_4.2H_2O$ in 1000 ml of water and 0.2 M solution of dibasic sodium phosphate was prepared by dissolving 71.7 g of $Na_2HPO_4.12H_2O$ in 1000 ml of water. Then 39.0 ml of 0.2M solution of monobasic sodium phosphate and 61.0 ml of 0.2 M solution of dibasic sodium phosphate were mixed and diluted to a total volume 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 (0.28 g), Tween 20 (0.28 g) and phosphate buffer (50 ml, 0.2 M, pH 7.0). Test samples were prepared in ethanol-water (6:4 v/v). Different test samples (0.5 ml each) were mixed with 2.5 ml of linoleic acid emulsion and 2.5 ml of phosphate buffer (0.2 M, pH 7.0) and incubated at 37ºC for 120 h (5 days). The mixture prepared as above without any test sample served as control. Aliquots (0.1ml) were drawn from the incubation mixture at intervals of 24 h and mixed with 5.0 ml of 75% ethanol, 0.1 ml of 300 gL^{-1} of ammonium thiocyanate and 0.1 ml of 20 mM ferrous chloride in 35 gL^{-1} HCl. After precisely 3 minute the absorbance at 500 nm was recorded. This method depends on peroxide formation in the aqueous emulsion of linoleic acid. In this method, the higher the increase in absorbance, more is the concentration of peroxide formed and hence, lower the antioxidant activity of the sample analysed.

Calculation

Antioxidant activity was expressed as

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

3.3.4.3 β-carotene bleaching method (BCBT)

Reagents

β-carotene

Linoleic acid

Tween 20

Chloroform

Butylated hydroxytoluene

Method

The β-carotene bleaching method of Hidalgo *et al*., 1994 was used to evaluate the antioxidant activity of the extract. β-carotene (0.2 mg), linoleic acid (20 mg) and tween 20 (200 mg) were mixed in 0.5 ml of chloroform. The solvent was subsequently evaporated at 40ºC on a vaccum evaporator and the mixture was diluted with 50 ml of triply distilled water. Aliquots (4 ml) of this emulsion were transferred into test tubes and then added 0.2 ml of aliquots of test samples in ethanol. Butylated hydroxytoluene (BHT) was used for comparative purposes. A control containing 0.2 ml of ethanol and 4 ml 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 470 nm was recorded using a Spectronic 20 spectrophotometer at the intervals of 30 minutes, 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

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

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.

Statistical analysis

Data obtained were expressed as means ± standard deviation and presented through bar charts and tables.

Chapter-IV

RESULTS

The evaluation of total phenols, flavonoids, minerals and antioxidant activity of different extracts from the six plants grown in Haryana has been undertaken by using three testing methods: β-carotene bleaching method (BCBT); 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method and ferric thiocyanate (FTC) method.

The extracts were obtained by three different solvents i.e. chloroform, methanol and water and the results obtained from the present investigations have been presented for medicinal plants and spices under the following headings:

4.1 Determination of total phenols

4.2 Determination of flavonoids

4.3 Determination of minerals

4.3.1 Copper

4.3.2 Manganese

4.3.3 Zinc

4.3.4 Iron

4.4 Anti-oxidant and antiradical activity of extracts

4.4.1 Anti-oxidant activity of extracts by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT)

4.4.2 Antiradical activity of extracts by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

Table 1: The medicinal plants and spices selected for study

Common name	Scientific name	Family	Part Used
Balam Kheera or Sausage tree	*Kigelia pinnata*	Bignoniaceae	Fruit
Arjuna	*Terminalia arjuna*	Combretaceae	Bark
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
Fennel	*Foeniculum vulgare*	Umbelliferae	Fruit
Cumin or Zeera	*Cuminum cyminum*	Umbelliferae	Fruit
Fenugreek or Methi	*Trigonella foenum*	Fabaceae	Seed

4.1 Determination of total phenols

Total phenolic content of the plants varied greatly. The results varied from 1.0 mg GAE/g (methanol extract of *T. foenum*) to 27.0 mg GAE/g (water extract of *F. vulgare*) of extract when Folin Cio-calteu method was used. A standard curve using gallic acid as standard was prepared for the determination of phenolic content of extracts.

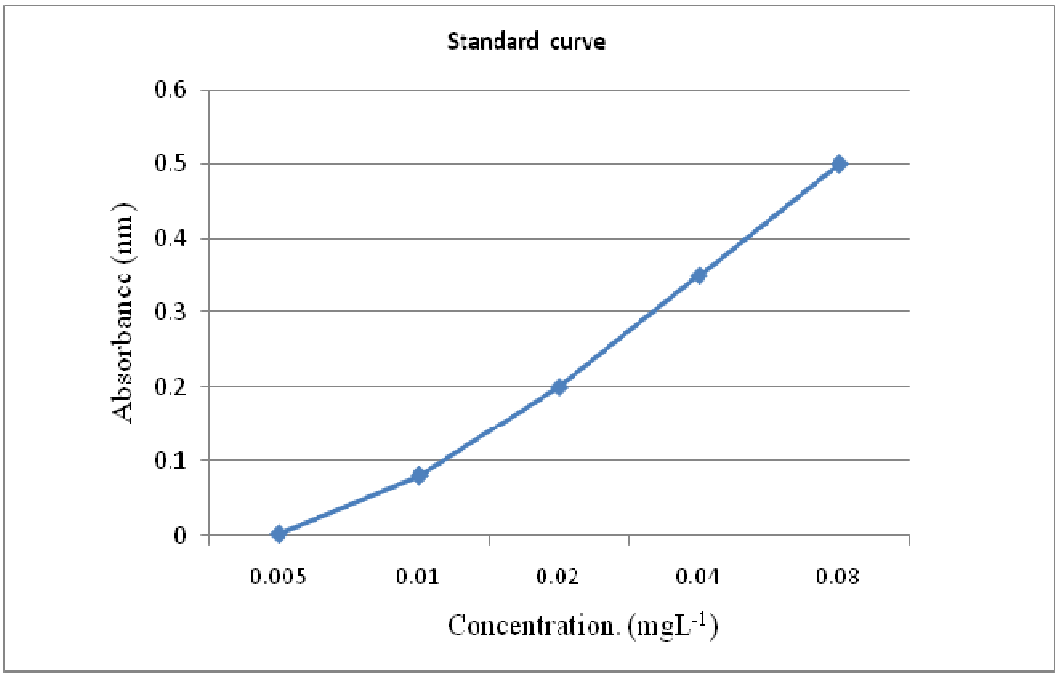

Figure 1: Standard curve for the determination of phenol content by using gallic acid as standard

Table 2: Total phenols (mg GAE/g)* in different extracts

Total Phenols (mg GAE/g ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	15.0±0.05	16.0±0.16	15.0±0.25
Kigelia pinnata (Balam Kheera)	8.0±0.09	6.0±0.14	4.0±0.25
Zingiber officinale (Ginger)	22.5±0.67	16.5±0.31	11.0±0.26
Foeniculum vulgare (Fennel)	4.0±0.32	24.0±0.23	27.0±0.15
Cuminum cyminum (Cumin)	18.0±0.12	19.0±0.21	18.0±0.17
Trigonella foenum (Methi)	1.5±0.26	1.0±0.19	22.0±0.36

***The values represent the average of three replicates.**

In *T. arjuna,* the maximum amount of phenol was present in the methanol extract (16.0 mg GAE/g), whereas the chloroform extract and water extract contained same amount of phenol (15.0 mg GAE/g). In *K. pinnata,* the maximum amount of phenol was found in chloroform extract (8.0 mg GAE/g) followed by methanol extract (6.0 mg GAE/g) and minimum amount of phenol was found in water extract (4.0 mg GAE/g). In *Z. officinale,* the maximum amount of phenol was found in chloroform extract (22.5 mg GAE/g) followed by

methanol extract (16.5 mg GAE/g), followed by water extract (11.0 mg GAE/g) and in *F. vulgare* maximum amount of phenol was present in water extract (27.0 mg GAE/g) followed by methanol extract (24.0 mg GAE/g) and minimum of phenol was found in chloroform extract (4.0 mg GAE/g). Maximum amount of phenol was present in methanol extract (19.0 mg GAE/g) of *C. cyminum* followed by chloroform extract and water extract (18.0 mg GAE/g). Whereas in *T. foenum* maximum amount of phenol was present in water extract (22.0 mg GAE/g) followed by chloroform extract (1.5 mg GAE/g) and minimum of phenol was found in methanol extract (1.0 mg GAE/g).

The following trend was observed in the phenol content of chloroform extract of plants as clear from Table 2 and Figures 2.

Zingiber officinale (mg GAE/g) (22.5) > *Cuminum cyminum* (18.0) > *Terminalia arjuna* (15.0) > *Kigelia pinnata* (8.0) > *Foeniculum vulgare* (4.0) > *Trigonella foenum* (1.5)

Thus, the maximum amount of phenol was present in *Z. officinale* i.e. 22.5 mg GAE/g and minimum in *T. foenum* i.e.1.5 mg GAE/g of the extract.

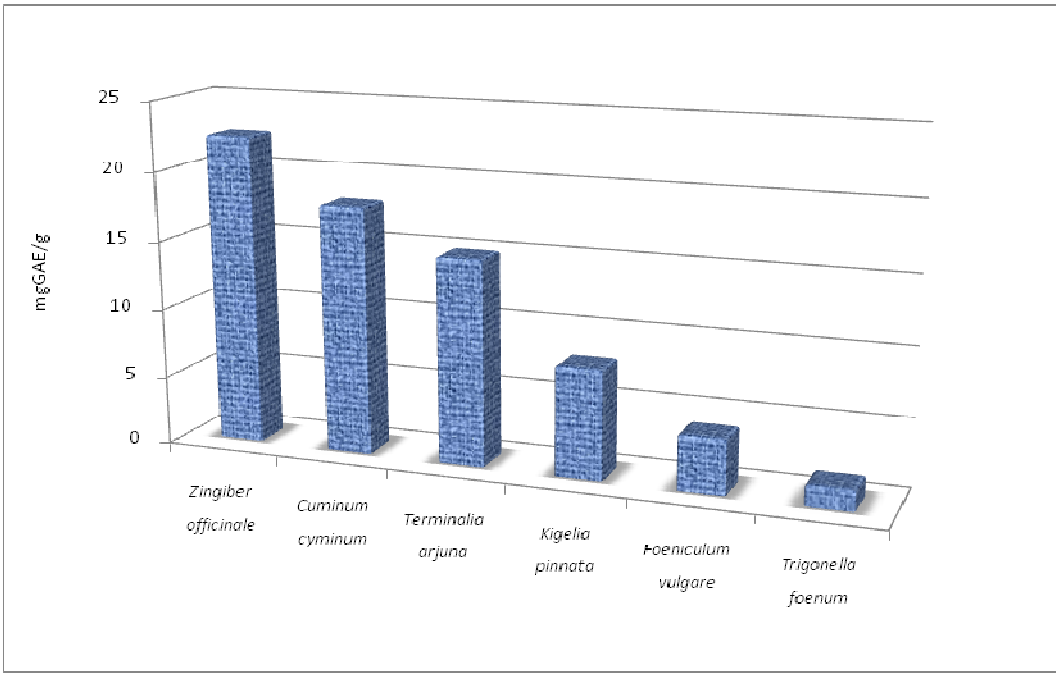

Figure 2: Total phenols in chloroform extract

In methanol extract, maximum amount of phenol was present in *F. vulgare* i.e.24.0 mg GAE/g of the extract and minimum in *T. foenum* i.e.1.0 mg GAE/g of the extract as shown in Table 2 and Figure 3.The following trend was observed in the phenol content:

Foeniculum vulgare (mg GAE/g) (24.0) > *Cuminum cyminum* (19.0) > *Zingiber officinale* (16.5) > *Terminalia arjuna* (16.0) > *Kigelia pinnata* (6.0) > *Trigonella foenum* (1.0)

Whereas in water extract, the maximum amount of phenol was present in *F. vulgare* i.e.27.0 mg GAE/g of the extract and minimum in *K. pinnata* i.e. 4.0 mg GAE/g of the extract as clear from Table 2 and Figure 4. The following trend was observed in the phenol content: *Foeniculum vulgare* (mg GAE/g) (27.0) > *Trigonella foenum* (22.0) > *Cuminum cyminum* (18.0) > *Terminalia arjuna* (15.0) > *Zingiber officinale* (11.0) > *Kigelia pinnata* (4.0)

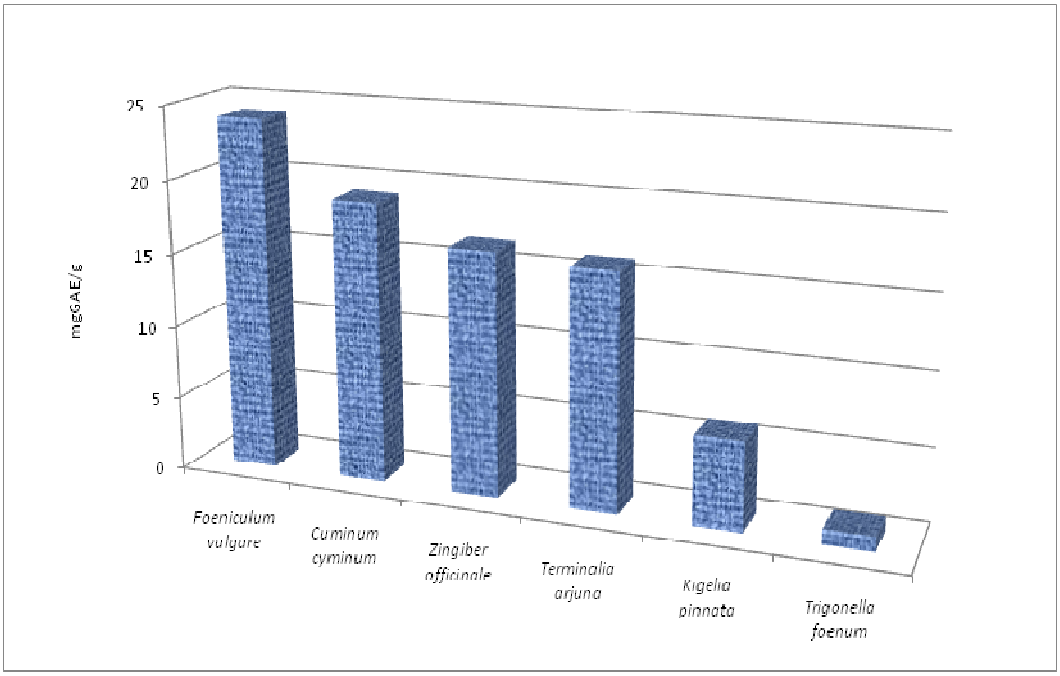

Figure 3: Total phenols in methanol extract

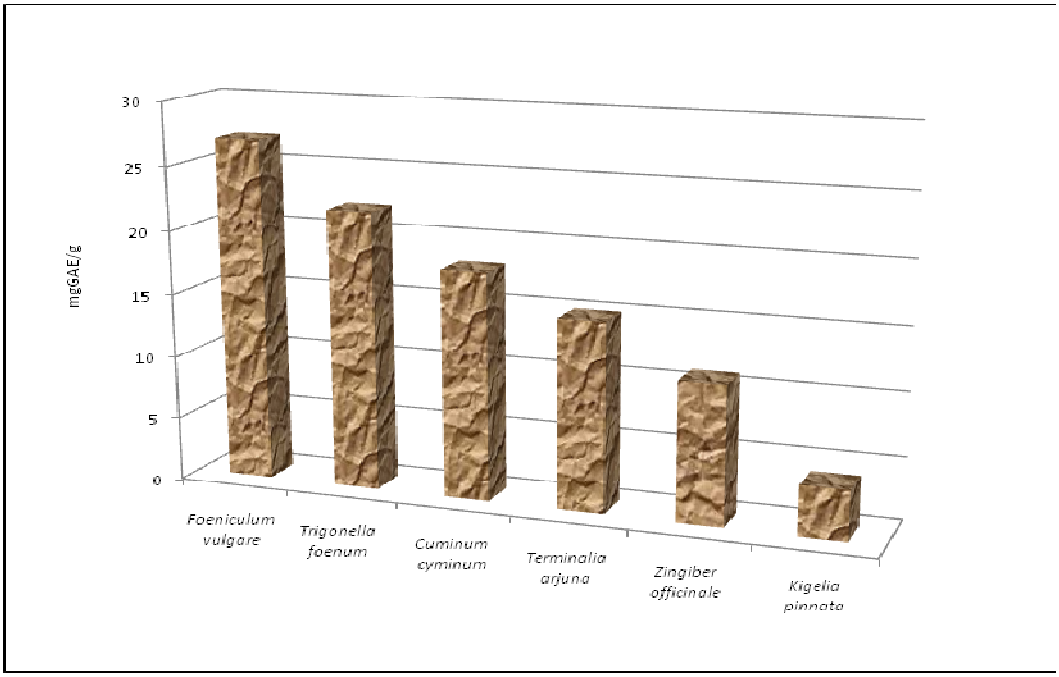

Figure 4: Total phenols in water extract

In Figure 5, comparison of the phenol contents have been depicted in all the three solvents i.e. chloroform, methanol and water.

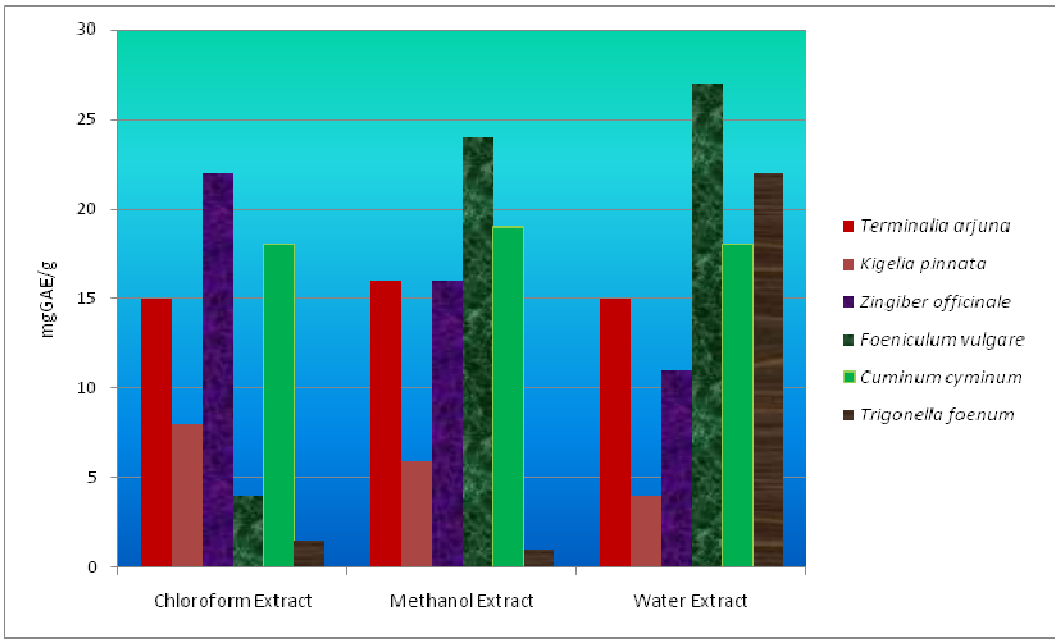

Figure 5: Total phenols in different extracts

4.2 Determination of flavonoids

The flavonoids were measured by aluminium chloride colorimetric method described by Marinova *et al*., 2005. Maximum amount of flavonoids were present in chloroform extract of *Z. officinale* and water extract of *K. pinnata* i.e. 60.0 mg CE/g of the extract and minimum in chloroform extract of *T. foenum* i.e. 1.5 mg CE/g of the extract as clear from Table 3.

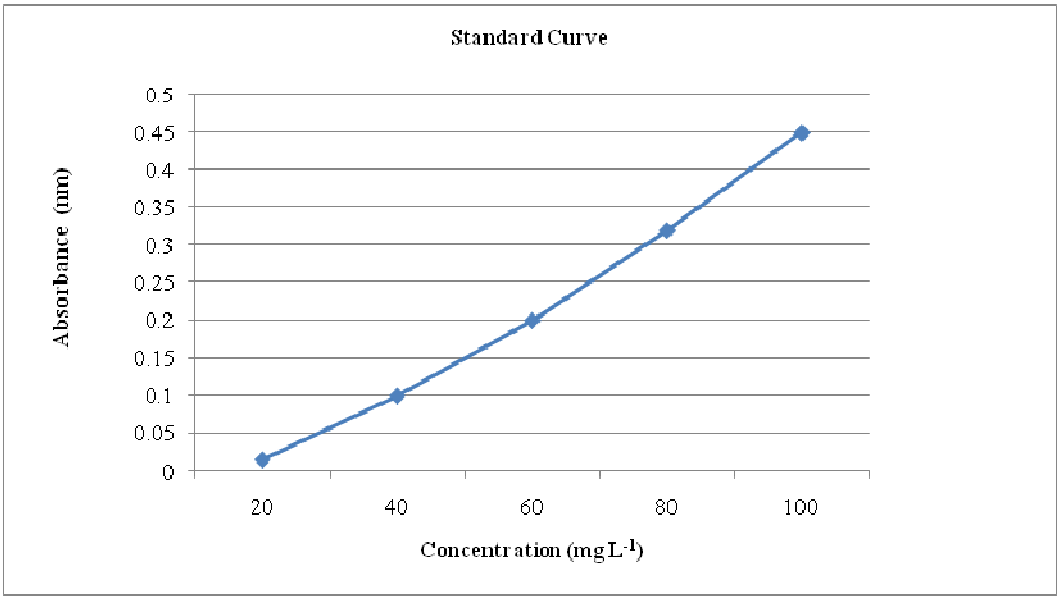

Figure 6: Standard curve for the determination of flavonoids by using catechin as standard

In *K. pinnata* maximum amount of flavonoids were detected in water extract (60.0 mg CE/g) followed by chloroform extract (10.0 mg CE/g) and minimum amount of flavonoids were found in methanol extract (9.5 mg CE/g). Whereas in *T. arjuna* maximum amount of flavonoids were detected in methanol extract (35.0 mg CE/g) followed by water extract (25.0 mg CE/g) and minimum of flavonoids were found in chloroform extract (3.0 mg CE/g). In *Z. officinale,* maximum amount of flavonoid was detected in chloroform extract (60.0 mg CE/g) followed by methanol extract (42.0 mg CE/g) and minimum of flavonoids were found in water extract (5.0 mg CE/g) and in *T. foenum* maximum amount of flavonoids were present in water extract (18.0 mg CE/g) followed by methanol extract (8.0 mg CE/g) and minimum of flavonoids werefound in chloroform extract (1.5 mg CE/g). In *C. cyminum,* maximum amount of flavonoid was detected in water extract (50.0 mg CE/g) of followed by methanol extract (22.0 mg CE/g) and minimum of flavonoids were found in chloroform extract (8.0 mg CE/g) whereas in *F. vulgare* maximum amount of flavonoids were detected in water extract (19.0 mg CE/g) followed by methanol extract (8.0 mg CE/g) and minimum of flavonoids were found in chloroform extract (3.0 mg CE/g) as clear from Table 3.

Table 3: Flavonoids (mg CE/g)* in different extracts

Flavonoids (mg CE/g ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	3.0±0.26	35.0±0.16	25.0±0.27
Kigelia pinnata (Balam Kheera)	10.0±0.13	9.5±0.13	60.0±0.26
Zingiber officinale (Ginger)	60.0±0.28	42.0±0.13	5.0±0.28
Foeniculum vulgare (Fennel)	3.0±0.45	8.0±0.32	19.0±0.37
Cuminum cyminum (Cumin)	8.0±0.31	22.0±0.27	50.0±0.38
Trigonella foenum (Methi)	1.5±0.32	8.0±0.25	18.0±0.29

***The values represent the average of three replicates.**

In chloroform extract, maximum amount of flavonoid was detected in *Z. officinale* i.e. 60.0 mg CE/g of the extract followed by *K. pinnata* (10.0 mg CE/g) followed by *C. cyminum* (8.0 mg CE/g) then *T. arjuna* and *F. vulgare* (3.0 mg CE/g); and minimum in *T. foenum* i.e. 1.5 mg CE/g of the extract as shown in Table 3 and Figure 7.

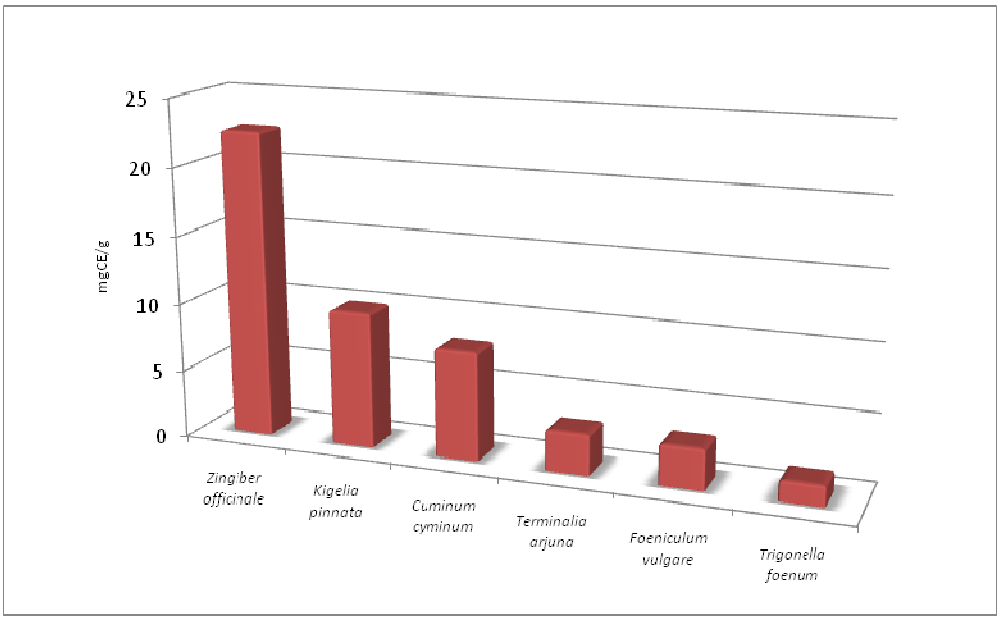

Figure 7: Flavonoids in chloroform extract

In methanol extract, maximum amount of flavonoid was detected in *Z. officinale* i.e. 42.0 mg CE/g of the extract and minimum amount of 8.0 mg CE/g of the extract in *T. foenum* and *F. vulgare* were at par as can be seen from Table 3 and Figure 8. The following trend was observed in the flavonoid content:

Z. officinale (mg CE/g) (42.0) > *T. arjuna* (35.0) > *C. cyminum* (22.0) > *K. pinnata* (9.5) > *F. vulgare* (8.0) = *T. foenum* (8.0)

In water extract, maximum amount of flavonoid was detected in *K. pinnata* i.e.60.0 mg CE/g of the extract and minimum in *Z. officinale* i.e.5.0 mg CE/g of the extract as can be seen from Table 3 and Figure 9. The following trend was observed in the flavonoid content:

K. pinnata (mg CE/g) (60.0) > *C. cyminum* (50.0) > *T. arjuna* (25.0) > *F. vulgare* (19.0) > *T. foenum* (18.0) > *Z. officinale* (5.0)

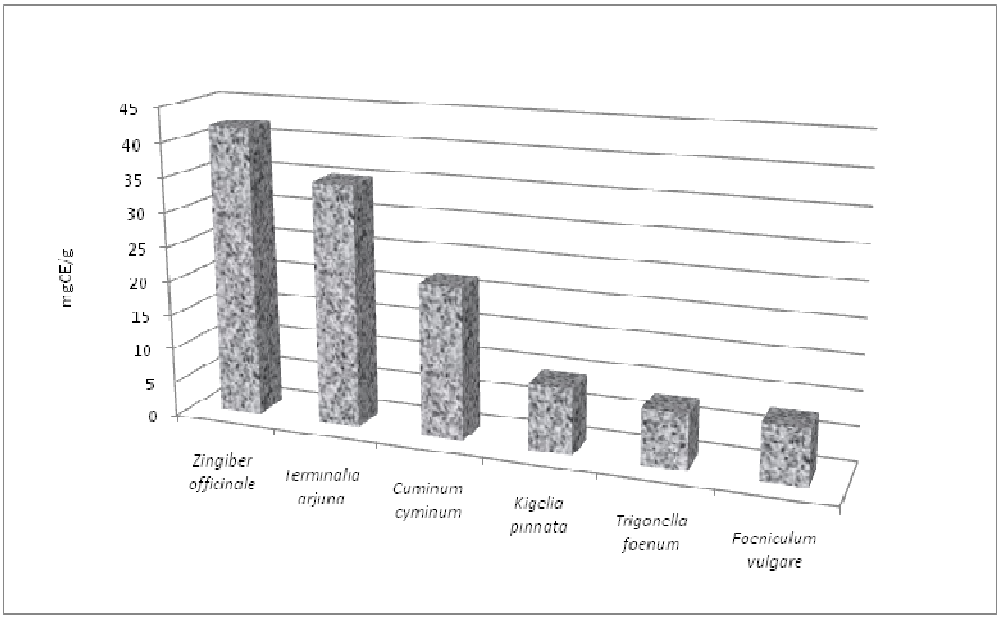

Figure 8: Flavonoids in methanol extract

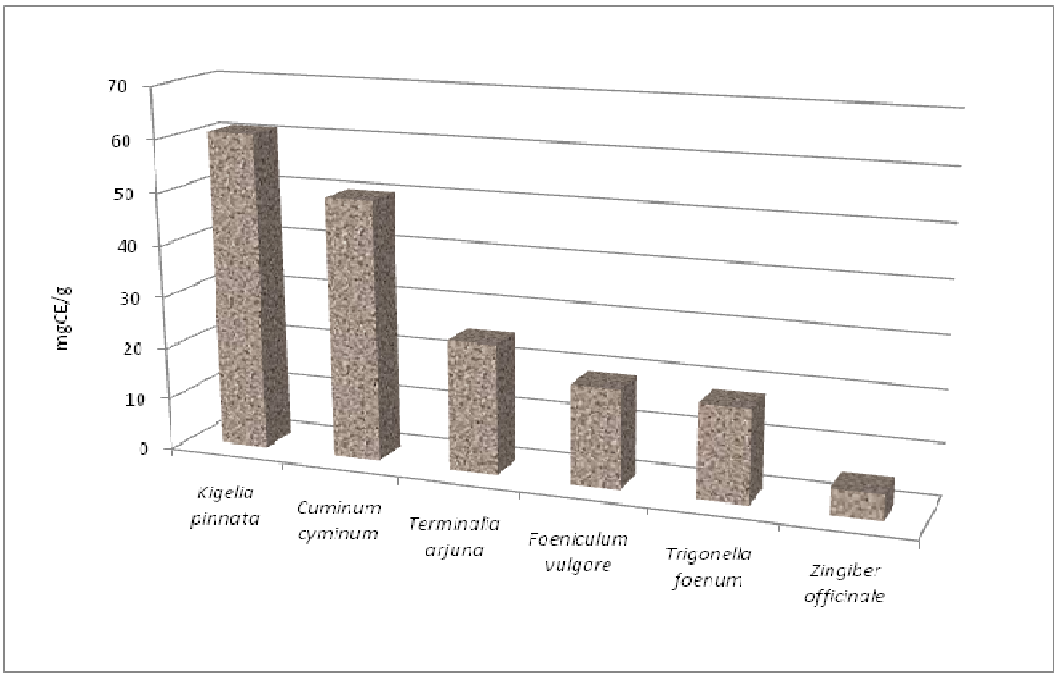

Figure 9: Flavonoids in water extract

In Figure 10, comparison of the flavonoid content have been depicted in all the three solvents i.e. chloroform, methanol and water.

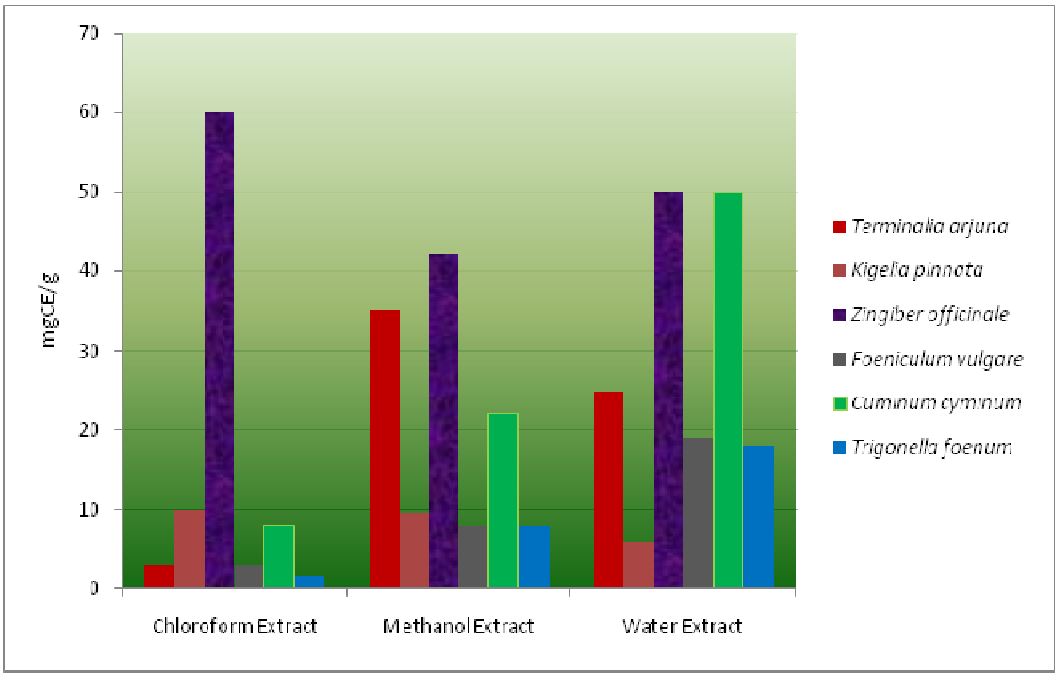

Figure 10: Flavonoids (mg CE/g) in different extracts

4.3 Determination of minerals

The minerals were determined by using Atomic Absorption Spectrophotometer. Following four minerals were detected:

4.3.1 Copper

4.3.2 Manganese

4.3.3 Zinc

4.3.4 Iron

The results show that maximum amount of copper, manganese, iron and zinc was present in water extract followed by methanol extract and minimum in chloroform extract.

Table 4: Copper content (ppm)* in different extracts

Copper content (ppm ± SD)			
Plants	**Chloroform extract ± S.D**	**Methanol extract ± S.D**	**Water extract ± S.D**
Terminalia arjuna (Arjuna)	1.0±0.15	2.0±0.24	4.0±0.45
Kigelia pinnata (Balam Kheera)	2.0±0.14	6.0±0.19	16.0±0.34
Zingiber officinale (Ginger)	2.0±0.16	14.0±0.26	20.0±0.44
Foeniculum vulgare (Fennel)	2.0±0.32	14.0±0.35	6.0±0.11
Cuminum cyminum (Cumin)	2.0±0.26	4.0±0.22	8.0±0.14
Trigonella foenum (Methi)	2.0±0.25	8.0±0.29	10.0±0.21

***The values represent the average of three replicates.**

Maximum amount of **c**opper was detected in water extract of *Z. officinale* (100.0 ppm) and minimum amount in chloroform extract of *T. arjuna* (1.0 ppm).

In chloroform extract, comparable amount of copper was found in five plants *Z. officinale*, *C. cyminum*, *T. foenum*, *K. pinnata* and *F. vulgare* i.e. nearly 2.0 ppm whereas *T. arjuna* contained less amount about 1.0 ppm as shown in Table 4 and Figure 11.

In methanol extract, significant amount of copper was found in *F. vulgare* and *Z. officinale* (14.0 ppm) while meager amount was found in rest of the four plants i.e. *T. foenum* (8.0ppm), *K. pinnata* (6.0 ppm) followed by *C. cyminum* (4.0 ppm) and minimum in *T. arjuna* (2.0 ppm) as shown in Table 4 and Figure 12.

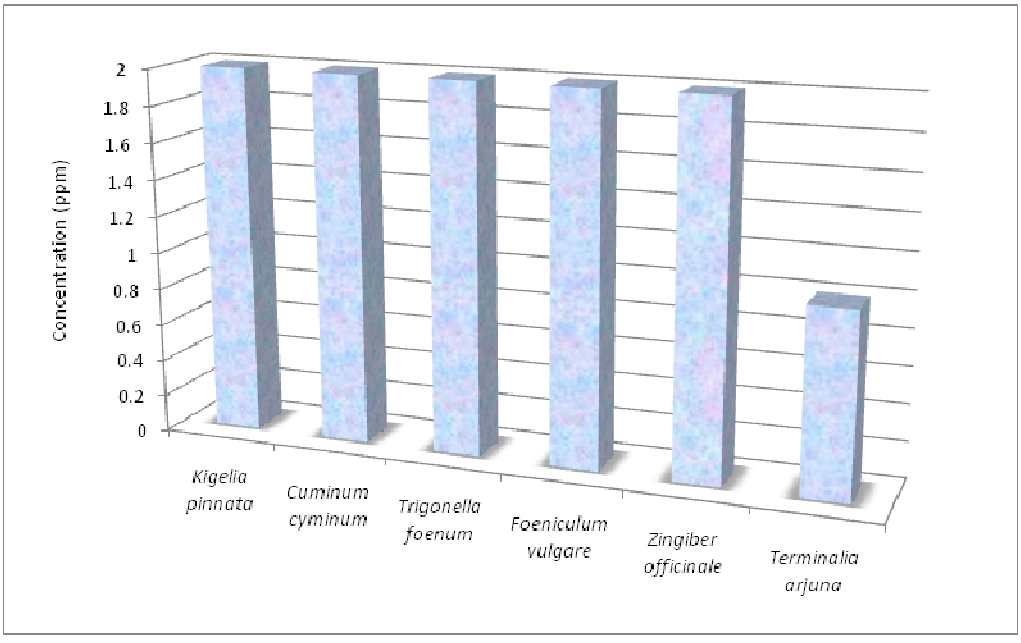

Figure 11: Copper content in chloroform extract

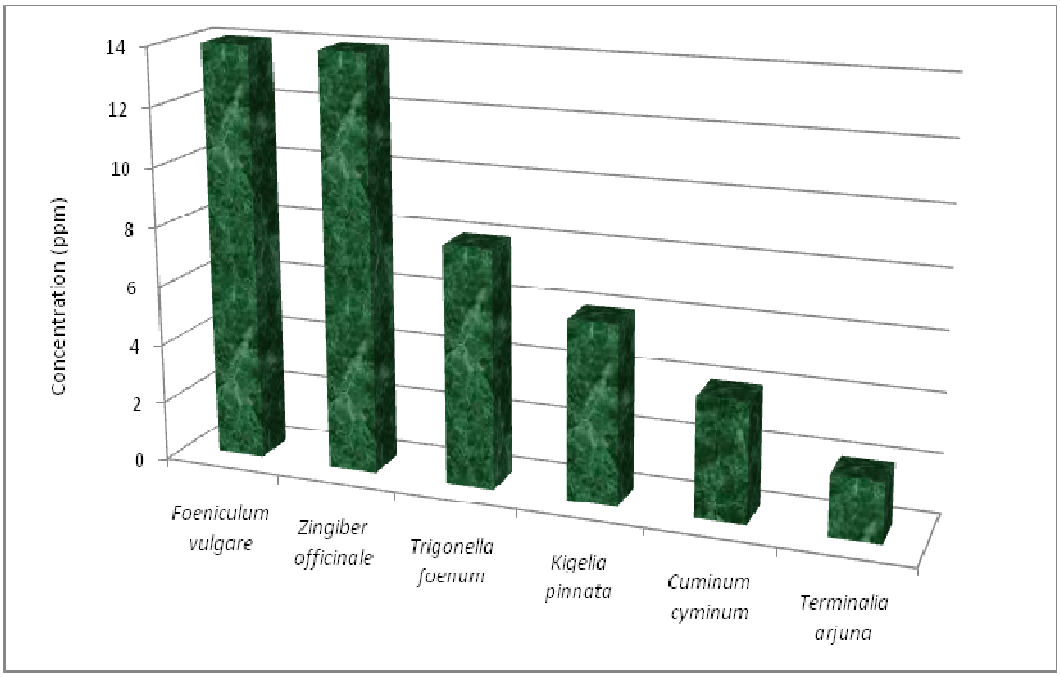

Figure12: Copper content in methanol extract

In water extract, maximum amount of copper was present in *Z. officinale* (20.0 ppm) and minimum in *T. arjuna* (4.0 ppm) as can be seen from Table 4 and Figure 13.

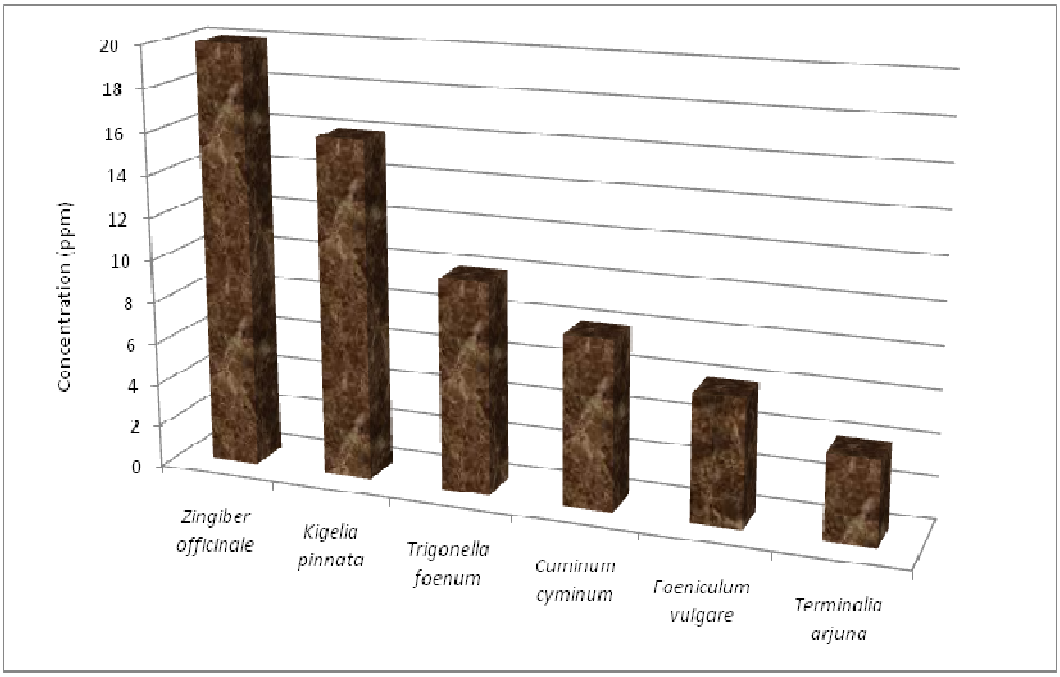

Figure 13: Copper content in water extract

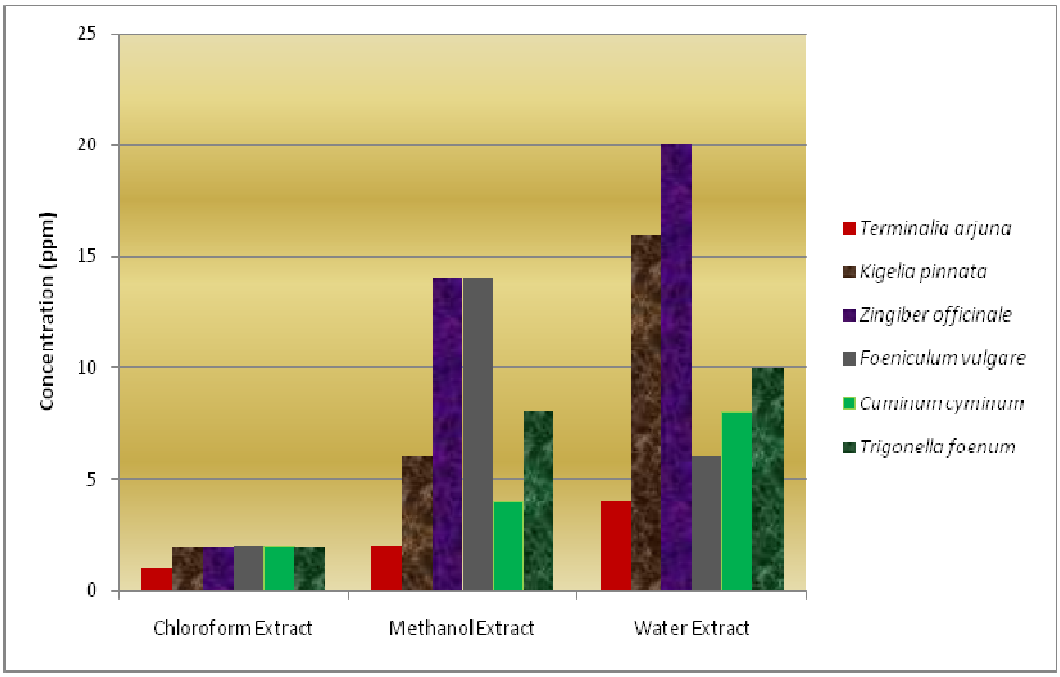

Figure 14: Copper content in different extracts

In Figure 14, comparison of the flavonoid content have been depicted in all the three solvents i.e. chloroform, methanol and water.

Table 5: Manganese content (ppm)* in different extracts

Manganese content (ppm ±SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	30.0±0.08	36.0±0.45	78.0±0.23
Kigelia pinnata (Balam Kheera)	80.0±0.11	88.0±0.75	90.0±0.33
Zingiber officinale (Ginger)	58.0±0.15	102.0±0.63	86.0±0.77
Foeniculum vulgare (Fennel)	64.0±0.07	88.0±0.35	68.0±0.34
Cuminum cyminum (Cumin)	78.0±0.44	76.0±0.35	136.0±0.15
Trigonella foenum (Methi)	8.0±0.24	13.0±0.03	15.8±0.33

***The values represent the average of three replicates**

Maximum amount of manganese was found in water extract of *C. cyminum* (136.0 ppm) and minimum amount in chloroform extract of *T. foenum* (8.0 ppm).

In chloroform extract, the manganese content was variable in each plant; maximum amount i.e. 80 ppm was present in *K. pinnata* and minimum amount was found in *T. foenum* i.e. 8 ppm as clear from Table 5 and Figure 15. The following trend was observed in the manganese content of chloroform extract of plants:

K. pinnata (ppm) (80.0) > *C. cyminum* (78.0) > *F. vulgare* (64.0) > *Z. officinale* (58.0) > *T. arjuna* (30.0) > *T. foenum* (8.0).

From figure 16 it is clear that in methanol extract the maximum amount of manganese was found in *Z. officinale* (102.0 ppm) and minimum in *T. foenum* (13.0 ppm). The following trend was observed in the manganese content of methanol extract of plants:

Z. officinale (ppm) (102.0) > *F. vulgare* (88.0) = *K. pinnata* (88.0) > *C. cyminum* (76.0) > *T. arjuna* (36.0) > *T. foenum* (13.0)

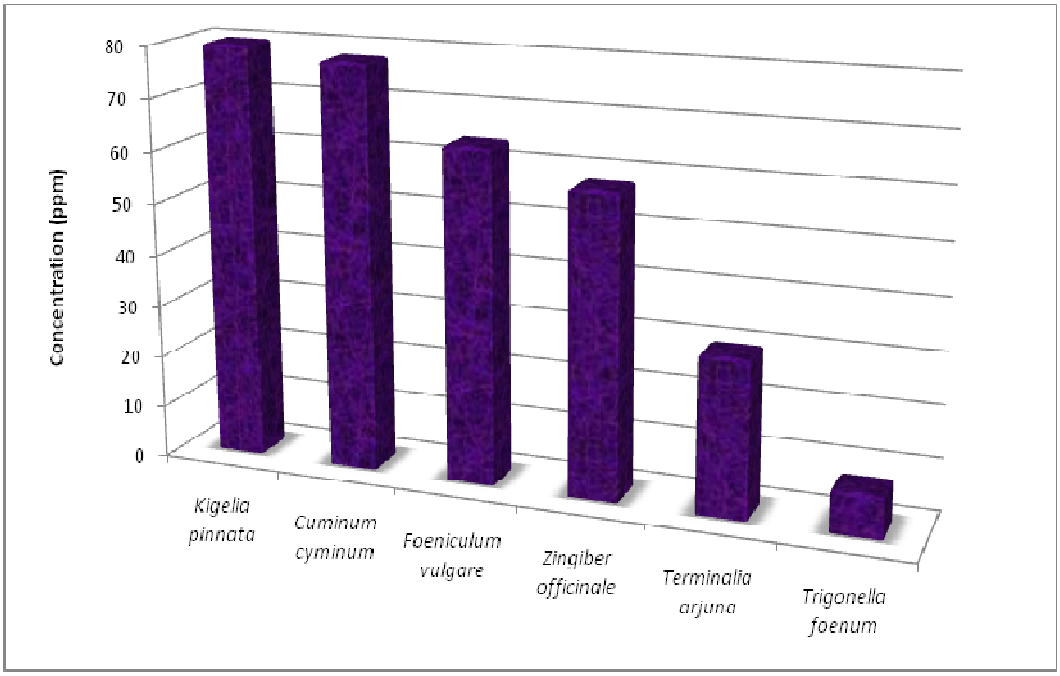

Figure 15: Manganese content in chloroform extract

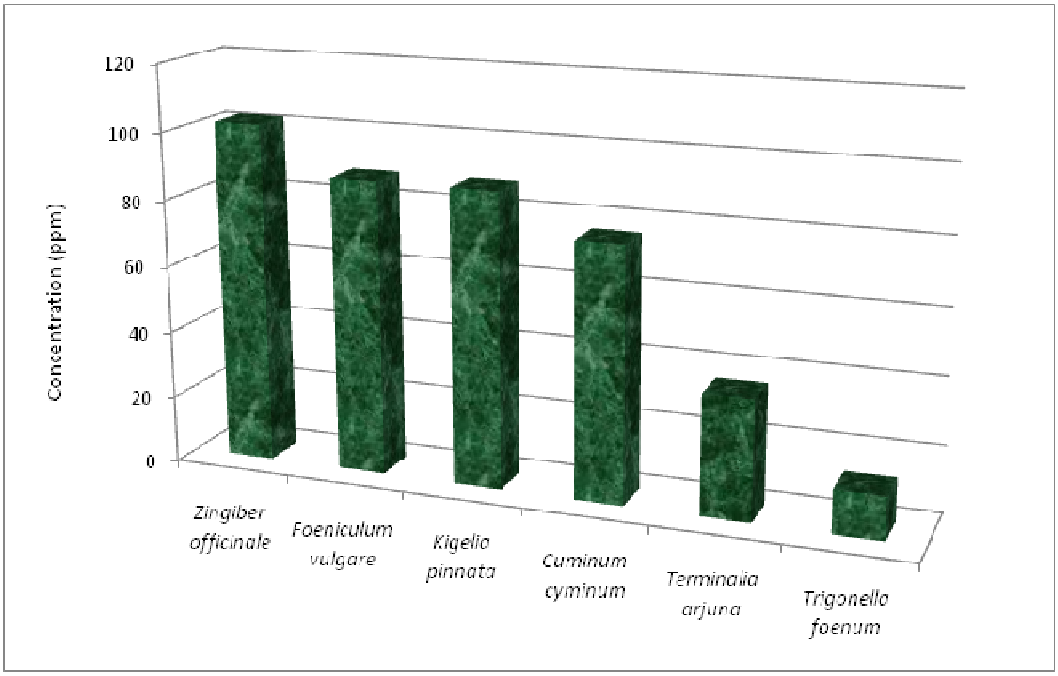

Figure 16: Manganese content in methanol extract

In water extract, manganese was present in maximum amount in *C. cymium* (136 ppm) and minimum in *T foenum* (15.8 ppm). The following trend was observed in the manganese content of water extract of plants:

C. cyminum (ppm) (136.0) > *K. pinnata* (90.0) > *Z. officinale* (86.0) > *T. arjuna* (78.0) > *F. vulgare* (68.0) > *T. foenum* (15.8)

In Figure 18, comparison of the manganese content have been depicted in all the three solvents i.e. chloroform, methanol and water.

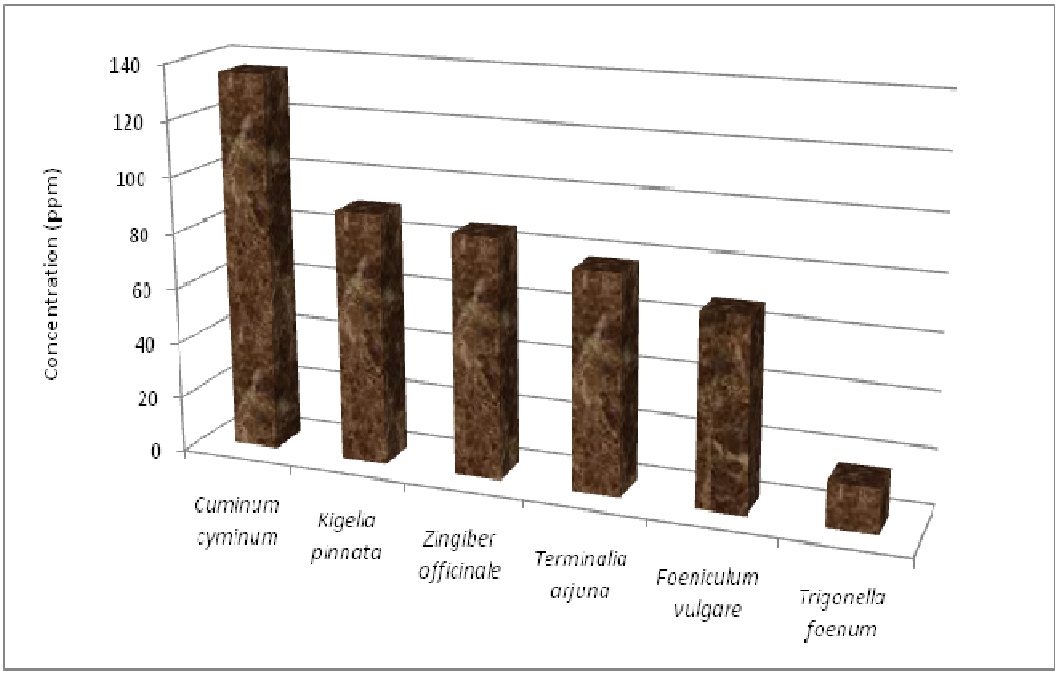

Figure 17: Manganese content in water extract

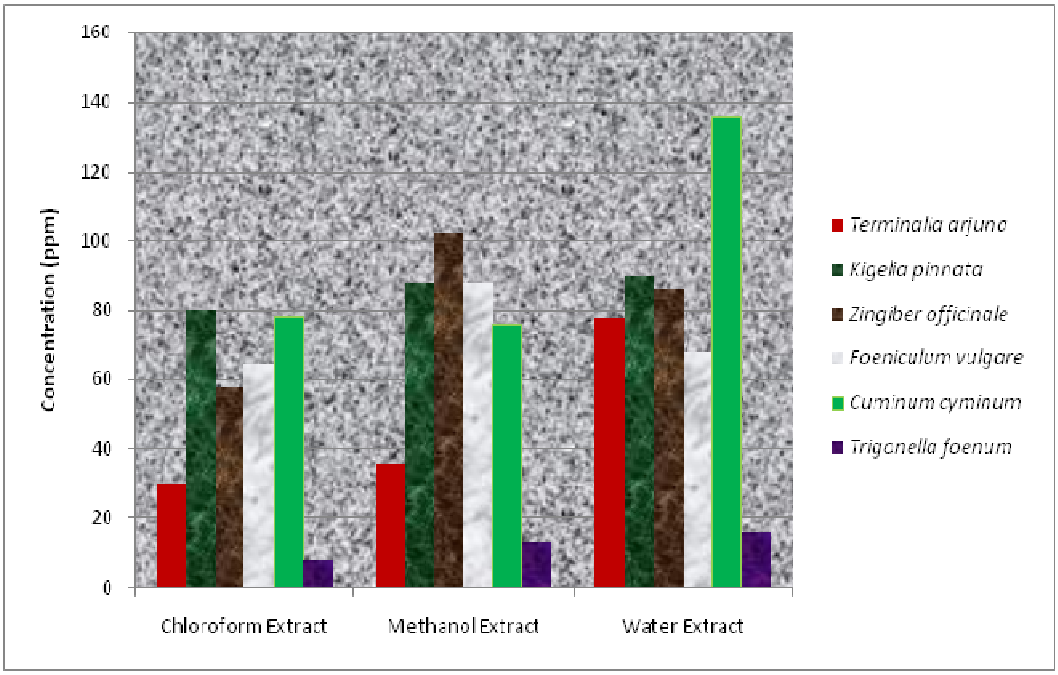

Figure 18: Manganese content in different extracts

Table 6: Zinc content (ppm)* in different extracts

Zinc content (ppm ±SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	10.0±0.24	15.0±0.33	46.0±0.25
Kigelia pinnata (Balam Kheera)	48.0±0.44	50.0±0.16	166.0±0.87
Zingiber officinale (Ginger)	28.0±0.24	76.0±0.21	94.0±0.25
Foeniculum vulgare (Fennel)	84.0±065	128.0±0.32	72.0 ±0.26
Cuminum cyminum (Cumin)	128.0±0.33	70.0±0.36	124.0±0.14
Trigonella foenum (Methi)	28.0±0.18	76.0±0.53	94.0±0.45

***The values represent the average of three replicates.**

Maximum amount of zinc was found in water extract of *K. pinnata* (166 ppm) and minimum amount in chloroform extract of *T. arjuna* (10 ppm).

In chloroform extract, maximum amount i.e. 128 ppm was present in *C. cyminum* and minimum amount was found in *T. arjuna* i.e. 10 ppm as clear from Table 6 and Figure 19. The following trend was observed in the zinc content of chloroform extract of plants:

C. cyminum (ppm) (128.0) > *F. vulgare* (84.0) > *K. pinnata* (48.0) > *T. foenum* (28.0) = *Z. officinale* (28.0) > *T. arjuna* (10.0)

From Figure 20 it is clear that in methanol extract the maximum amount of zinc was found in *F. vulgare* (128.0 ppm) and minimum in *T. arjuna* (15.0 ppm). The following trend was observed in the zinc content of methanol extract of plants:

F. vulgare (ppm) (128.0) > *Z. officinale* (76.0) = *T. foenum* (76.0) > *C. cyminum* (70.0) > *K. pinnata* (50.0) > *T. arjuna* (15.0)

In water extract, zinc was present in maximum amount in *K. pinnata* (166 ppm) and minimum in *T. arjuna* (46 ppm). The following trend was observed in the zinc content of methanol extract of plants:

K. pinnata (166) (ppm) > *C. cyminum* (124.0) > *Z. officinale* (94.0) = *T. foenum* (94.0) > *F. vulgare* (72.0) > *T. arjuna* (46.0)

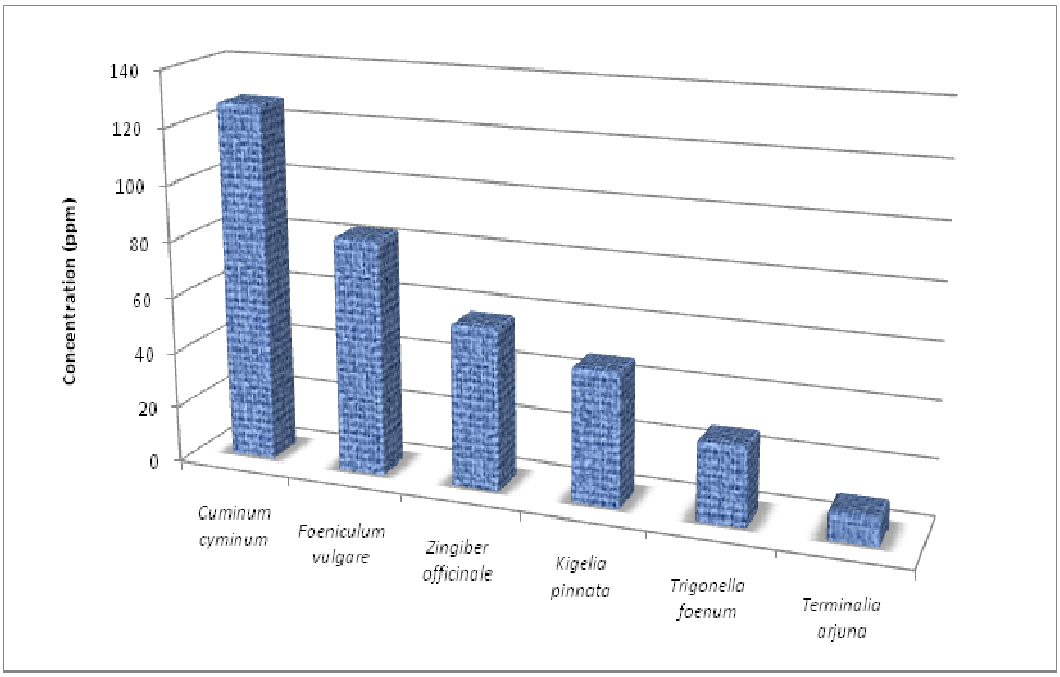

Figure 19: Zinc content in chloroform extract

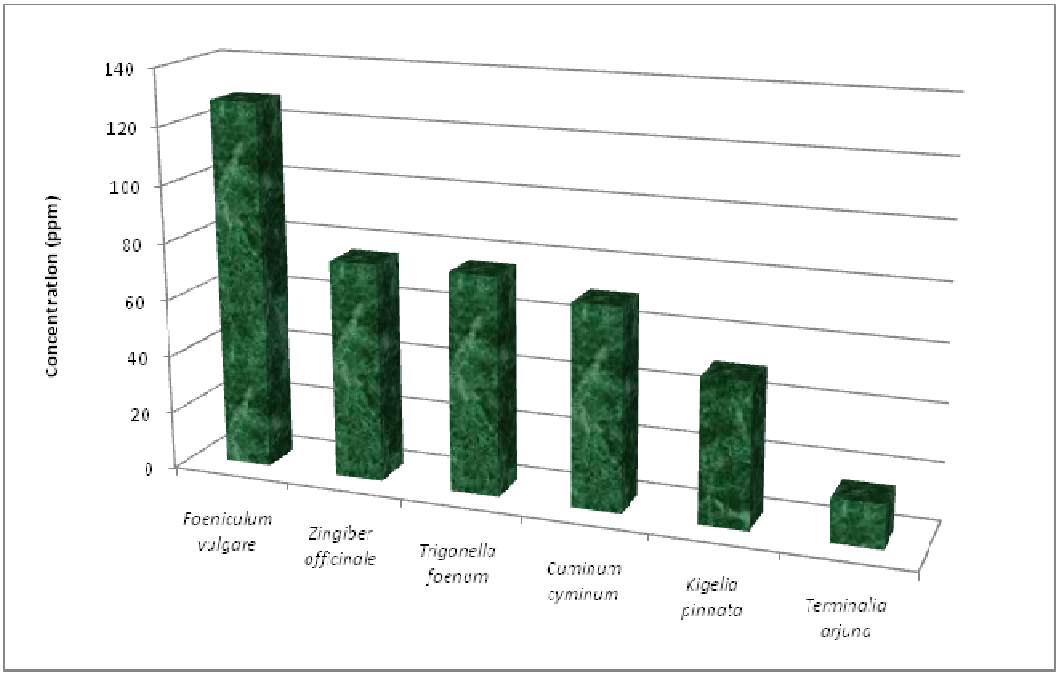

Figure 20: Zinc content in methanol extract

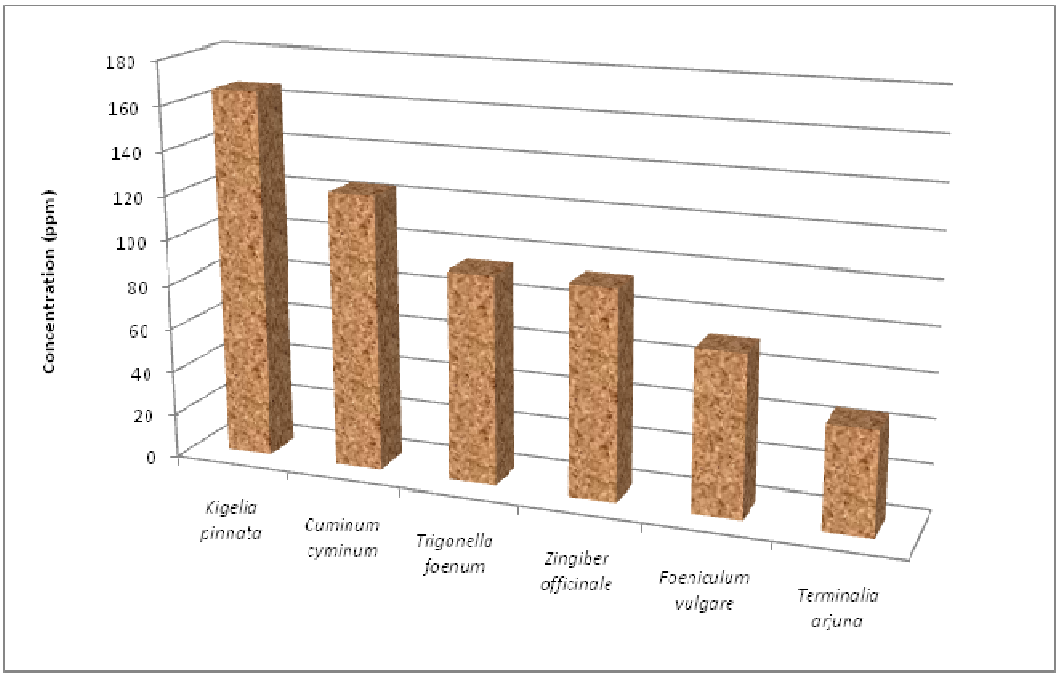

Figure 21: Zinc content in water extract

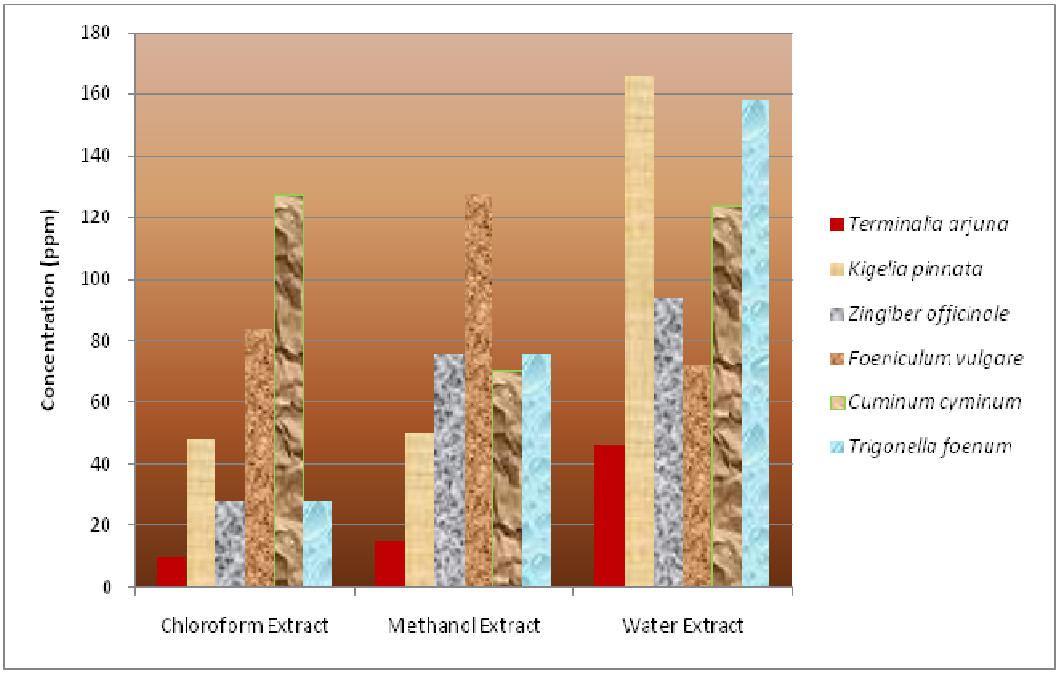

Figure 22: Zinc content in different extracts

In Figure 22, comparison of the manganese content have been depicted in all the three solvents i.e. chloroform, methanol and water.

Table 7: Iron content (ppm)* in different extacts

Iron content (ppm ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	1.0±0.08	1.8±0.08	8.8±0.05
Kigelia pinnata (Balam Kheera)	4.0±0.08	6.2±0.09	9.6±0.05
Zingiber officinale (Ginger)	7.0±0.04	15.4±0.87	130.0±0.02
Foeniculum vulgare (Fennel)	4.0±0.76	11.4±0.03	24.0±0.35
Cuminum cyminum (Cumin)	4.0±0.35	11.2±0.43	52.0±0.15
Trigonella foenum (Methi)	2.8±0.46	3.8±0.54	28.0±0.13

***The values represent the average of three replicates.**

Maximum amount of iron was found in water extract of *Z. officinale* (130 ppm) and minimum amount in chloroform extract of *T. arjuna T. arjuna* (1.0 ppm).

In chloroform extract, maximum amount i.e. 7.0 ppm was present in *Z. officinale* and minimum amount was found in *T. arjuna* i.e. 1.0 ppm as clear from Table 7 and Figure 23. The following trend was observed in the manganese content of chloroform extract of plants:

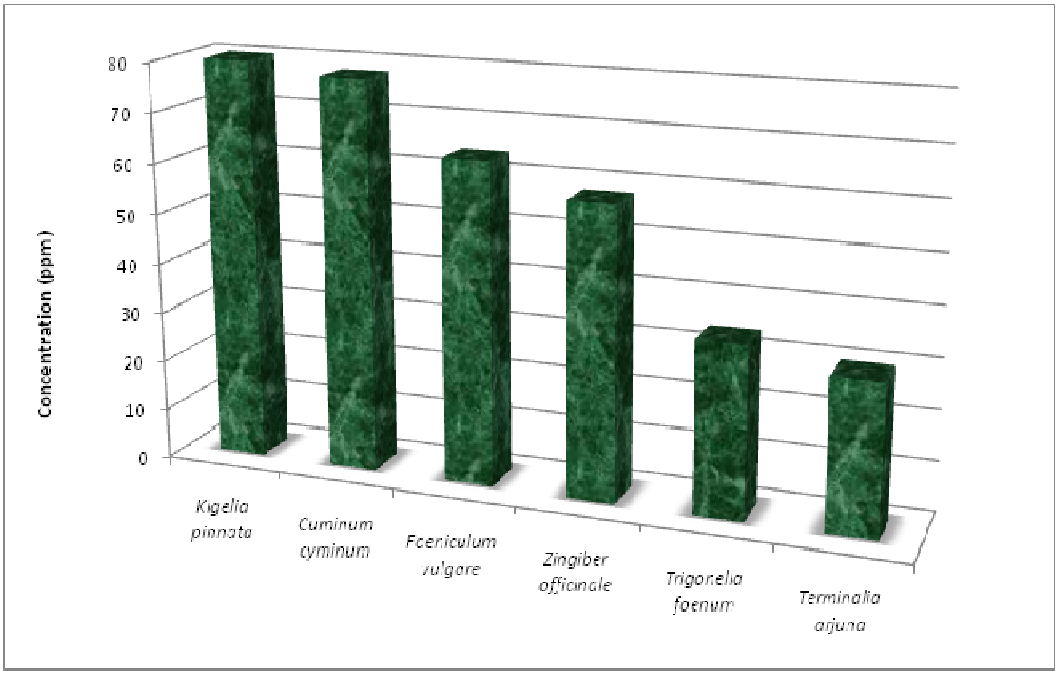

Figure 23: Iron content in chloroform extract

Z. officinale (ppm) (7.0) > *C. cyminum* (4.0) = *K. pinnata* (4.0) = *F. vulgare* (4.0) > *T. foenum* (2.8) > *T. arjuna* (1.0)

From Figure 24, it is clear that in methanol extract the maximum amount of iron was found in *Z. officinale* (15.4 ppm) and minimum in *T. arjuna* (1.8 ppm). The following trend was observed in the zinc content of methanol extract of plants:

Z. officinale (ppm) (15.4) > *F. vulgare* (11.4) > *C. cyminum* (11.2) > *K. pinnata* (6.2) > *T. foenum* (3.8) > *T. arjuna* (1.8)

In water extract, iron was present in maximum amount in *Z. officinale* (130 ppm) and minimum in *T. arjuna T. arjuna* (8.8 ppm). The following trend was observed in the iron content of methanol extract of plants:

Z. officinale (ppm) (130.0) > *C. cyminum* (52.0) > *T. foenum* (28.0) > *F. vulgare* (24.0) > *K. pinnata* (9.6) > *T. arjuna T. arjuna* (8.8)

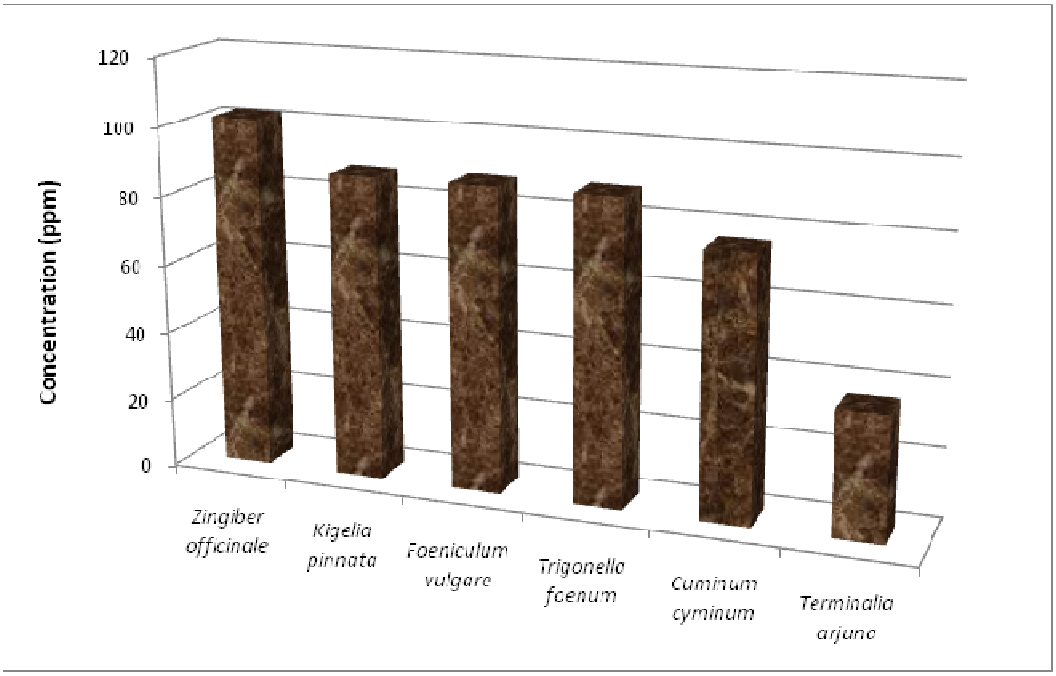

Figure 24: Iron content in methanol extract

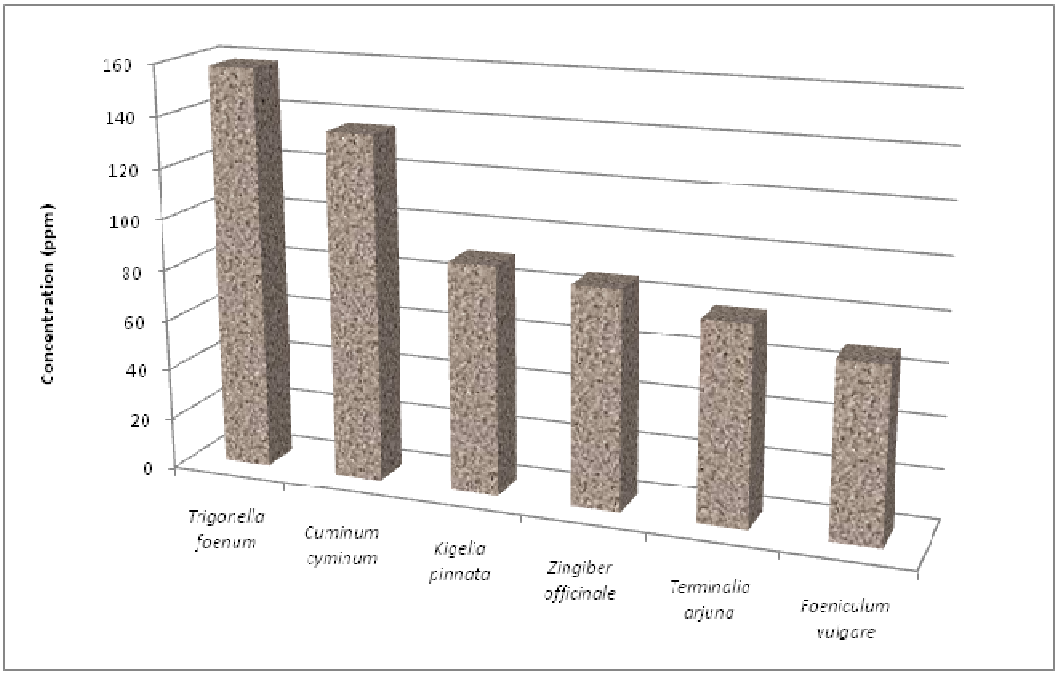

Figure 25: Iron content in water extract

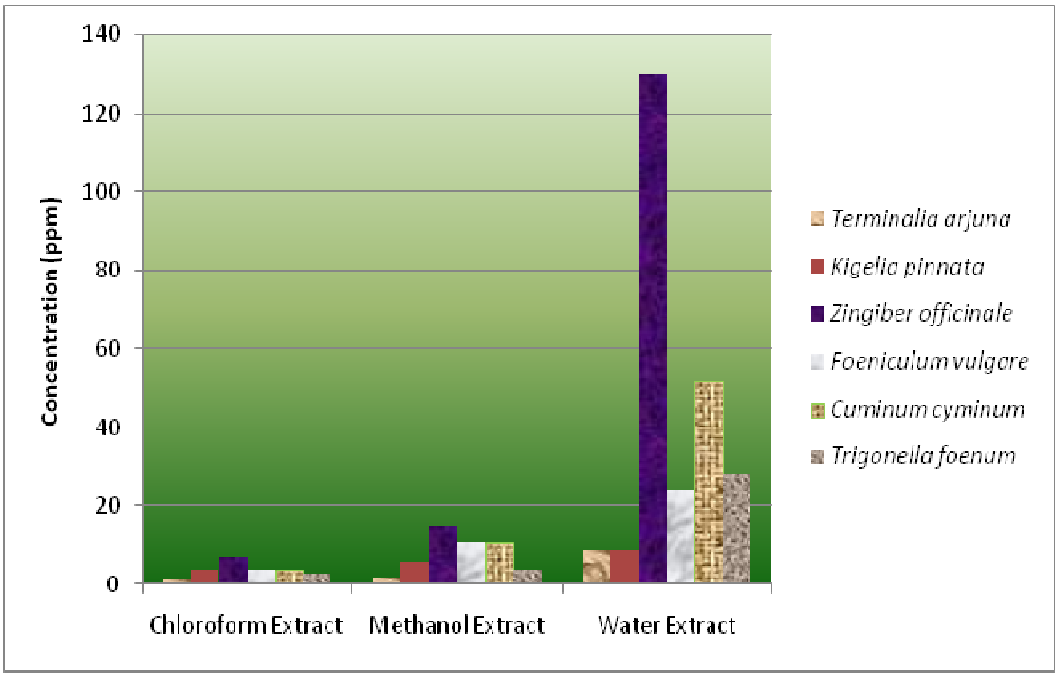

Figure 26: Iron content in different extracts

In Figure 26, comparison of the Iron content have been depicted in all the three solvents i.e. chloroform, methanol and water.

4.4 Anti-oxidant and antiradical activity of extracts

The antioxidant activity and anti-radical activity of different extracts of these plants was measured by three different methods viz; β-carotene bleaching method (BCBT), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric thiocyanate (FTC) method.

4.4.1 Anti-oxidant activity of extracts by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT)

Antioxidant activity of extracts was measured by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT).

4.4.1.1 FTC method

According to FTC method **,** methanol extract of *K. pinnata,* methanol extract of *T. arjuna ,* chloroform extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 85.7% ; chloroform extract of *T. foenum* and methanol extract of *C. cyminum* showed minimum activity i.e. 28.5%.

Table 8: Antioxidant activity (%)* of different extracts by ferric thiocyanate (FTC) method

Antioxidant activity (% ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	42.8±0.24	85.7±0.09	71.4±0.09
Kigelia pinnata (Balam Kheera)	71.4±0.44	85.7±0.24	71.4±0.18
Zingiber officinale (Ginger)	85.7±0.09	42.8±0.18	71.4±065
Foeniculum vulgare (Fennel)	71.4±0.24	85.7±0.35	57.1±0.35
Cuminum cyminum (Cumin)	57.1±0.18	28.5±0.18	57.1±0.24
Trigonella foenum (Methi)	28.5±0.35	57.1±0.24	42.8±0.33

***The values represent the average of three replicates**

In chloroform extract, maximum amount of antioxidant activity i.e. 85.7% was present in *Z. officinale* and minimum amount was found in *T. foenum* i.e. 28.5% as clear from Table 8 and Figure 27. The following trend was observed in the antioxidant activity of chloroform extract of plants:

Z. officinale (%) (85.7) > *F. vulgare* (71.4) = *K. pinnata* (71.4) > *C. cyminum*(57.1) > *T. arjuna T. arjuna* (42.8) > *T. foenum*(28.5)

From Figure 28, it is clear that in methanol extract the maximum and equal amount of antioxidant activity was found in *K. pinnata*, *T. arjuna T. arjuna* and *F. vulgare* (85.7%) and minimum in *C. cyminum* (28.5%). The following trend was observed in the antioxidant activity of methanol extract of plants:

K. pinnata (%) (85.7) = *T. arjuna* (85.7) = *F. vulgare* (85.7) > *T. foenum* (57.1) > *Z. officinale* (42.8) > *C. cyminum* (28.5)

In water extract, antioxidant activity was present in maximum amount in *T.arjuna, K. pinnata* and *Z. officinale* (71.4%) and minimum in *T. foenum* (42.8%). The following trend was observed in the antioxidant activity of water extract of plants:

T.arjuna= K. pinnata = Z. officinale (%) (71.4) > *F. vulgare* (57.1) =*C. cyminum* (57.1) > *T. foenum* (42.8)

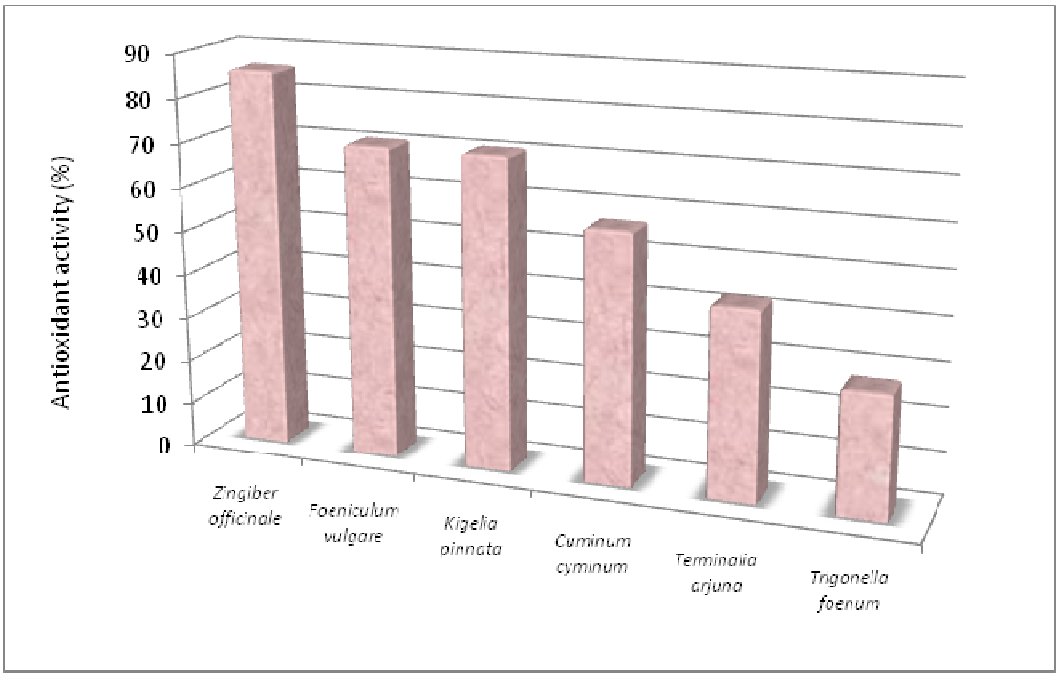

Figure 27: Antioxidant activity (%) by FTC method in chloroform extract

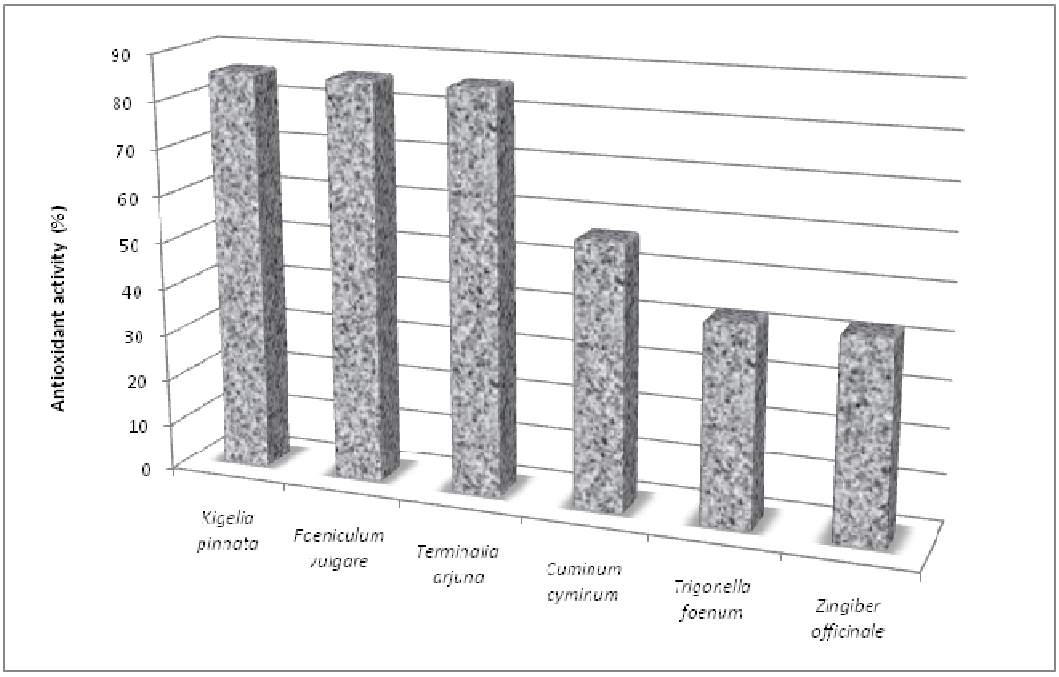

Figure 28: Antioxidant activity (%) by FTC method in methanol extract

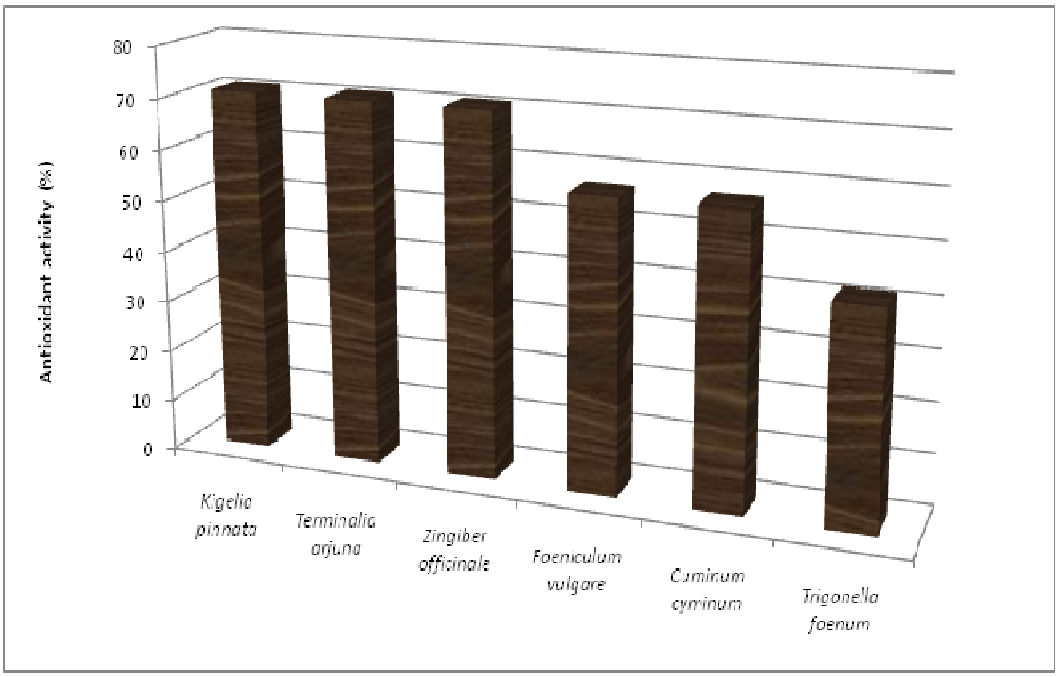

Figure 29: Antioxidant activity (%) by FTC method in water extract

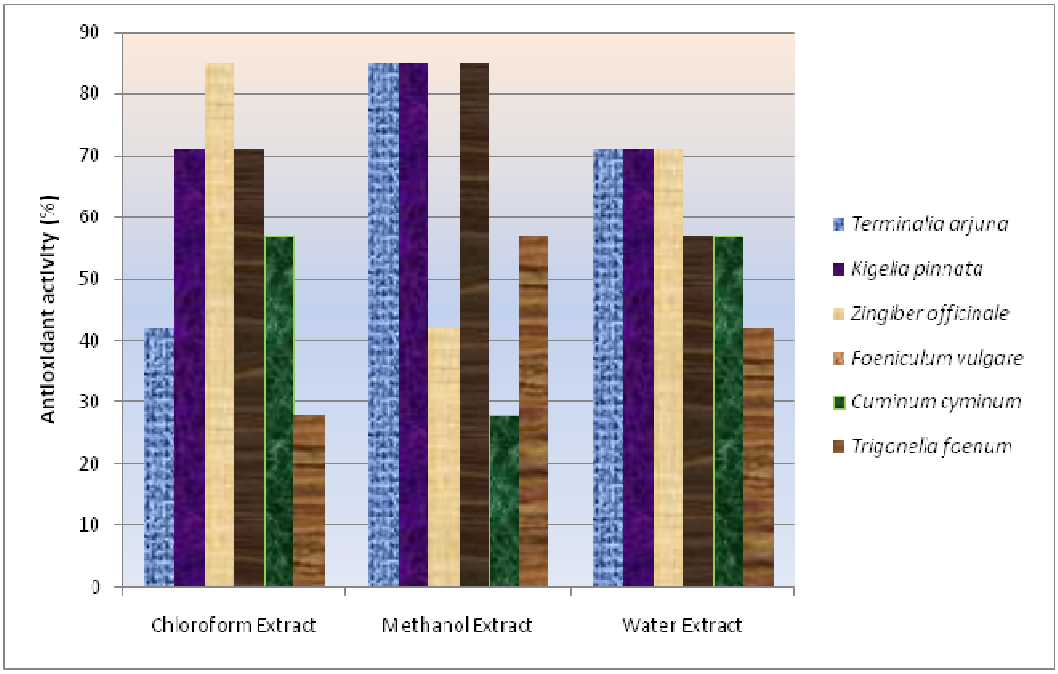

Figure 30: Antioxidant activity (%) of different extracts by FTC method

In Figure 30, comparison of the antioxidant activity have been depicted in all the three solvents i.e. chloroform, methanol and water.

4.4.1.2 β-carotene bleaching method (BCBT) method

β-carotene bleaching method (BCBT) method showed quiet different results. According to this method, the water extract of *T.arjuna,* methanol extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 83.3% while chloroform and water extract of *C. cyminum* showed minimum activity i.e. 33.3%.

In chloroform extract, the maximum amount of antioxidant activity was present in *Z. officinale* (50%) and *K. pinnata* while minimum amount was found in *T.arjuna, F. vulgare* and *T. foenum* i.e. 16.6% as clear from table 9 and figure 31. The following trend was observed in the antioxidant activity of chloroform extract of plants:

Z. officinale (%) (50.0) = *K. pinnata* (50.0) > *C. cyminum* (33.3) > *F. vulgare* (16.6) = *T. arjuna T. arjuna* (16.6) = *T. foenum* (16.6)

Table 9: Antioxidant activity (%)* of different extracts by beta carotene method

Antioxidant activity (% ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	16.6±0.09	66.6±0.18	83.3±0.35
Kigelia pinnata (Balam Kheera)	50.0±0.24	66.6±0.35	33.3±0.18
Zingiber officinale (Ginger)	50.0±0.18	83.3±0.35	33.3±0.09
Foeniculum vulgare (Fennel)	16.6±0.24	83.3±0.35	16.6±0.18
Cuminum cyminum (Cumin)	33.3±0.09	16.6±0.35	33.3±0.09
Trigonella foenum (Methi)	16.6±0.35	16.6±0.24	66.6±0.35

***The values represent the average of three replicates**

From Figure 32, it is clear that in methanol extract the maximum amount of antioxidant activity was found in *Z. officinale* and *F. vulgare* (83.3%) and minimum in *C. cyminum* and *T. foenum* (16.6%). The following trend was observed in the antioxidant activity of methanol extract of plants:

Z. officinale (%) (83.3) = *F. vulgare* (83.3) > *K. pinnata* (66.6) = *T. arjuna T. arjuna* (66.6) > *C. cyminum* (16.6) = *T. foenum* (16.6)

In water extract, antioxidant activity was present in maximum amount in *T. arjuna* (83.3%) and minimum in *F. vulgare* (16.6%). The following trend was observed in the antioxidant activity of water extract of plants:

T. arjuna (%) (83.3) > *T. foenum* (66.6) > *C. cyminum* (33.3) = *K. pinnata* (33.3) = *Z. officinale* (33.3) > *F. vulgare* (16.6)

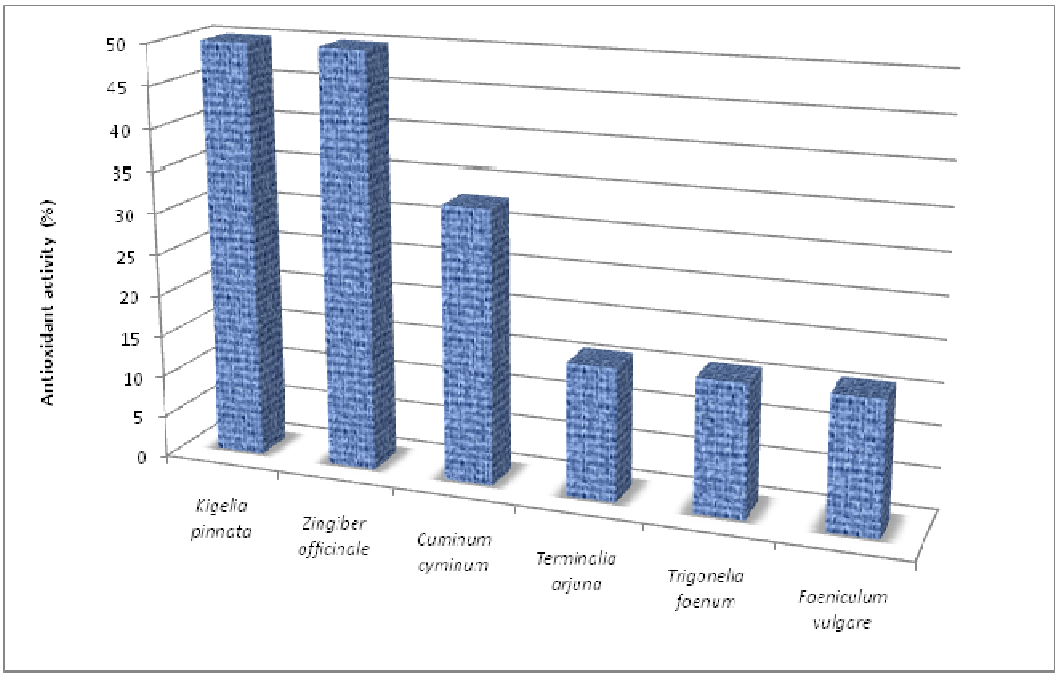

Figure 31: Antioxidant activity (%) by beta carotene method in chloroform extract

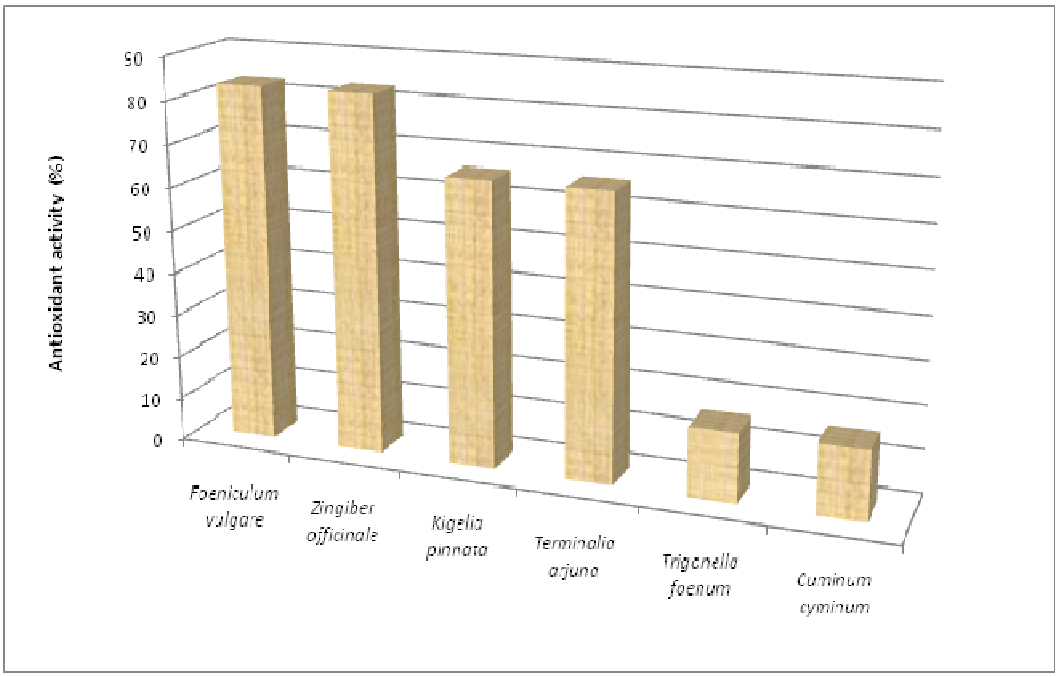

Figure 32: Antioxidant activity (%) by beta carotene method in methanol extract

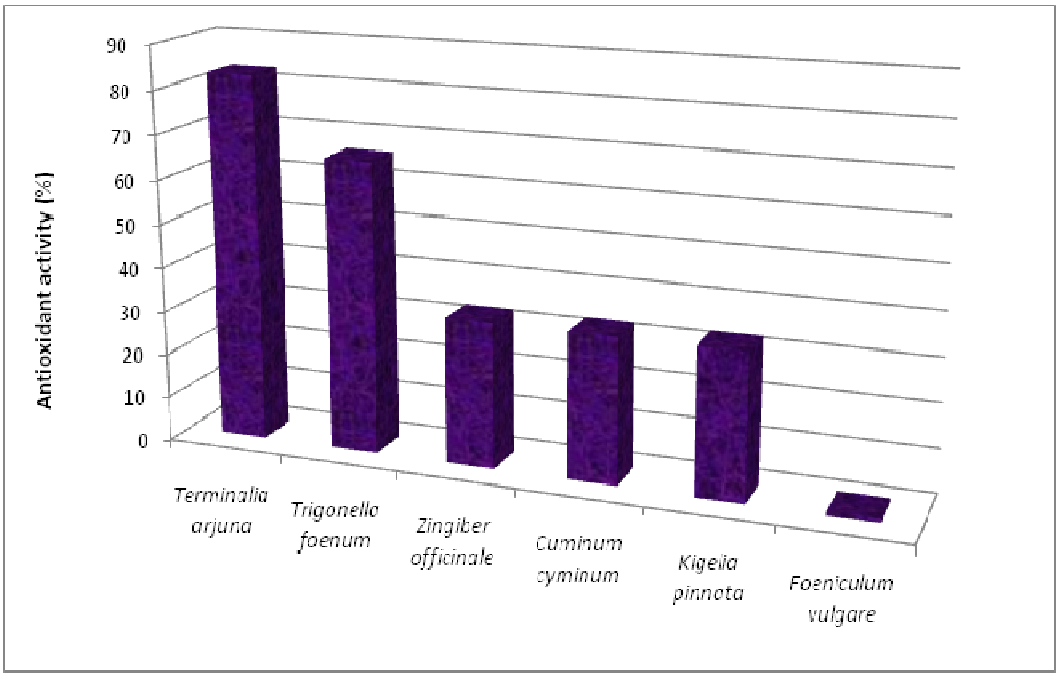

Figure 33: Antioxidant activity (%) by beta carotene method in water extract

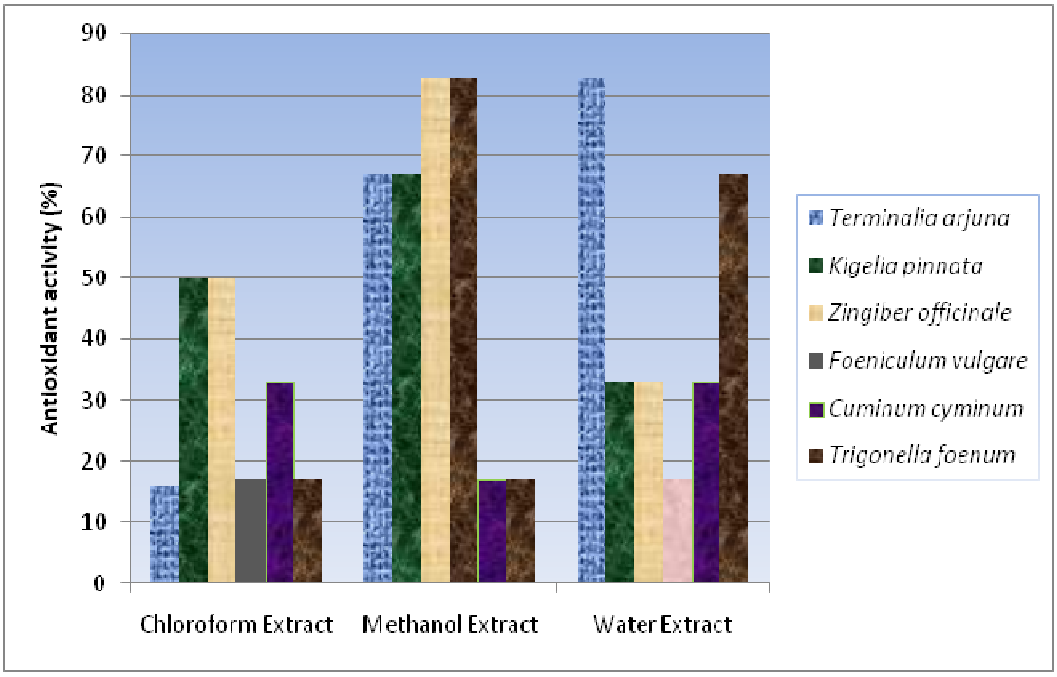

Figure 34: Antioxidant activity (%) of different extracts by Beta carotene method

In Figure 34, comparison of the antioxidant activity by BCBT method have been depicted in all the three solvents i.e. chloroform, methanol and water.

4.4.2 Antiradical activity of extracts by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

Antiradical activity of extracts was measured according to 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method. The following extracts showed maximum activity, chloroform extract of *K. pinnata,* and water extract of *T. arjuna* i.e. 91.7% , chloroform extract of *Z. officinale* , chloroform and water extract of *C. cyminum* showed 91% activity while methanol extract of *T. foenum* showed minimum activity.i.e. 58%.

Table 10: Antiradical activity (%)* of different extracts by DPPH radical scavenging method

Antiradical activity (% ± SD)			
Plants	**Chloroform extract**	**Methanol extract**	**Water extract**
Terminalia arjuna (Arjuna)	75.0±0.86	83.3±0.14	91.7±0.27
Kigelia pinnata (Balam Kheera)	91.7±0.9	85.0±0.22	83.3±0.18
Zingiber officinale (Ginger)	91.0±0.35	83.3±0.35	66.7±0.23
Foeniculum vulgare (Fennel)	66.7±0.09	75.0±0.98	83.3±0.37
Cuminum cyminum (Cumin)	91.0±0.13	91.0±0.58	75.0±0.35
Trigonella foenum (Methi)	66.7±0.24	58.0±0.14	75.0±0.25

***The values represent the average of three replicates.**

In chloroform extract, maximum amount of antiradical activity i.e. 91% was present in *K. pinnata, Z. officinale and C. cyminum* and minimum amount was found in *F. vulgare* and *T. foenum* i.e. 66.7% as clear from Table 10 and Figure 35. The following trend was observed in the antiradical activity of chloroform extract of plants:

Z. officinale = C. cyminum = K. pinnata (%) (91.7) > *T. arjuna* (75) > *F. vulgare = T. foenum* (66.7).

From Figure 36, it is clear that in methanol extract the maximum amount of antiradical activity was found in *C. cyminum* (91%) and minimum in *T. foenum* (58%). The following trend was observed in the antiradical activity of methanol extract of plants:

C. cyminum (%) (91.0) > *K. pinnata* (85.0) > *T. arjuna* (83.3) = *Z. officinale* (83.3) > *F. vulgare* (75.0) > *T. foenum* (58.0)

In water extract, antiradical activity was present in maximum amount in *T. arjuna* (91.7%) and minimum in *Z. officinale* (66.7%). The following trend was observed in the antiradical activity of water extract of plants:

T. arjuna (91.7) (%) > *F. vulgare* (83.3) = *K. pinnata* (83.3) > *C. cyminum* (75.0) = *T. foenum* (75.0) > *Z. officinale* (66.7)

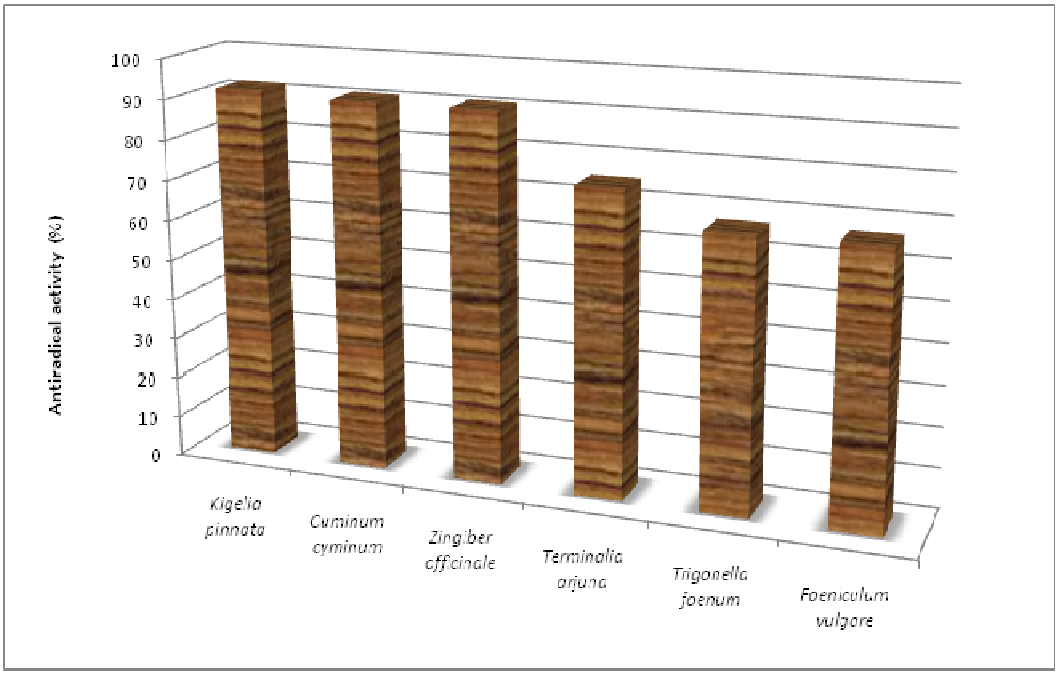

Figure 35: Antiradical activity (%) by DPPH radical scavenging method in chloroform extract

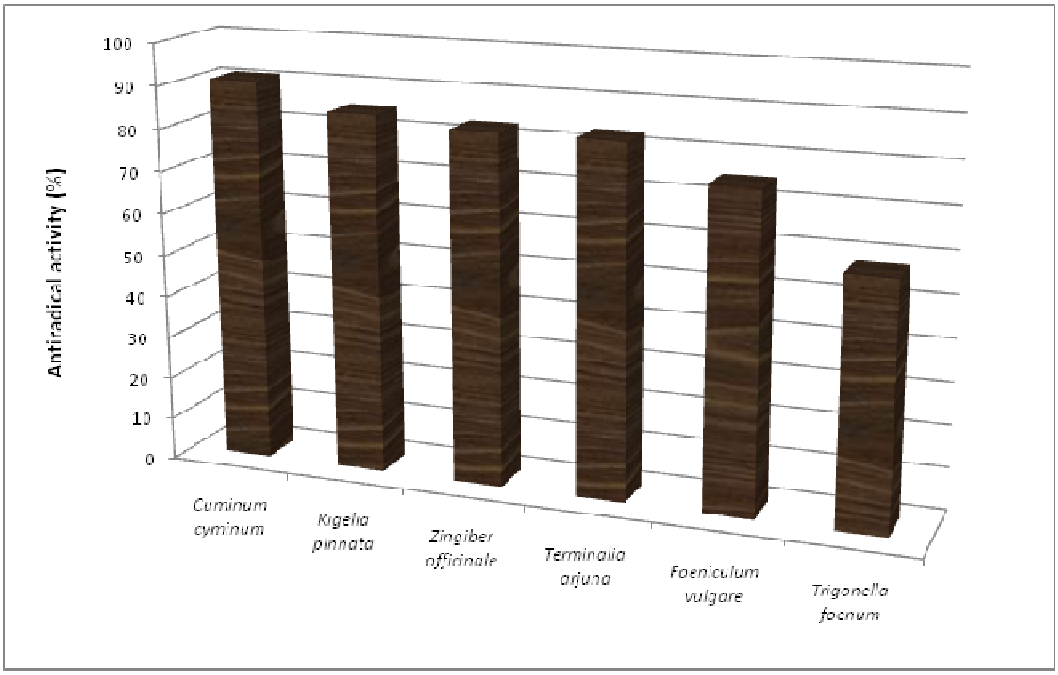

Figure 36: Antiradical activity (%) by DPPH radical scavenging method in methanol extract

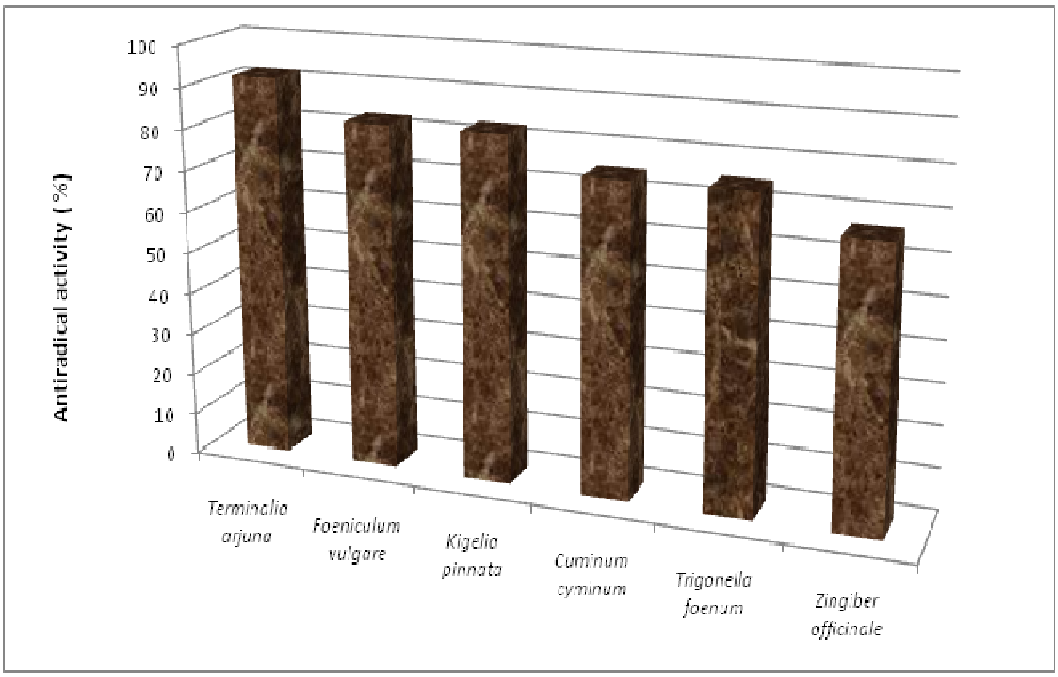

Figure 37: Antiradical activity (%) by DPPH radical scavenging method in water extract

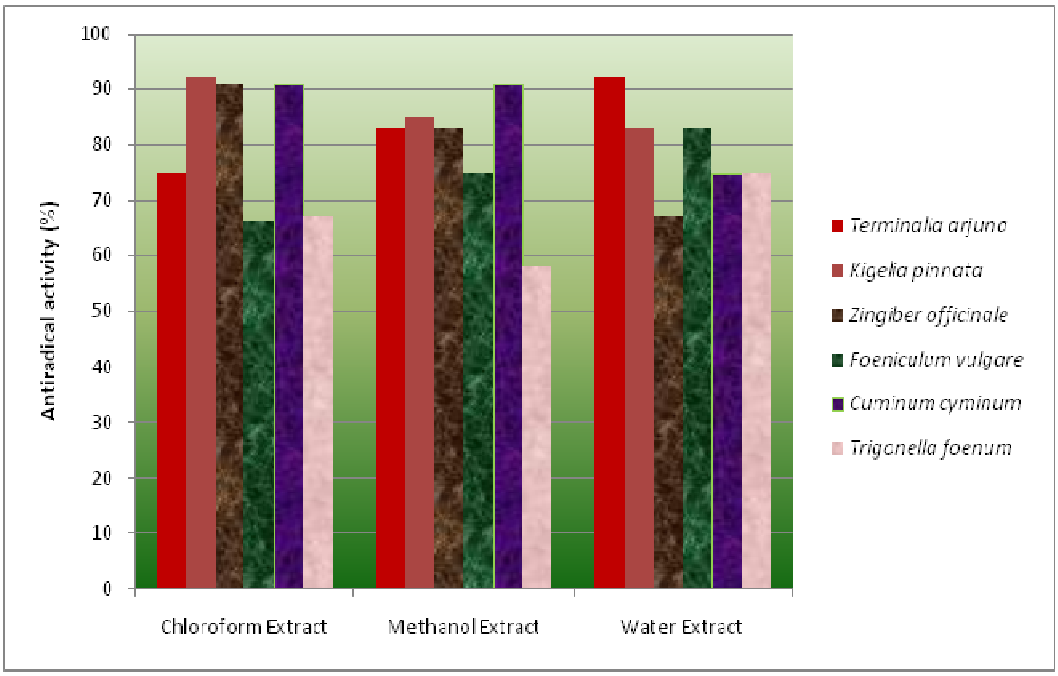

Figure 38. Antiradical activity (%)* of different extracts by DPPH radical scavenging method

In Figure 38, comparison of the antiradical activity have been depicted in all the three solvents i.e. chloroform, methanol and water.

In case of *T.arjuna*, according to DPPH radical scavenging method, the water extract of *T. arjuna* showed the maximum activity followed by methanol extract while chloroform extract showed the minimum activity. Whereas according to Beta carotene method**,** water extract of *T.arjuna,* showed highest activity followed by methanol extract followed by chloroform extract. FTC method revealed that methanol extract of *T. arjuna* showed the maximum activity followed by water extract while chloroform extract showed the minimum activity.

In case of *K. pinnata*, according to DPPH radical scavenging method, the chloroform extract of *K. pinnata* showed the maximum activity followed by methanol extract while water extract showed the minimum activity. Whereas according to beta carotene method**,** methanol extract of *K. pinnata,* showed highest activity followed by chloroform extract and water extract showed minimum activity. FTC method also confirms that methanol extract has the maximum activity followed by chloroform extract and water extract.

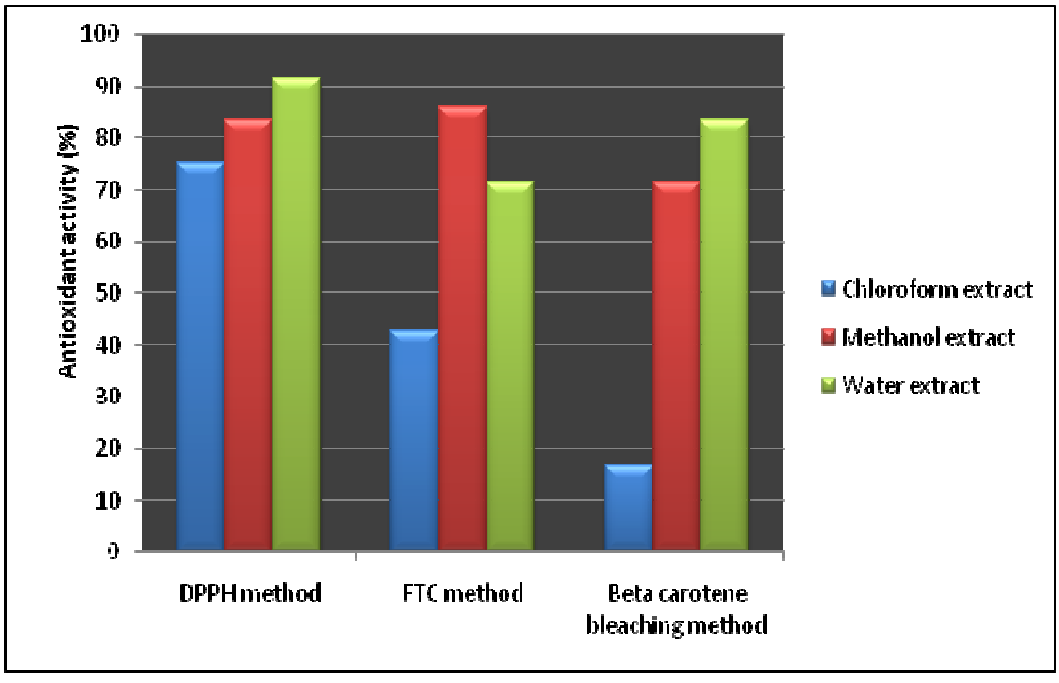

Figure 39: Antioxidant activity (%) of different extracts of *Terminalia arjuna* bark

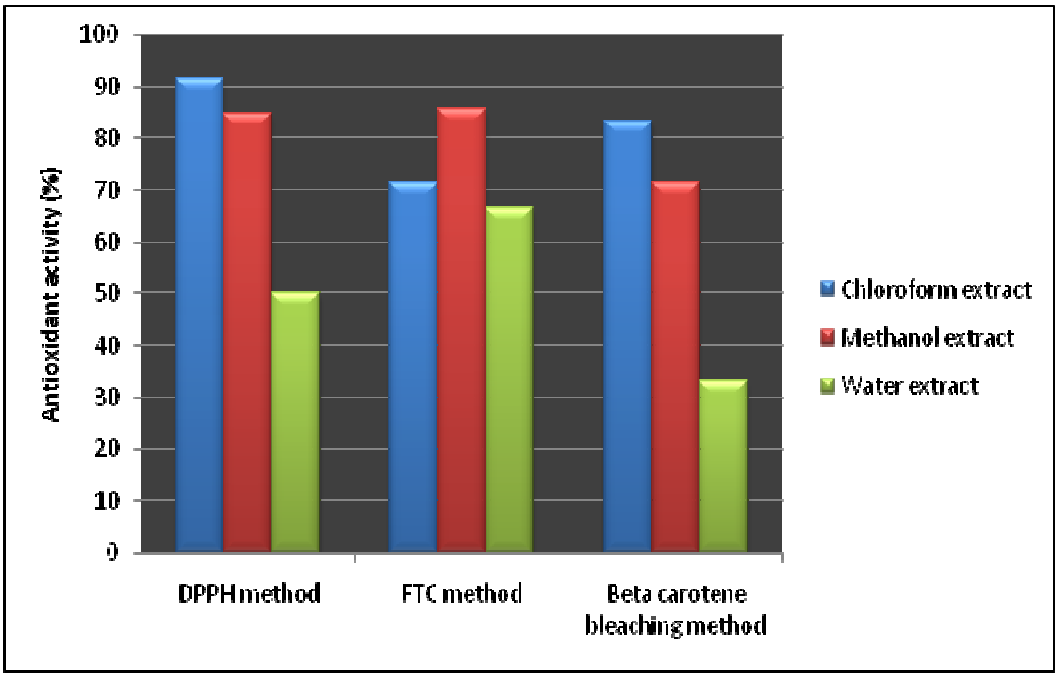

Figure 40: Antioxidant activity (%) of different extracts of *Kigelia pinnata* fruits

According to DPPH radical scavenging method, the chloroform extract of *Z. officinale* showed the maximum activity followed by methanol extract while water extract showed the minimum activity. Whereas according to beta carotene method**,** methanol extract, showed highest activity followed by chloroform extract followed by water extract. FTC method showed different results according to this method, chloroform extract showed the maximum activity followed by water extract while methanol extract showed the minimum activity

In case of *T. foenum*, according to DPPH radical scavenging method, the water extract of *T. foenum* showed the maximum activity followed by chloroform extract while methanol extract showed the minimum activity. Whereas according to beta carotene method**,** water extract showed highest activity followed by methanol and chloroform extract, which showed similar activity. FTC method showed quiet different results according to this method, methanol extract showed the maximum activity followed by water extract followed by chloroform extract which showed the minimum activity.

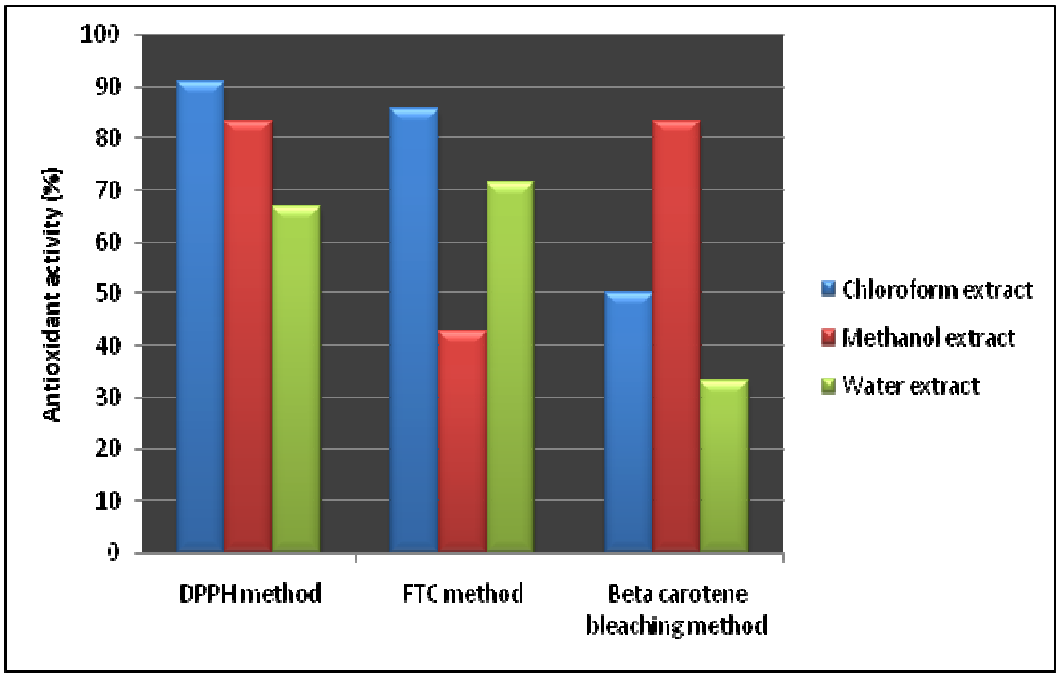

Figure 41: Antioxidant activity (%) of different extracts of *Zingiber officinale* root

In case of *Cuminum cyminum,* according to DPPH radical scavenging method, the chloroform and methanol extract showed the maximum activity followed by water extract. Whereas according to beta carotene method**,** chloroform and water extract showed more activity as compared to methanol extract. FTC method showed similar results.

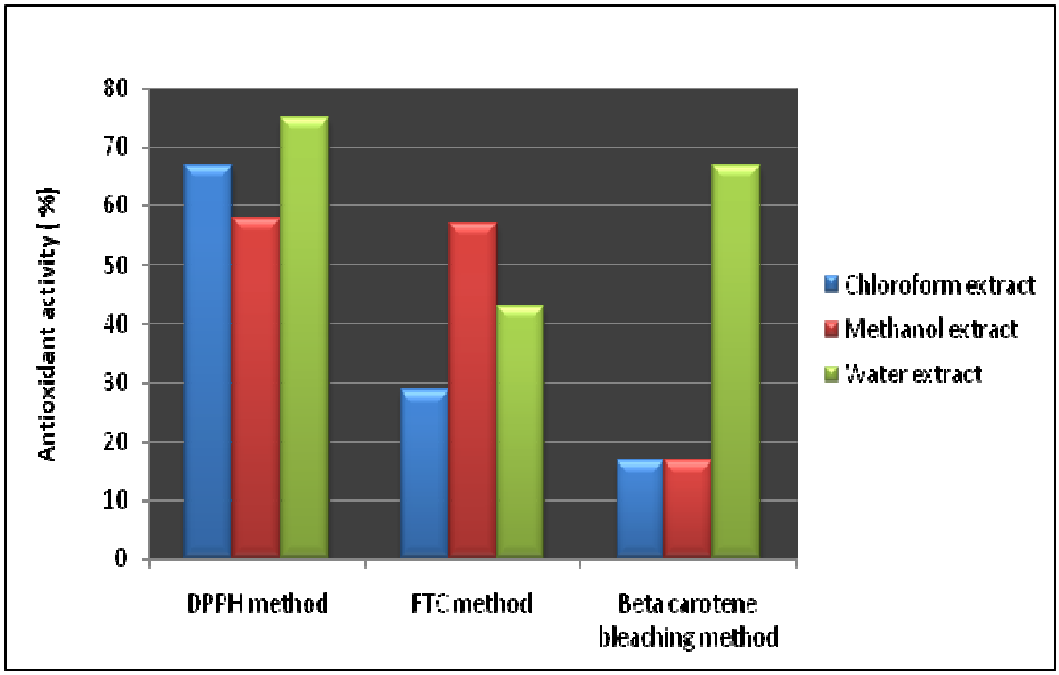

Figure 42: Antioxidant activity (%) of different extracts of *Trigonella foenum* seeds

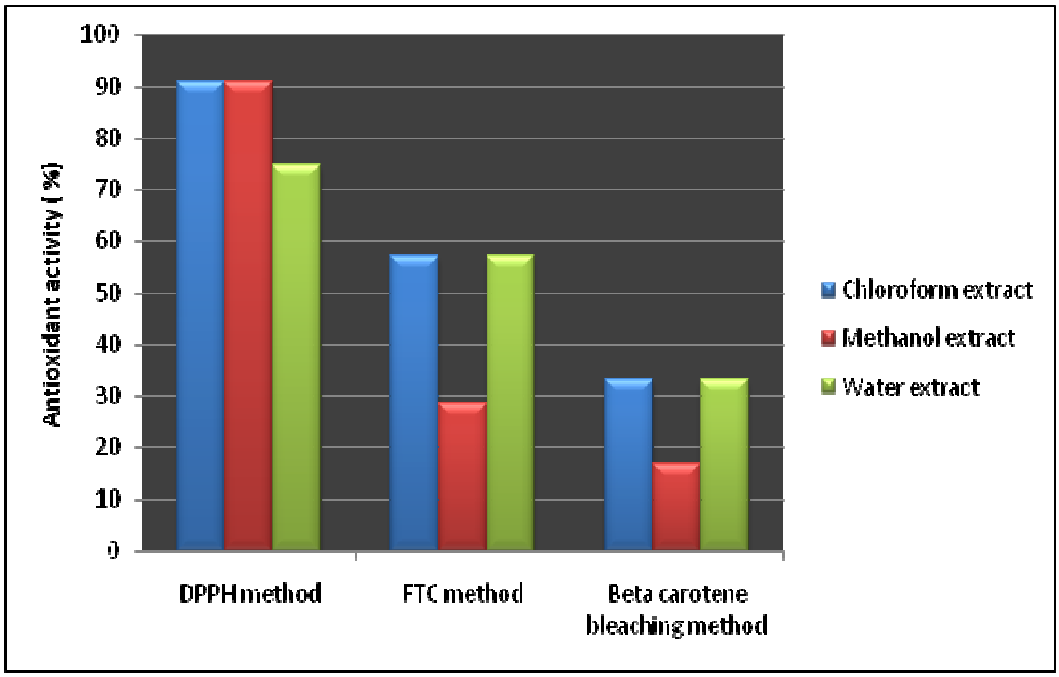

Figure 43: Antioxidant activity (%) of different extracts of *Cuminum cyminum* fruit

According to DPPH radical scavenging method, the water extract of *F. vulgare* showed the maximum activity followed by methanol extract while chloroform extract showed the minimum activity. Whereas according to Beta carotene method**,** methanol extract, showed highest activity followed by chloroform and water extract. FTC method showed quiet different results according to this method, methanol extract showed the maximum activity followed by chloroform extract while water extract showed the minimum activity

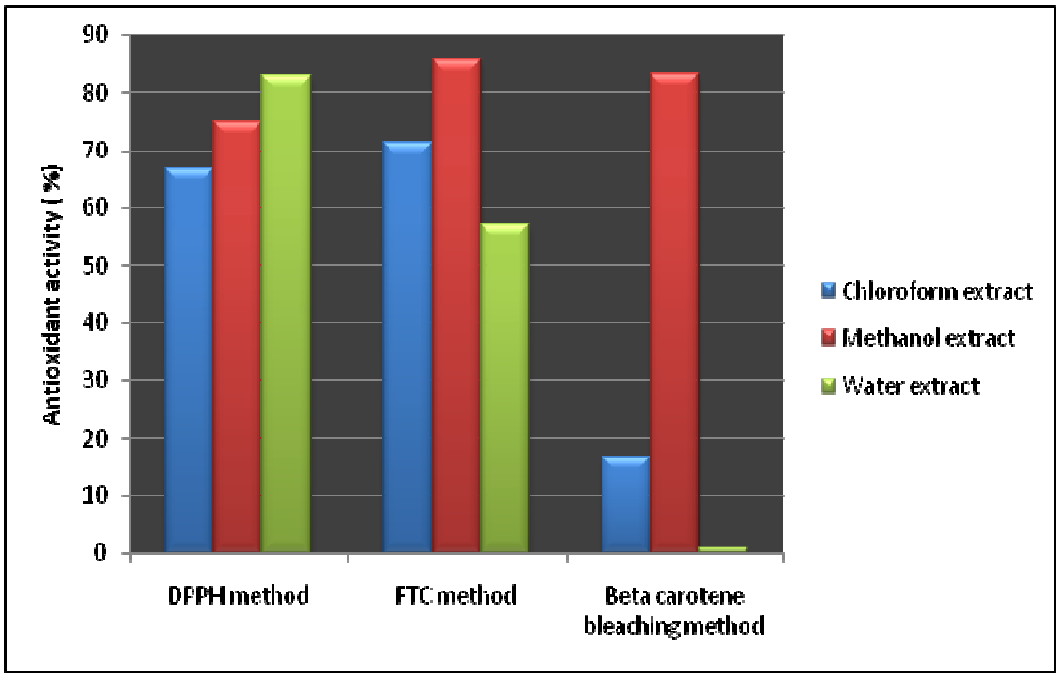

Figure 44: Antioxidant activity (%) of different extracts of *Foeniculum vulgare* fruit

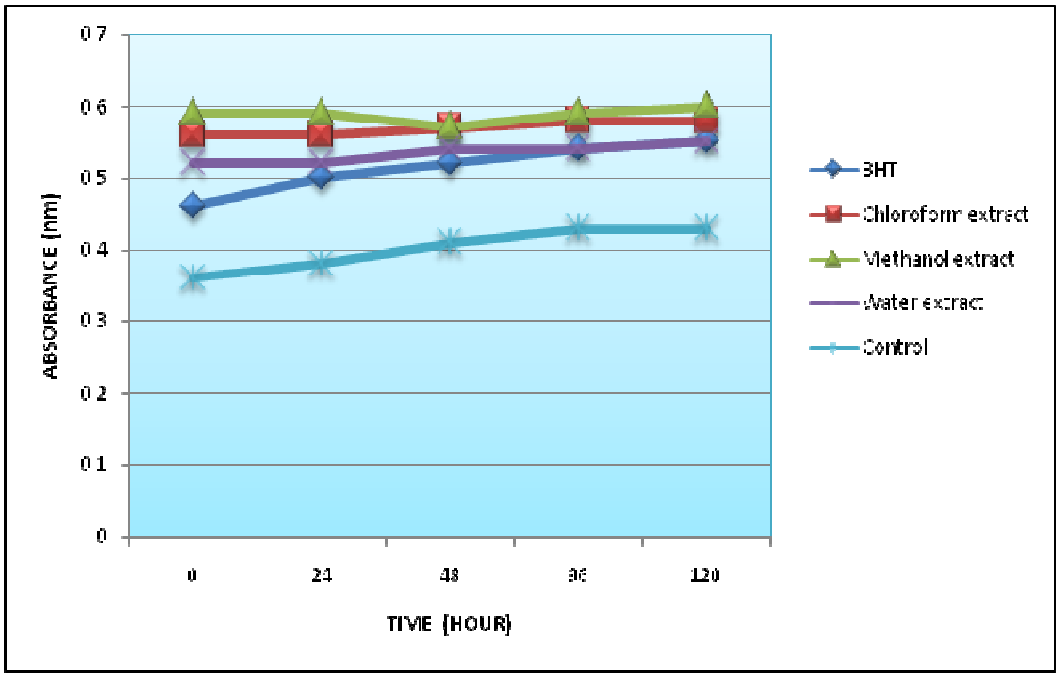

Figure 45: Antioxidant activity of various extracts of *Kigelia pinnata* and BHT (Standard) at 0.1mg/ml by FTC method

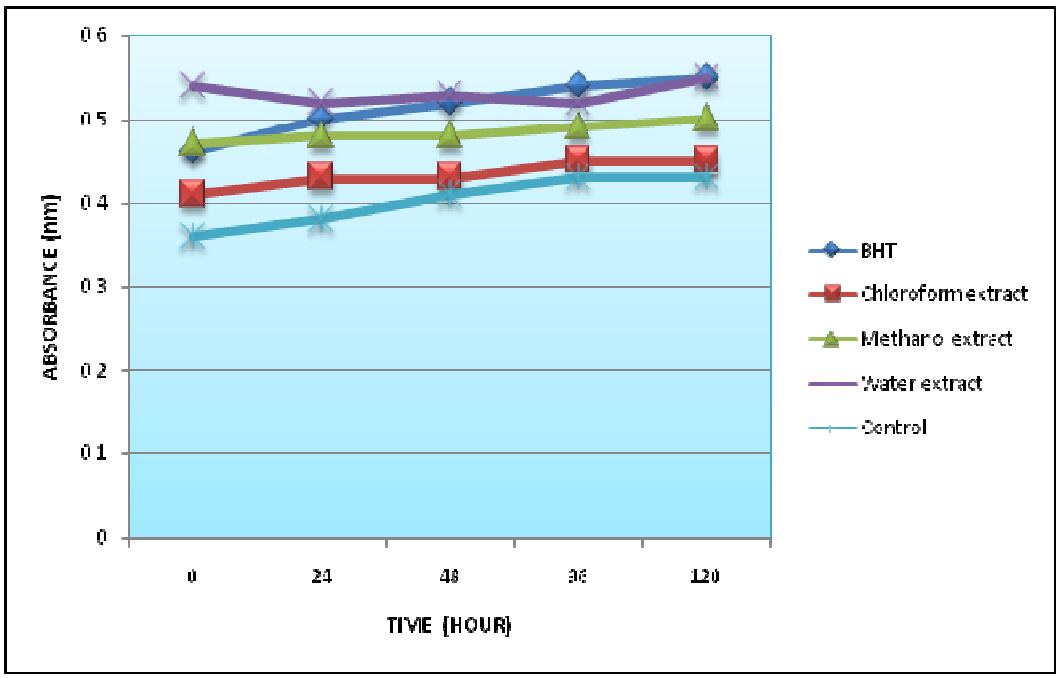

Figure 46: Antioxidant activity of various extracts of *Terminalia arjuna* and BHT at 0.1mg/ml by FTC method

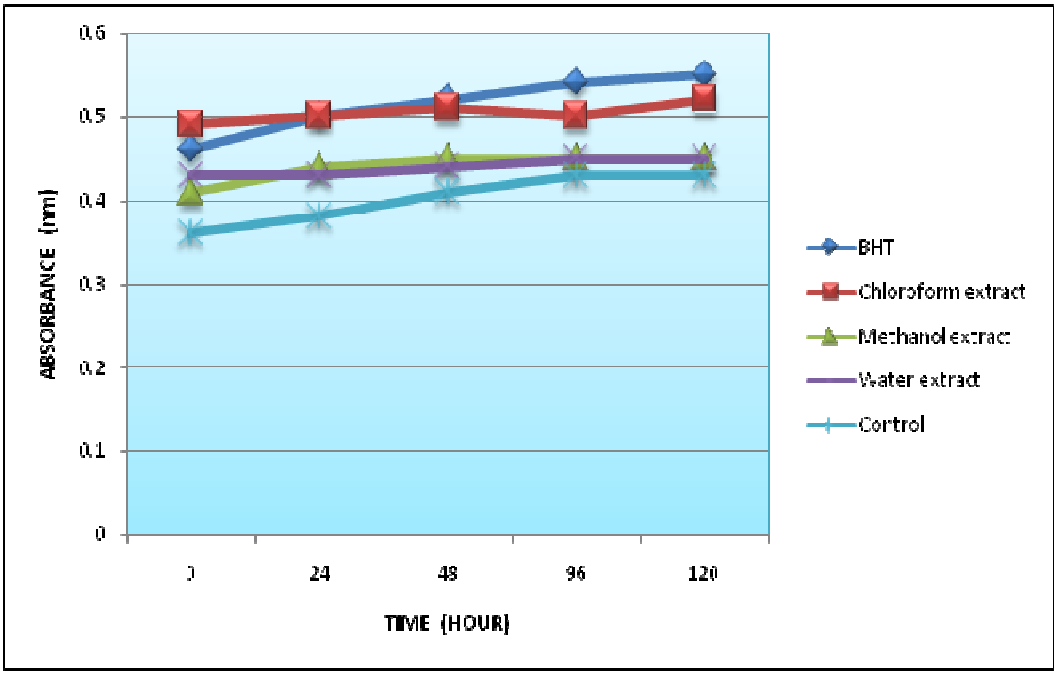

Figure 47: Antioxidant activity of various extracts of *Zingiber officinale* and BHT at 0.1mg/ml by FTC method

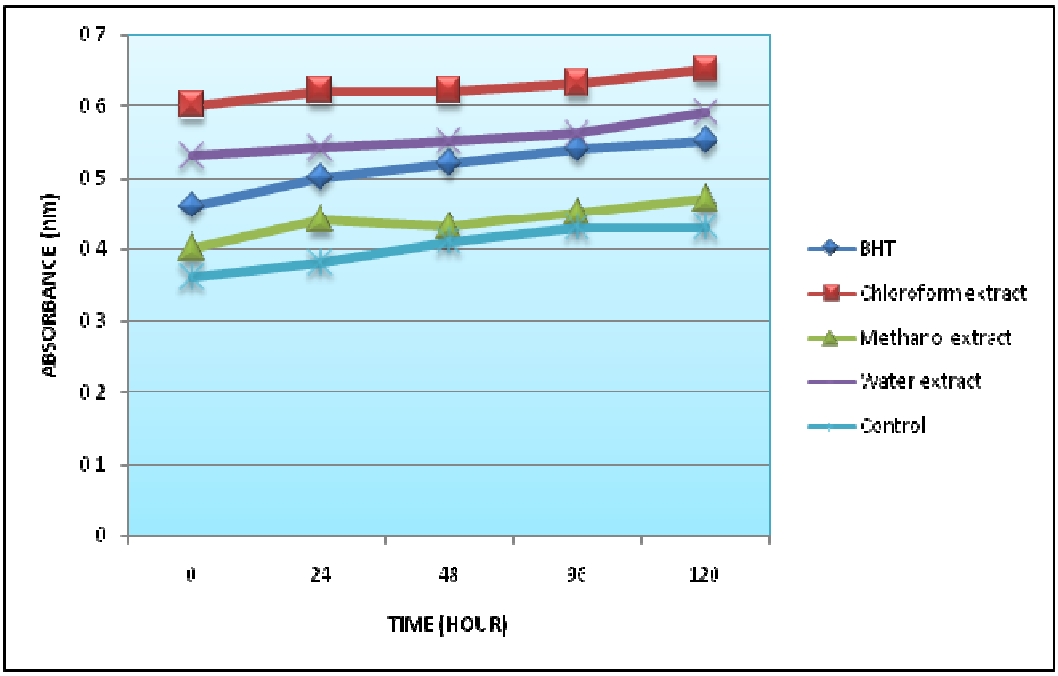

Figure 48: Antioxidant activity of various extracts of *Cuminum cyminum* and BHT at 0.1mg/ml by FTC method

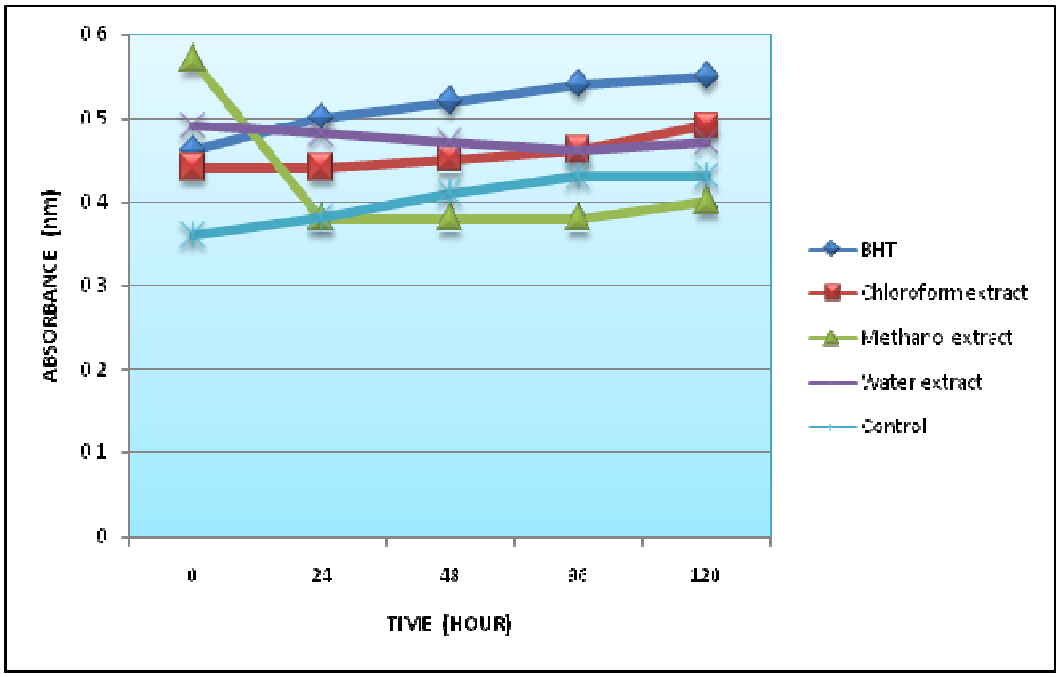

Figure 49. Antioxidant activity of various extracts of *Foeniculum vulgare* and BHT at 0.1mg/ml by FTC method

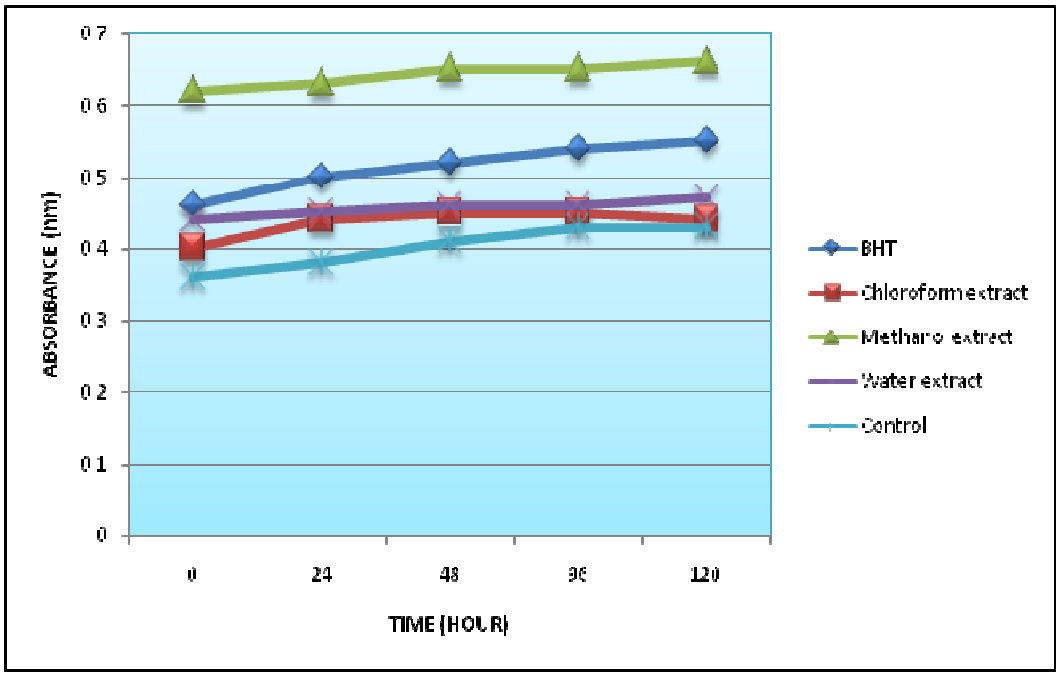

Figure 50: Antioxidant activity of various extracts of *Trigonella foenum* and BHT at 0.1mg/ml by FTC method

Chapter-V

DISCUSSION

The findings and important results obtained in the present investigation entitled 'Studies on chemical composition of some Indian medicinal plants and their antioxidant activity'have been discussed below under appropriate headings:

5.1 Determination of total phenols

5.2 Determination of flavonoids

5.3 Determination of minerals

5.3.1 Copper

5.3.2 Manganese

5.3.3 Zinc

5.3.4 Iron

5.4 Anti-oxidant and antiradical activity of extracts

5.4.1 Anti-oxidant activity of extracts by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT)

5.4.2 Antiradical activity of extracts by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

5.1 Determination of total phenols

The phenols were determined by Folin Ciocalteu method. The amount of phenols ranged from 1.0 mg GAE/g (methanol extract of *T. foenum*) to 27.0 mg GAE/g (water extract of *F. vulgare*). The data is shown in Table 2.

In *T.arjuna,* the maximum amount of phenol was present in the methanol extract (16.0 mg GAE/g), whereas the chloroform extract and water extract contained almost same amount of phenol (15.0 mg GAE/g).The result was in corroboration with Padma Sree *et al*., 2007, according to which the ethanol extract showed the highest phenolic content among various extracts of different polarity of *T.arjuna.*

The maximum amount of phenol was found in chloroform extract (8.0 mg GAE/g) followed by methanol extract (6.0 mg GAE/g) and minimum amount in water extract (4.0 mg GAE/g) in *K. pinnata*. Similar trend was found in *Z. officinale ,* i.e. maximum amount of phenol was found in chloroform extract (22.5 mg GAE/g) followed by methanol extract (16.5 mg GAE/g). The water extract contained 11.0 mg GAE/g phenols. However, Moraes-de-Souza *et al.* in 2007 reported, 2.3 mg GAE/g of phenol in water extract of *Z. officinale* . It

may be due to different species used, different stage of ripening or different method of extract preparation in our study.

The water extract of *F. vulgare* contained highest amount of phenol i.e. 27.0 mg GAE/g, whereas methanol extract contained 24.0 mg GAE/g and chloroform extract contained minimum amount of phenol i.e. 4.0 mg GAE/g. In *C. cyminum,* the maximum amount of phenol was found in methanol extract i.e. 19.0 mg GAE/g and similar amount of phenol was found in chloroform extract and water extract i.e. 18.0 mg GAE/g.

Whereas in *T. foenum,* maximum amount of phenol was present in water extract (22.0 mg GAE/g) followed by chloroform extract (1.5 mgGAE/g) and minimum of phenol was found in methanol extract (1.0mg GAE/g).

In the present investigation, maximum amount of phenol was found in water and methanol extract, because the solubility of phenolic compounds is higher in polar solvents (Mavi *et al.,* 2003). This is in agreement with other report where the ethanol extract showed the highest phenolic content among various extracts studied of different polarity from *Maydis stigma* (Zoran *et al.,* 2005).

Nevertheless, it is important to consider that several factors might affect the content of phenolic compounds found in extracts, such as the way of preparation (plant processing, concentration, time and temperature of extract and the method of analysis), the herb (species, part used, stages of development) and the cultivation characteristics.

5.2 Determination of flavonoids

The data presented in Table 3 revealed that maximum amount of flavonoid was present in chloroform extract of *Z. officinale* i.e. 60.0 mg CE/g and minimum amount was found in chloroform extract of *T. foenum* i.e.1.5 mg CE/g. Whereas in *T. arjuna* maximum amount of flavonoids were present in methanol extract (35.0 mg CE/g) followed by water extract (25.0 mg CE/g) and minimum of flavonoids were found in chloroform extract (3.0 mg CE/g). However, in earlier studies (Padma Sree *et al*., 2007), the chloroform extract showed the highest flavonoid content whereas ethanol and water extract showed negligible amount of flavonoid in *T.arjuna.*

The water extract of *K. pinnata* contained maximum amount of flavonoid i.e. 60.1 mg CE/g, followed by chloroform extract which contained 10.0 mg CE/g. Minimum amount was present in methanol extract i.e. 9.5 mg CE/g.

In *Z. officinale* maximum amount of flavonoid was present in chloroform extract (60.0 mg CE/g) of followed by methanol extract (50.0 mg CE/g) and minimum of flavonoid was found in water extract (42.0 mg CE/g) and in *T. foenum,* maximum amount of flavonoid was present in water extract (18.0 mg CE/g) followed by methanol extract (8.0 mg CE/g) and minimum amount of flavonoid was found in chloroform extract (1.5 mg CE/g).

Maximum amount of flavonoid was present in water extract (50.0 mg CE/g) followed by methanol extract (22.0 mg CE/g) and minimum amount of flavonoid was found in chloroform extract (8.0 mg CE/g) in *C. cyminum.*

Whereas in *F. vulgare* maximum amount of flavonoid was present in water extract (19.0 mg CE/g) followed by methanol extract (8.0 mg CE/g) and minimum of flavone was found in chloroform extract (3.0 mg CE/g).

The concentration of flavonoids in plant extracts depends on the polarity of solvents used in the extract preparation. The data obtained revealed that values of the concentration of flavonoids in the examined extracts, it was found that the highest concentration of these compounds was in the extracts obtained using solvents of high polarity.

5.3 Determination of minerals

The mineral content was determined by using Atomic Absorption Spectrophotometer. Following four minerals were detected:

5.3.1 Copper

5.3.2 Manganese

5.3.4 Zinc

5.3.3 Iron

5.3.1 Copper

According to the data presented in Table 4, in *T.arjuna,* the maximum amount of copper was found in the water extract (4.0 ppm), followed by methanol extract (2.0 ppm) and minimum amount was found in chloroform extract (1.0 ppm).The result was in corroboration with Padma Sree *et al*., 2007, according to which the ethanol extract showed the highest phenolic content among various extracts of *T. arjuna* studied of different polarity.

Water extract of *K. pinnata* showed the maximum amount of copper i.e. 16.0 ppm, followed by methanol extract (6.0 ppm) and minimum amount was found in chloroform extract (2.0 ppm) (Table 4).

The maximum amount of copper was found in the water extract (20.0 ppm), followed by methanol extract (14.0 ppm) and minimum amount was found in chloroform extract (2.0 ppm) in *Z. officinale* . According to Ujowundu *et al*. 2011, water extract of ginger contains 3.52 mg kg^{-1} copper. The variation in result may be due to different species used or different stage of ripeness or different method of extract preparation.

The methanol extract of *F. vulgare* contained highest amount of copper i.e. 14.0 ppm whereas water extract contained 6.0 ppm copper and cholorform extract contained minimum amount of copper i.e. 2.0 ppm. In *C. cyminum* the maximum amount of copper was found in water extract (8.0 ppm) at minimum amount of copper was found in cholorform extract (2.0 ppm) whereas methanol extract of *C. cyminum* contained 4.0 ppm of copper.

Whereas in *T. foenum,* the maximum amount of copper was found in the water extract (10.0 ppm), followed by methanol extract (8.0 ppm) and minimum amount was found in chloroform extract (2.0 ppm). The result was in corroboration with Yuksel *et al*., 2005, according to which *T. foenum* contains 9.44 µg/g of copper in its water extract.

5.3.2 Manganese

The data presented in Table 5 revealed that the maximum amount of manganese was found in the water extract (78.0 ppm), followed by methanol extract (36.0 ppm) and minimum amount was found in chloroform extract (30.0 ppm) in *T.arjuna*.

In *K. pinnata,* the maximum amount of manganese was found in the water extract (90.0 ppm), followed by methanol extract (88.0 ppm) and minimum amount was found in chloroform extract (80.0 ppm).

The methanol extract of *Z. officinale* contained highest amount of manganese (102.0 ppm), whereas water extract contained 86.0 ppm manganese and choloroform extract contained minimum amount i.e. 58.0 ppm. Similar in *F. vulgare,* also highest amount of manganese was found in the methanol extract (88.0 ppm), followed by water extract (68.0 ppm) and minimum amount was found in chloroform extract (64.0 ppm).

In *C. cyminum* the maximum amount of manganese was found in the water extract (136.0 ppm), followed by chloroform extract (78.0 ppm) and minimum amount of manganese was found in methanol extract (76.0 ppm). Whereas in *T. foenum,* the maximum amount of manganese was found in the water extract (15.8 ppm), followed by methanol extract (88.0 ppm) and minimum amount was found in chloroform extract (34.0 ppm). The result was in corroboration with Yuksel *et al*., 2005, according to which *T. foenum* contains 15.8 µg/g of manganese in its water extract.

5.3.3 Zinc

According to the data given in Table 6 in *T.arjuna,* the maximum amount of zinc was found in the water extract (46.0 ppm), followed by methanol extract (15.0 ppm) and minimum amount was found in chloroform extract (10.0 ppm).

In *K. pinnata,* the maximum amount of zinc was found in the water extract (166.0 ppm), followed by methanol extract (50.0 ppm) and minimum amount was found in chloroform extract (48.0 ppm) and in *Z. officinale ,* the maximum amount of zinc was found in the water extract (94.0 ppm), followed by methanol extract (76.0 ppm) and minimum amount was found in chloroform extract (28.0 ppm).

The maximum amount of zinc was found in the methanol extract (128.0 ppm), followed by chloroform extract (84.0 ppm) and minimum amount was found in water extract (72.0 ppm) in *F. vulgare* and in *C. cyminum* the maximum amount of zinc was found in the chloroform extract (128.0 ppm), followed by water extract (124.0 ppm) and minimum amount was found in methanol extract (70.0 ppm).

Whereas in *T. foenum,* the maximum amount of zinc was found in the water extract (94.0 ppm), followed by methanol extract (76.0 ppm) and minimum amount was found in chloroform extract (28.0 ppm). Yuksel *et al.,* 2005 reported 54.6µg/g of zinc in it water extract of *T. foenum*. Cu and Mn contents in *T. foenum*, were found in accordance with the results of previous studies on Fenugreek seed but Fe and Zn contents of Fenugreek seed, determined in this study were low with respect to results obtained by Yuksel *et al.,* 2005.

The results showed that maximum amount of minerals i.e. copper, manganese, iron and zinc were present in water extract followed by methanol extract and minimum in chloroform extract. This indicates that water is a very efficient solvent for extraction of minerals. Many factors affect the elemental contents of plants, viz. variety, state of ripeness, soil type, soil condition, fertilization, irrigation and weather (Hardisson *et al*., 2001).

5.3.4 Iron

From Table 7, it has been concluded that in *T.arjuna,* the maximum amount of iron was found in the water extract (8.8 ppm), followed by methanol extract (1.8 ppm) and minimum amount was found in chloroform extract (1.0 ppm).

The maximum amount of iron was found in the water extract (9.6 ppm), followed by methanol extract (6.2 ppm) and minimum amount was found in chloroform extract (4.0 ppm) in *K. pinnata* and in *Z. officinale* , the maximum amount of iron was found in the water extract (130.0 ppm), followed by methanol extract (15.4 ppm) and minimum amount was found in chloroform extract (7.0 ppm). The result was in corroboration with Ujowundu *et al*. 2011, according to which *Z. officinale* contains 138 mg kg^{-1} of iron in its water extract.

In *F. vulgare,* the maximum amount of iron was found in the water extract (24.0 ppm), followed by methanol extract (11.4 ppm) and minimum amount was found in chloroform extract (4.0 ppm).

The maximum amount of iron was found in the water extract (52.0 ppm), followed by methanol extract (11.2 ppm) and minimum amount was found in cholorform extract (4.0 ppm) in *C. cyminum*.

Whereas in *T. foenum,* the maximum amount of iron was found in the water extract (28.0 ppm), followed by methanol extract (3.8 ppm) and minimum amount was found in chloroform extract (2.8 ppm). The result was not in corroboration with Yuksel *et al.,* 2005, according to which *T. foenum* contains 62.6µg/g of iron in its water extract.

5.4 Anti-oxidant and Antiradical activity of extracts

The antioxidant activity and anti-radical activity of different extracts of these plants was measured by three different methods viz; β-carotene bleaching method (BCBT), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric thiocyanate (FTC) method. The use of simplified models to evaluate antioxidant activity is very important for studies aiming to determine this biological property of foods. Among the various models

of antioxidant activity, both in terms of scavenging activity of radicals and oxidation inhibition in lipidic system; three are frequently used because they are quick, sensitive and do not need equipment or reagents that are difficult to find: DPPH method, Ferric Thiocyanate (FTC) method and β-carotene bleaching method.

5.4.1 Anti-oxidant activity of extracts by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT) and 5.4.2. Antiradical activity of extracts by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

According to FTC method (Table 8)**,** methanol extract of *K. pinnata,* methanol extract of *T. arjuna ,* chloroform extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 85.7% while chloroform extract of *T. foenum* and methanol extract of *C. cyminum* showed minimum activity i.e. 28.5%.

As revealed from the data presented in Table 9, β-carotene bleaching method (BCBT) method showed quiet different results. According to this method, the water extract of *T.arjuna,* methanol extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 83.3% while chloroform and water extract of *C. cyminum* showed minimum activity i.e. 33.3%.

The data given in Table 10, of DPPH radical scavenging method revealed that the following extracts showed maximum antioxidant activity chloroform extract of *K. pinnata,* and water extract of *T. arjuna* i.e. 91.7%*,* chloroform extract of *Z. officinale* , chloroform and water extract of *C. cyminum* showed 91% activity while water extract of *T. foenum* showed minimum activity.i.e. 75%.

The results obtained for antioxidant activity may vary due to variation in reagent quality, envoirnmental conditions, sample concentration, method of determination and ways to present the results.

Chapter-VI

SUMMARY AND CONCLUSION

Medicinal plants possessing natural antioxidants polyphenolics such as anthraquinones, flavonoids, aromatic acids, and tannins have been shown to have reactive oxygen species (ROS) scavenging and lipid peroxidation prevention effects. The commercial development of plants as sources of antioxidants to enhance health and food preservation is of current interest. Epidemiological studies have suggested positive assoc-iations between the consumption of phenolic-rich foods or beverages and the prevention of diseases. These effects have been attributed to antioxidant components such as plant phenolics, including flavonoids and phenylpropanoids among others. Evidence is mounting for the role of these dietary phytochemicals, including flavonoids, ascorbic acid, ∞-tocopherol, and carotenoids, in the maintenance of health and protection from disease. As plants produce antioxidants to control the oxidative stress caused by sunlight and oxygen, they became a source of useful new compounds with antioxidant activity.

Different anti-oxidant substances occur in plant tissues especially fruits and vegetables. This makes it relatively difficult to measure each anti-oxidant component separately. Therefore several methods including oxygen radical absorption capacity method, ferric reducing antioxidant capacity method, liposome assay, lipid peroxidation and total oxyradical scavenging capacity assay have been developed in recent years to calculate the total anti-oxidant activity of biological samples. Antioxidant activity represents the capability of scavenging free radical and offering hydrogen atom.

Little literature data is available regarding anti-oxidant activity of *Terminalia arjuna, Kigelia pinnata, Zingiber officinale, Foeniculum vulgare, Cuminum cyminum* and *Trigonella foenum* therefore; the research was undertaken with the following objectives:

1. Quantitative estimation of total phenols, flavonoids and minerals.
2. Contribution of these compounds to anti-oxidant and antiradical activity.

The bark of *T. arjuna* and fruit of *K. pinnata* were procured from the university campus of Chaudhary Charan Singh Haryana Agricultural University, Hisar.While fruit of *F. vulgare, C. cyminum;* rhizome of *Z. officinale* and seed of *T. foenum* were purchased from the local market of Hisar.

Samples of commonly used parts of each plant material the bark of *Terminalia arjuna,* fruits of *Kigelia pinnata, Foeniculum vulgare* and *Cuminum cyminum;* rhizome of *Zinziber officinale* and seeds of *Trigonella foenum* was have been extracted separately with

chloroform, methanol and distilled water.The antioxidant activity and phenols, flavonoids and minerals of these extracts were than determined using various methods.

6.1 Determination of total phenols

There was large variation in the phenol content. Total phenolic content varied from 1.0 mg GAE/g (methanol extract of *T. foenum*) to 27.0 mg GAE/g (water extract of *F. vulgare*) of extract when Folin Cio-calteu method was used. A standard curve using gallic acid as standard was prepared for the determination of phenol content of extracts.

1. Chloroform extract

Zingiber officinale (mg GAE/g) (22.5) > *Cuminum cyminum* (18.0) > *Terminalia arjuna* (15.0) > *Kigelia pinnata* (8.0) > *Foeniculum vulgare* (4.0) > *Trigonella foenum* (1.5)

2. Methanol extract

Foeniculum vulgare (mg GAE/g) (24.0) > *Cuminum cyminum* (19.0) > *Zingiber officinale* (16.5) > *Terminalia arjuna* (16.0) > *Kigelia pinnata* (6.0) > *Trigonella foenum* (1.0)

3. Water extract

Foeniculum vulgare (mg GAE/g) (27.0) > *Trigonella foenum* (22.0) > *Cuminum cyminum* (18.0) > *Terminalia arjuna* (15.0) > *Zingiber officinale* (11.0) > *Kigelia pinnata* (4.0)

6.2 Determination of flavonoids

The flavonoids were measured by aluminium chloride colorimetric method described by Marinova *et al*., 2005. Maximum amount of flavonoids were present in chloroform extract of *Z. officinale* i.e.60.0 mg CE/g of the extract and minimum in chloroform extract of *T. foenum* i.e.1.5 mg CE/g of the extract.

1. Chloroform extract

Z. officinale (mg CE/g) (60.0) > *K. pinnata* (10.0) > *C. cyminum* (8.0) > *T. arjuna* = *F. vulgare* (3.0) > *T. foenum* (1.5)

2. Methanol extract

Z. officinale (mg CE/g) (42.0) > *T. arjuna* (35.0) > *C. cyminum* (22.0) > *K. pinnata* (9.5) > *Z. officinale* (8.0) = *T. foenum* (8.0)

3. Water extract

K. pinnata (mg CE/g) (60.0) > *C. cyminum* (50.0) > *T. arjuna* (25.0) > *F. vulgare* (19.0) > *T. foenum* (18.0) > *Z. officinale* (5.0)

6.3 Determination of minerals

The minerals were determined by using Atomic Absorption Spectrophotometer (AAS). Following four minerals were detected:

6.3.1 Copper

Maximum amount of **c**opper was detected in water extract of *Z. officinale* (20.0 ppm) and minimum amount in chloroform extract of *T. arjuna* (1.0 ppm).

1. Chloroform extract

Z. officinale = *C. cyminum* = *T. foenum* = *K. pinnata* = *F. vulgare* (ppm) (2.0) > *T. arjuna* (1.0)

2. Methanol extract

F. vulgare = *Z. officinale* (ppm) (14.0) > *T. foenum* (8.0) > *K. pinnata* (6.0) > *C. cyminum* (4.0) > *T. arjuna* (2.0)

3. Water extract

Z. officinale (ppm) (20.0) > *K. pinnata* (16.0) > *T. foenum* (10.0) > *C. cyminum* (8.0) > *F. vulgare* (6.0) > *T. arjuna* (4.0)

6.3.2 Manganese

Maximum amount of manganese was found in water extract of *C. cymium* (136.0 ppm) and minimum amount in chloroform extract of *T. foenum* (8.0 ppm).

1. Chloroform extract

K. pinnata (ppm) (80.0) > *C. cyminum* (78.0) > *F. vulgare* (64.0) > *Z. officinale* (58.0) > *T. arjuna* (30.0) > *T. foenum* (8.0)

2. Methanol extract

Z. officinale (ppm) (102.0) > *F. vulgare* (88.0) = *K. pinnata* (88.0) > *C. cyminum* (76.0) > *T. arjuna* (36.0) > *T. foenum* (13.0)

3. Water extract

C. cyminum (ppm) (136.0) > *K. pinnata* (90.0) > *Z. officinale* (86.0) > *T. arjuna* (78.0) > *F. vulgare* (68.0) > *T. foenum* (15.8)

6.3.3 Zinc

Maximum amount of zinc was found in water extract of *K. pinnata* (166 ppm) and minimum amount in chloroform extract of *T. arjuna* (10 ppm).

1. Chloroform extract

C. cyminum (128.0) (ppm) > *F. vulgare* (84.0) > *K. pinnata* (48.0) > *T. foenum* (28.0) =*Z. officinale* (28.0) > *T. arjuna* (10.0)

2. Methanol extract

F. vulgare (128.0) (ppm) > *Z. officinale* (76.0) =*T. foenum* (76.0) > *C. cyminum* (70.0) > *K. pinnata* (50.0) > *T. arjuna* (15.0)

3. Water extract

K. pinnata (166) (ppm) > *C. cyminum* (124.0) > *Z. officinale* (94.0) = *T. foenum* (94.0) > *F. vulgare* (72.0) > *T. arjuna* (46.0)

6.3.4 Iron

Maximum amount of iron was found in water extract of *Z. officinale* (130.0 ppm) and minimum amount in chloroform extract of *T. arjuna* (1.0 ppm).

1. Chloroform extract

Z. officinale (ppm) (7.0) > *C. cyminum* (4.0) =*K. pinnata* (4.0) =*F. vulgare* (4.0) > *T. foenum* (2.8) > *T. arjuna* (1.0)

2. Methanol extract

Z. officinale (ppm) (15.4) > *F. vulgare* (11.4) > *C. cyminum* (11.2) > *K. pinnata* (6.2) > *T. foenum* (3.8) > *T. arjuna* (1.8)

3. Water extract

Z. officinale (ppm) (130.0) > *C. cyminum* (52.0) > *T. foenum* (28.0) > *F. vulgare* (24.0) > *K. pinnata* (9.6) > *T. arjuna* (8.8)

6.4 Anti-oxidant and antiradical activity of extracts

The antioxidant activity and anti-radical activity of different extracts of these plants was measured by three different methods viz; β-carotene bleaching method (BCBT), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric thiocyanate (FTC) method.

6.4.1 Anti-oxidant activity of extracts by ferric thiocyanate (FTC) method and β-carotene bleaching method (BCBT)

6.4.1.1 FTC method

The following trend was observed in the antioxidant activity of plants:

1. Chloroform extract

Z. officinale (%) (85.7) > *F. vulgare* (71.4) =*K. pinnata* (71.4) > *C. cyminum* (57.1) > *T. arjuna* (42.8) > *T. foenum* (28.5)

2. Methanol extract

K. pinnata (%) (85.7) =*T. arjuna* (85.7) =*F. vulgare* (85.7) > *T. foenum* (57.1) > *Z. officinale* (42.8) > *C. cyminum* (28.5)

3. Water extract

T. arjuna = K. pinnata = Z. officinale (%) (71.4) > *F. vulgare* (57.1) =*C. cyminum* (57.1) > *T. foenum* (42.8)

6.4.1.2 β-carotene bleaching method (BCBT) method

The following trend was observed in the antioxidant activity of plants:

1. Chloroform extract

Z. officinale (%) (50.0) =*K. pinnata* (50.0) > *C. cyminum* (33.3) > *F. vulgare* (16.6) =*T. arjuna* (16.6) =*T. foenum* (16.6)

2. Methanol extract

Z. officinale (%) (83.3) = *F. vulgare* (83.3) > *K. pinnata* (66.6) =*T. arjuna* (66.6) > *C. cyminum* (16.6) =*T. foenum* (16.6)

3. Water extract

T. arjuna (%) (83.3) > *T. foenum* (66.6) > *C. cyminum* (33.3) = *K. pinnata* (33.3) =*Z. officinale* (33.3) > *F. vulgare* (16.6)

6.4.2 Antiradical activity of extracts by 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method

1. Chloroform extract

Z. officinale = *C. cyminum*=*K. pinnata* (%) (91.7) > *T. arjuna* (75) > *F. vulgare* = *T. foenum* (66.7).

2. Methanol extract

C. cyminum (91.0) (%) > *K. pinnata* (85.0) > *T. arjuna* (83.3) = *Z. officinale* (83.3) > *F. vulgare*(75.0) > *T. foenum* (58.0)

3. Water extract

T. arjuna (91.7) (%) > *F. vulgare* (83.3) = *K. pinnata* (83.3) > *C. cyminum* (75.0) = *T. foenum* (75.0) > *Z. officinale* (66.7)

In the present study, the phenols and flavonoids of different extracts were found to be directly related to the antioxidant capacity suggesting that the antioxidant activity in these spices is largely due to the presence of phenolic and flavonoid components. The maximum amount of copper, manganese, iron and zinc was present in water extract followed by methanol extract and minimum in chloroform extract. Water extract of *K. pinnata*, methanol extract of *F. vulgare* and chloroform and water extract of *C. cyminum* have substantial amount of zinc. Water extract of *C. cyminum* and methanol extract of *Z. officinale* are rich in manganese. Water extract of *Z. officinale* is rich in copper content. Water extract of *Z. officinale* has high iron conent (130ppm).

The following extracts showed maximum antioxidant activity according to DPPH radical scavenging method, chloroform extract of *K. pinnata,* and water extract of *T. arjuna* i.e. 91.7%, chloroform extract of *Z. officinale* , chloroform and methanol extract of *C. cyminum* showed 91% activity while water extract of *T. foenum* showed minimum activity.i.e. 75%.

According to FTC method **,** methanol extract of *K. pinnata,* methanol extract of *T. arjuna ,* chloroform extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 85.7%, while chloroform extract of *T. foenum* and methanol extract of *C. cyminum* showed minimum activity i.e. 28.5%.

β-carotene bleaching method (BCBT) method showed quiet different results. According to this method, the water extract of *T.arjuna,* methanol extract of *Z. officinale* , methanol extract of *F. vulgare* showed highest activity i.e. 83.3% while chloroform extract of *C. cyminum,* water extract of *K. pinnata, C. cymium* and *Z. officinale* showed minimum activity i.e. 33.3%.

The variations in the results may occur due to variation in reagent quality, envoirnmental conditions, sample concentration, method of determination and ways to present the results. Nevertheless, these results indicate that spices containing high phenols provide a source of dietary anti-oxidants and in addition to imparting flavor to the food, they possess potential health benefits.

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ABSTRACT

Title of Thesis	:	**Studies on chemical composition of some Indian medicinal plants and their antioxidant activity**
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Total number of Thesis pages	:	67+vii
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Key words: Phenols, flavonoids, minerals, antioxidant activity and anti radical activity

The total phenols, flavonoids and minerals (Cu, Mn, Zn, Fe) in six Indian medicinal plants viz.; *Terminalia arjuna, Kigelia pinnata, Zinziber officinale, Foeniculum vulgare, Cuminum cyminum* and *Trigonella foenum* were determined using chloroform, methanol and water as solvents. Total phenols were estimated by Folin Cio-calteu method, flavonoids by aluminum chloride colorimetric method and minerals by using Atomic Absorption Spectrophotometer (AAS). The antioxidant activity and antiradical activity of different extracts of these plants were measured by three different methods viz; β-carotene bleaching method (BCBT), 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity and ferric thiocyanate (FTC) method. Maximum concentration of phenols in chloroform extract was estimated from *Z. officinale* (22.5 mg GAE/g), 24.0 mg GAE/g from *F. vulgare* in methanol and 27.0 mg GAE/g in water extract from *F. vulgare* using Folin Cio-calteu method. Colorimetric method found effective in extracting the flavonoids to the extent of 60 mg CE/g from *Z. officinale* in chloroform, 42.0 mg CE/g in methanol from *Z. officinale* and 60.1 mg CE/g from *K. pinnata* in water. Among minerals, maximum concentration of Cu, Mn, Zn and Fe were detected in water extract of *Z. officinale* (20.0 ppm), water extract of *C. cyminum* (136 ppm), water extract of *K. pinnata* (166 ppm), water extract of *Z. officinale* (130 ppm), respectively. Best result of antioxidant activity (85.7%) was shown by FTC method in chloroform extract of *Z. officinale* and methanol extract of *K. pinnata*. Antiradical activity of extracts was determined by DPPH free radical scavenging method. Highest activity (91.7%) was shown by chloroform extract of *K. pinnata* and water extract of *T.arjuna.*

MAJOR ADVISOR **SIGNATURE OF THE DEGREE HOLDER**

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