ANTIOXIDATIVE AND FREE RADICAL SCAVENGING ACTIVITIES OF FEW PLANTS USED AS SPICES

By

JAMSHINA K K

(16PBO004)

Dissertation submitted to

AVINASHILINGAM INSTITUTE FOR HOME SCIENCE AND HIGHER EDUCATION FOR WOMEN

COIMBATORE-43

In partial fulfillment of the requirements for the

DEGREE OF MASTER OF SCIENCE IN BOTANY

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Signature of the Head of the Department

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Signature of the Supervisor

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Introduction

Introduction

Plants are the lungs of nature. They are the backbone of all life on Earth and an essential resource for human well-being. Man in his struggle against disease or towards healthy life has always tried to use plants in a traditional way. The herbal medicines of ancient times practiced by the Assyrians (4000 BC), Sumerians (3500 BC), Indians(3500 BC),Chinese(3000BC)and Egyptians(2500 BC), etc. Thousands of years ago, several civilizations have started to use natural products in order to treat various diseases. Despite the big advances observed in modern medicine, the natural products continue to be used and 30% of all the drugs evaluated as therapeutical agents result of secondary metabolites (Calixto, 2005).

Food plays a major role in ayurvedic practice by supporting the body's healing processes. Metabolic diseases and diseases of the gastrointestinal tract are directly influenced by food. Spice is a strongly flavoured or aromatic substance of vegetable origin, obtained from tropical plants, which is commonly used as a condiment. From ancient times to present, Asia has been well known as the 'Land of Spices'. In India, kerala state is the leading which produces and exports the majority of spices for the world markets and so it is also known as Spice Bowl of the World (Chomchalow, 1996). Valuable spices present in India attracted explorers like Columbus which paved way for trade routes (Brown, 2003). The story of Indian Spices is more than 7000 years old. Various parts of the plants are used as spices Eg: *Cinnamon* – bark and leaves; *Murraya koengii* – leaves and *Mentha* – leaves (Parthasarathy, 2008)

Spices have medicinal properties. Herbs and spices are widely used as flavouring agents in cooking in oriental countries owing to their taste and aroma (Azzouz and Bullerman, 1982 and Hannah *et al*., 2010). Spices are well known appetizers and digestives having various phytochemicals in them and considered essential in the culinary art all over the world. Phytochemicals are defined in the strictest sense, as chemicals produced by plants. The name phytochemicals comes from the Greek word *phyton*, meaning plant. Tough Some phytochemicals have been used as poisons and others as medicine. However, the term is generally used to describe chemicals from plants that may enhance health status of

organisms, but are not essential nutrients (Srivastava *et al*., 2011). There is ample evidence to support the health benefits of the diet in the form of fruits, vegetable, legumes, whole grains and nuts (Mojab *et al*., 2003). Many of the indigenous medicinal plants are used as spices and food plants. They are also sometimes added to food for medicinal purposes for pregnant and nursing mothers (Okwu, 1999 and 2001). More than 2000 phytochemicals have been identified from plants (Taiz and Zeiger, 2006). Over 100 plant species are consumed world wide as vegetables, but of these, only about 20 species are grown globally and account for most of the vegetables produced and consumed (Siemonsma and Kasem, 1996).The phytochemical constituents of the medicinal plants were recorded by a number of workers (Joshi, 2000, Syed and Usha, 2005 and Ramasubbu 14 and Chandra Prabha, 2009). In India thousands of plant especially the angiosperms that all being exploited by the natives in tribal in variety of ways. The most important utilization of these plants is their application in medicines (Camciuc *et al*., 1998 and Felter *et al*., 2007). Because plant based foods are complex mixtures of bioactive compounds, information on the potential health of individual phytochemical is linked to information on the health effects of foods that contain those phytochemicals (Manjula *et al*., 2009).

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et a*l., 2005). Medicinal compounds from them are used all over the world as the most natural way to intake of phytochemicals. In this study three plants (*Murraya, Mentha and Cinnamon*) which are used for the purpose of seasoning in food material were taken to study the antioxidant and free radical scavenging activities in them.

Curry leaves (*M. koenigii*) belonging to the family Rutaceae are highly aromatic when rubbed or bruised. They are best used fresh in cooking (dried leaves may be used but have significantly diminished flavor). Aroma/flavor of the fresh leaves is enhanced when the leaves are fried in oil or butter. Curry leaves are often added to vegetable dishes. They add subtle flavors to many other dishes, including meat, seafood, chutneys, coconut sauces, relishes, marinades and omelettes. They are being used in traditional medicine, with various parts of the plants used to treat fever, pain, and dysentery. Compounds isolated from *Murraya* include many types of coumarins and alkaloids. It has yielded a vast array of compounds, including carbazoles and carotenoids. The leaves alone have been found to contain such compounds as koenimbine, koenigine, koenine, koenidine, koenimbidine, murrayacine, murrayanine, murrayazoline, and murrayazolidine.

Mentha species, (*Mentha arvensis*) locally known as pudina (Khan and Khatoon, 2008) belongs to the family Lamiaceae (mint family). It constitutes the most important source of therapeutic agents and is used in the alternative systems of medicine (Naeem *et al.*, 2011). Mints are aromatic, almost exclusively perennial, rarely annual herbs. Mint was originally used as a medicinal herb to treat stomach ache and chest pains. There are several uses in traditional medicine and preliminary research for possible use in treating irritable bowel syndrome. Menthol from mint essential oil (40–90%) is an ingredient of many cosmetics and some perfumes. Menthol and mint essential oil are also used in aromatherapy which may have clinical use to alleviate post-surgery nausea. The leaf, fresh or dried, is the culinary source of mint. Fresh mint is usually preferred over dried mint when storage of the mint is not a problem. The leaves have a warm, fresh, aromatic, sweet flavor with a cool aftertaste, and are used in teas, beverages, jellies, syrups, candies, and ice creams. In Middle Eastern cuisine, mint is used on lamb dishes, while in British cuisine and American cuisine, mint sauce and mint jelly are used, respectively. Mint is a necessary ingredient in Touareg tea, a popular tea in northern African and Arab countries. Mint essential oil and menthol are extensively used as flavorings in breath fresheners, drinks, antiseptic mouth rinses, toothpaste, chewing gum, desserts, and candies, such as mint (candy) and mint chocolate.

Cinnamomum tamala, Indian bay leaf, also known as *tejpat*, *tejapatta*, Malabar leaf, Indian bark, Indian cassia, or malabathrum, is a tree within the Lauraceae family which is native to India, Bangladesh, Nepal, Bhutan, and China. It is thought to have been one of the major sources of the medicinal plant leaves known in classic and medieval times as malabathrum (or malobathrum). The dried leaves of *Cinnamomum tamala* called tejpatta are used as a spice in Indian homes for seasoning (Hannah *et al.*, 2010) and in ayurvedic medicine in curing a number of ailments since ancient time (Gill *et al.*, 2015). Tejpatta (Indian Bay Leaf) is an important ingredient of Trijataka, which is an excellent remedy for the abdominal diseases. Individually, Tejpatta is also used due to its action on Vata Dosha and Kapha Dosha. In the ancient India, spices were used to prevent water-borne diseases and one of these spices is Tejpatta. It also induces sweating and digestive stimulant in higher dosage. Tejpatta is an excellent remedy to remove the gas from the human gut. It is anti-flatulent, antispasmodic, and digestive. It is likely to prevent gas formation in the gut when added to the food. You should remember it should only be used in cases of Vata and Kapha predominance. In people having intestinal gas along with gastritis or acidity, then it is not the suitable remedy. Tejpatta has antispasmodic and carminative action, which helps to treat

abdominal pain and cramps. It is more likely to be effective when pain or cramps are due to accumulated gas in the abdomen. Tejpatta reduces cholesterol and helpful in the treatment of Hyperlipidemia.

The objectives of the present study are as follows:

1. To investigate the twenty three phytochemicals present present in methanolic leaf extract of *Murraya koenigii, Cinnamon tamala and Mentha arvensis*.
2. To analyse and compare the biochemical parameters carbohydrates, proteins, total phenolics and flavonoids present in methanolic leaf extract of *M. koenigii*, *C.tamala* and *M.arvensis*.
3. To study the antioxidant activity of *M. koenigii*, *C.tamala* and *M. arvensis*.
4. To study and compare the free radical scavenging activities of the methanolic leaf extract of *M. koenigii*, *C.tamala* and *M.arvensis*.

Review of Literature

2. Review of Literature

Plant is known for having several phytochemicals, including polyphenols that are highly effective antioxidants and are less toxic than the synthetic ones. This property makes it of great interest to the Food Industry, since the phenolic compounds retard the oxidative degradation of lipids improving the quality and nutritional value of food. Herbs and spices are considered an important part of human diet and have been used for thousands of years in traditional medicine. As part of our diet, Spices and herbs have been used as functional food in addition to fruits and vegetables. Spices are common food additives, which are always used as flavoring, seasoning, and coloring agents, and sometimes as preservatives (Andrade and Ferreira 2013), due to their antimicrobial phenolic constituents (Zarai *et al*., 2012), but they can also protect people from acute and chronic diseases, due to their high antioxidant activity. It may also be used as medicines as many spices have been recognized to have medicinal properties and possess many beneficial effects on human health which include anti-sclerotic, antithrombotic, anti-carcinogenic, anti-inflammatory, antiarrhythmic, anti-rheumatic, gastroprotective, and lipid-lowering action. In addition, spices have radioprotective (protects against radiation), anti-allergic, and antimalarial effects. Spices inhibit the oxidation of low-density lipoprotein and protein glycation along with antibiotics.

Spices and culinary herbs are rich in antioxidants. Presence of active components in spices has been demonstrated over the last 30 years and their therapeutic properties have been demonstrated by the presence of a large group of bioactive compounds which consist of flavonoids, phenolic compounds, sulfur-containing compounds, tannins, alkaloids, phenolic diterpenes, and vitamins (Shan *et al.*, 2005; Charles, 2013; Yesilogu and Audin, 2013; Choi, 2014; Srinivasan, 2014 and McCormic, 2017). Antioxidants are referred to as compounds which are able to inhibit the oxidation of different bio molecules and helps in repairing the damages caused to the body tissues due to oxidation processes. Oxidative metabolism is essential for the survival of cells. A side effect of this dependence is the production of free radicals and other reactive oxygen species that cause oxidative changes.

The by-product of normal metabolism, drugs or ionizing radiations leads to the formation of free radicals or reactive oxygen species (ROS), or activated oxygen species

(AOS) (Freeman and Crapo, 1982). In plants ROS generated performs various functions like programmed cell death, pathogen defence, and stomatal behaviour (Apel and Hirt, 2004). There is a greater impact on humans both from within the body and the environment due to free radicals, particularly ROS. Moreover, environmental factors such as pollution, radiation, cigarette smoke and herbicides can also generate free radicals. The body possesses defence mechanisms in the form of enzymes and antioxidant nutrients, which prevents the damaging properties of ROS (Wu *et al*., 1998; Liao and Yin, 2000; Halliwell, 1999; Halliwell, 1995 and Sies, 1993). The amount of free radicals is increased in the body beyond its ability to control and cause irreversible oxidative damages due to continuous exposure to chemicals and contaminants (Tseng *et al*., 1997). This results in damage of essential proteins, DNA and lipids can be damaged by ROS and cause various human diseases like atherosclerosis, cancer, liver injury, cardiovascular disease, neurodegenerative disorders and rheumatism. Therefore, the ability of antioxidants to scavenging free radicals may have great role to play in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated (Soares *et al*., 1997). In this respect, the correlation between polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity (Brown and Rice-Evans, 1998; Gil *et al*., 1999; Kahkonen *et al*., 1999 and Vinson *et al*., 1995).

Various studies have focused on use of antioxidants from natural sources so that they could be used as healthy additives and potential antioxidant in our daily food intake habits (Msaada *et al*., 2012). Antioxidants either synthetic or natural are potent scavengers of free radicals and have beneficial effects on human health and disease prevention (Mohamed and l-okbi, 2004). The most widely used synthetic antioxidants in food i.e., butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) are very effective in their role as antioxidants. However, their use in food products has been failing off due to their instability or their suspected action as promoters of carcinogenesis. For this reason, there is a growing interest in the studies of natural, healthy (nontoxic) additives as potential antioxidants**1** especially of plant origin, has greatly increased in recent years (Jayaprakash and Rao, 2000). It is known that constituents of plants are associated in reducing the risk of many chronic diseases, in which antioxidants play a major role in their protective effects **(**Saeed *et al.*, 2012).

Natural antioxidants can protect lipids and oils in food against oxidative degradation. When added to food, antioxidants control rancidity development, retard the formation of toxic oxidation products, maintain nutritional quality, and extend the shelf-life of products. Therefore, spices could potentially be used as ameliorative or preventive agents for some health issues (Lim and Henry, 2017; Serafini and peluso, 2016; Surh, 2016; Coiandcha, 2014; Yesilogu *et al.*, 2013). The antioxidants are an important category of food preservatives, natural or synthetic, designed to prevent food from spoiling through oxidation, thus reducing loss of nutrients, and maintaining texture, colour pigments, taste, freshness, functionality, and aroma (Ahuja *et al.*, 2012).

Antioxidants of natural and synthetic origin prevent the free radical damage by its protective role such as reacting with them, chelating catalytic metals and by playing as oxygen scavengers. In the intervening time, the ingestion of several synthetic antioxidants such as BHT and BHA has been reported to have various possible toxicities to man (Lobo *et al*., 2010). Recently, due to the harmful side effects of synthetic antioxidants, and also importance towards natural antioxidant has now drawn attention of mankind. Different kinds of plants and its parts have already been proved as natural antioxidant sources (Lobo *et al*., 2010). It is generally assumed that frequent consumption of plant derived phytochemicals like phenolics and flavonoids from vegetables, fruit, tea, and herb provides good natural antioxidants (Halliwell, 1996). These natural antioxidants present offer health benefits in preventing various diseases by fighting cellular damage caused by free radicals in the body **15**. Free radicals have been implicated in the pathogenesis of various diseases such as cancer, diabetes, cardiovascular diseases, aging, oxidative stress and metabolic syndrome. The important radicals include superoxide anions, hydroxyl, nitric oxide, hydrogen peroxide radicals and singlet oxygen (Raghuveer & Tandon, 2009 and Hosseinimehr *et al*.,). Herbal drugs containing radical scavengers are gaining importance in treating such diseases1. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain by donating a hydrogen atom and may have great relevance in the prevention and treatment of diseases associated with oxidants or free radicals (Anindya *et al*., 2016).

Cinnamomum tamala

The genus *Cinnamomum* belongs to the family Lauraceae, comprising of many commercial spices. *C. tamala* (Tamil – Talishapattiri; Malayalam-Ilavannam, paccila; Hindi – tej patra, tejpat) It is the main ingredient of gram masala which is the main spice used in north Indian kitchens daily. It is also one among the spices that is commonly used as flavouring agents in Indian homes for seasoning.

Geographical Distribution

The genus *Cinnamomum* belonging to the family *lauraceae* comprises 270 species which occur naturally in Asia and Australia is indigenous to the Asian minor and southern Europe (Hannah *et al*., 2010). They are ever green plants and shrubs and most species are aromatic and many are economically important about 20 species occur in India (Anonymous 1950). *Cinnamomum tamala* Nees. and Eberm. (Lauraceae) is a moderate sized evergreen tree, found in India along the North - Western Himalayas, in Sikkim, Assam, Mizoram and Meghalaya . It is also found in tropical Asia, Australia, Pacific region and South Asia (Brandis 1998; Showkat *et al*., 2004).

It is the source of *tejpat* leaves, used extensively in India as spice and also yields an oil known as Indian cassia lignes oil (Annonymous 1950). Medicinally, its leaves are reported to be hypoglycaemic, stimulant, carminative, anti-rheumatic, anti-diarrhoeal and antidote for scorpion sting and also used in colic (Hussain *et al., 1992).* *Cinnamomum* leaves and bark are aromatic and traded as a spice (Anonymous 2006; Dhar *et al*., 2002 and Edwards, 1993). In Kashmir it is used as a substitute for betel leaves. The bark of *C. tamala* is coarser than the bark of *C. zeylanicum* and is one of the common adulterants of true *cinnamomum* (Datta and Datta 1955).

C.*tamala* mainly used for flavouring food and widely used in pharmaceutical preparation because of its hypoglycemic, stimulant and carminative properties3, (Hussain *et al,*1980). Tejpata dried leaves are used as a common ingredient of Indian cooking. The leaves of this tree have a clove like taste and a faintly pepper like odour.(Dhar *et al* 2003). Until now , the antidiabetic activity5 (Gupta Rahul *et al* 2008), anti-bacterial activity6(Gurdip Singh 2007), antioxidant activity (Anuradha *et al*., 2007), antimicrobial (Parekh and Chanda,

2007)8, anti-inflammatory activity(Gambhire *et al*2009), antidiarrhoeal activity (Rao Chandana 2008) 10, of CT extracts have been ssevaluated.

Table: 1.a)List of Phytochemicals present in *Cinnamomum tamala*		
Plant Parts	**Phytochemicals present**	**Reference**
Leaves and Barks	Flavonoides, Glycosides, Carbohydrates Phenol, terepinoides, alkaloides, steroids, steroids, tannins, saponins, triteripinoides, protein and aminoacids, anthraquinones, cardiac glycosides.	Kharkwal *et al*.,2013; Nagaraju *et al*.,2016
Essential oil from fresh leaves	furanosesquiterpenoids , a-Pinene, Camphene-Pinene, Benzaldehyde, Myrcene, Limonene-Cymene, a-Iinalool, Benzylacetate a-terpineol, Cinnamic aldehyde, Geraniol, Linalyl acetate Eugenol, Furanogermenone, is β- caryophyllene, sabinene, germacrene D, curcumenol, sesquiterpenoids, curzerene, curzerenone,	Nath *et al*.,1994 and Simic *et al*.,2004

Folk remedies

Cinnamomum tamala is a widely used medicinal plant to treat various diseases. The leaves of this plant have also been extensively used extensively as spice in the foods industry due to its special aroma (Chang and Cheng 2002). Medicinal uses of *C. tamala* is listed in table.2

Antioxidant properties

Various experiments were conducted to evaluate the presence of antioxidants in them. An experiment was conducted to evaluate *in-vitro* antioxidant activity of Indian Bay Leaf on rat by S. Lakshmi Devi *et al* (2007) and reported increases in the levels of lipid and lipid peroxidation products and a decline in antioxidant potential in diabetic rat brain synaptosomes when induced with methanolic extract of bay leaf (Devi *et al*.,2007)

Table 1.b).: List of Ethanobotanical uses of *Cinnamomum tamala*		
Plant Parts	**Ethanobotanical uses**	**References**
Whole plant	Used as anti-inflammation pharmacological activity. used against gastrointestinal conditions.	Mazimba *et al*.,2015**.**
Leaves	Used as aromatic, astringent, stimulant and carminative qualities. Used in Rheumatism, colic, diarrhoea, nausea and vomiting.	Edwards.,1993.
Seeds	Used to cure dysentry or cough	
Leaves and barks	Used as Antidiabetic activity, anti-bacterial activity, antioxidant activity, antimicrobial8, anti-inflammatory activity, antidiarrhoeal activity	Gupta *et al*.,.2008; . Gurdip *et al*., 2007; Anuradha *et al*.,2007; Manoj *et al*.,2009.
Barks and Leaves	Used as Flavouring agents in cooking	Kirtikar.,1999.
Leaves	Used as antioxidative, antiviral, antimicrobial and antiplatelet activities. Used to cure diabetes, cancer and coronary heart disease.	Middletonavd, Kandaswami., 1993. Broadhurst *et al*.,2000; Fabri *et al*.,2009.
Leaves	aromatic, carminative, stimulant, diuretic, diaphoretic, lactagogue, and deobstruent.	Rahman *et al*., 2013.

Katti, director of Cancer Nanotechnology Platform (2010), discovered that phytochemicals present in *Cinnamomum* have the ability to treat cancer when combined with gold nanoparticles because active pharmaceutical ingredients are carried by gold nanoparticles into cancer cells and assist in the destruction or imaging of malignancies. Chanda *et al* (2011) showed that *Cinnamon* coated gold nanoparticles have the ability to detect cancer cells (Chanda *et al*., 2011). It shows antioxidant property which can be proved by several studies. Studies also showed that synaptosomes from diabetic rats are susceptible to oxidative damage and bay leaf extract can show inhibition to it (Devi *et al*., 2007).

Mentha arvensis

The genus *Mentha* belongs to the family Lamiaceae consisting of about 25 to 30 species (Brickell and Zuk, 1997). Mints grow 10 -120 centimeters tall and can spread over an indeterminate sized area. Mint is the common name for any of the various herbaceous plants and perennial aromatic herbs that are cultivated for their essential oils and culinary purposes. The genus *Mentha* produces secondary metabolites such as alkaloids, flavanoids, phenols, gummy polysaccharides. Terpens and quinines are used in food and pharmaceutical, cosmetics and pesticide industries (Khanuja *et al*., 2000). Some members of this genus are also used as herbal teas and condiments both in fresh and dried form due to their distinct aroma (Baser *et al*., 1995).

Mentha arvensis (Japanese mint or Corn mint or Field mint), belonging to the family lamiaceae, which is popularly known as pudina, is native to the temperate regions of Europe and western and central Asia, East to the Himalaya and eastern Siberia, and America. It is widely distributed throughout India and is a common household remedy. It is a herbaceous perennial plant growing to 10–60 cm (rarely to 100 cm) tall. *Mentha* species are known for kitchen and medicinal herbs since time immemorial. It is either used as herb or its essential oil form is used for flavoring, perfume production and medicinal purposes (Dorman *et al*., 2003). It is valued for its multipurpose uses in the field of pharmaceuticals, cosmetics as well as for flavouring foods beverages and tobacco. Mint leaves have been included in cooking as a flavouring agent in many parts of the world. The fresh or dried leaves are the substances that give the mints their characteristic aromas and flavors are menthol (African pharmacopoeia, 1985; Briggs, 1993; Hoffmann and Lunder,1984). Frequent and continued

intake of mint leaves in daily diet may prove beneficial in keeping the pathogenic microbes below the threshold level (*Pramila et al*., 2012).

The leaves of the plant are extensively used in traditional system of medicine. It has found many uses in the Indian indigenous system of medicine against several ailmets as an anaesthetic, antiphlogistic, antidepresent (Coutinho *et al*.,2009), antiseptic, antispasmodic carminative, digestive, expectorant, nervine, stomachic, tonic and antifertility drug (Kanjanpothi *et al*.,1981 and Sharma and Jacob, 2009) and reversible contraceptive efficacy (Sharma and Jacob, 2002), abortificient and antinidational properties in various mammalian species**,** cardiotonic, diuretic, dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhea, bronchitis and skin diseases It has beta-galactosidase activity and it protects against radiation induced lethality (Kiritikar and Basu, 1982; Chopra andChopra,1994; Sola,1995 and Khare, 2004).

The plant has been shown to possess anti–inflammatory and sedative–hypnotic activity, hepatoprotective and antioxidant activity, antibacterial and antifertility action (Verma *et al*., 2003; Kowti *et al*., 2013; Coutinho *et al*., 2009; Kanjanapothi *et al*., 1981).The plant consist essential oils of monoterpenes like menthol, menthone, carvone and pulegone major constituents. This plant also possesses anti-Candida and also radio protective activity against gamma radiation (Marta *et al*., 2005; Ganesh and Manjeshwar, 2002). In Kashmir, the powder of aerial parts mixed with dilute curd is given to cure cough, sore throat, indigestion and constipation; also the leaves are used in Diarrhoea and Asthma (Akhtar *et al*., 2011; Towseef *et al*., 2012).

Folk remedies

Folks in local medicine use of mint, including carminative, antiinflammatory, antispasmodic antiemetic, diaphoretic, analgesic, stimulant, emmenagogue, and anticatharrhal application. It is also used against nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints.

Table2: List of Ethanobotanical uses of *Mentha arvensis*		
Plant Parts	**Ethanobotanical uses**	**References**
Whole plant	Used as Stimulant, Hair tonic, Bloodpurifier, Antidepressant, Antidysenteric, Antidiarrheal, Antifungal, Anti-inflammatory, Antiemetic, Febrifuge, Stomachic and Anti-periodic To cure Diabetes mellitus, Leucoderma, Body aches, Kidney pain, Vomiting	Rao *et al*., 2011; Rana *et al*., 2004; Kumar *et al*., 1999; Purohit *et al*., 2009; Iyer and Mani, 1990; Nutan *et al*., 1998; Chakrabarthy *et al*., 1997; Ponnusamy *et al*., 2010; Adebajo *et al*., 2004; Gandhi *et al*., 2004; Mandal *et al*., 2010; Ningappa *et al*., 2010; Khuntia *et al*., 2011; Xie *et al*., 2006; Purthi, 1976; Mhaskar *et al*., 2000; Parota, 2001.
essential oils	Used as anti-inflammatory, antimicrobial, antioxidant, anticarcinogenic, insecticide and analgesic used in perfumery, pharmaceutical and food industries	Shaikh *et al*., 2014 and Thawkar *et al*., 2016
Leaves	Used as carminative, digestive, expectorant, cardiotonic, diuretic To cure dentifrice, jaundice, hepatalgia, inflammation of liver, peptic ulcer, diarrhea, bronchitis and skin diseases	Sola AV.,1975; Kiritikar & Basu.,1998; Chopra & Chopra.,1994; . Khare .,2004. Kanjanapothi *et al*.,1981 Bodhanka *et al*., 1995.

Murraya koennigii

The history of curry leaves are seen in early 1st to 4th century AD. The word now popularly used for the *Murraya koenigii* is curry leaf which is originated from Tamil word Kari which means as 'spiced sauce' (Parrota 2001). In the early literatures of Tamil and Kannada the use of *Murraya koenigii* is described as the flavouring agent for the vegetables(Prajapati *et al* 2003). For therapeutic or prophylactic purposes medicinal plant are used. For the therapeutic properties of medicinal plants presence of secondary metabolites plays a very important role such as alkaloids, flavonoids, terpinoids, vitamins, tannins etc., these all are the secondary metabolites of the plant as active constituent (Gupta 2009). These all secondary metabolites of plant physiologically affect the body at different stages of body development and make the body disease free.

GEOGRAPHICAL DISTRIBUTION

Murraya koenigii is distributed from south and East Asia to Australia (Joshi and Gawd, 1970; Narasimha *et al* ,1975; . It grows throughout India up to the height of 1500 to 1655 m from sea level and in the Andaman Islands (Khosa 1974). Traditionally, the plant is used as tonic, stomachic, and carminative. (Muthumani, 2009) Fresh juice of the root is taken to relieve pain associated with kidney (Nayak and Baner, 2009). Spices are dried parts of herbs used as flavouring agents in cooking in oriental countries owing to their taste and aroma. Indian bay leaf (*Cinnamomum tamala* Nees.) is one among them. The dried leaf of this plant is a spice commonly used in Indian homes for seasoning (Hannah and Parameswari 2010)

Plant extracts are rich source of natural antioxidants and are being used as therapeutic agents (Tamokou *et al*2013), () Saafi-Ben *et al*2012)), nutraceuticals ()Srivastava *et al*2006), and food preservatives Preethi, ., and Loganathan, 2010). Laurus nobilis (Bay Leaves). It is also used in cooking due to its peculiar flavor and fragrance in either fresh or dried form (Vardapetyan,*et al* 2013)

Curry leaves obtained from the leaves of the plant *murraya koenigii*. It is a very important part of south Indian dishes. It also has antioxidant property.study showed the protective nature of curry leaves in diabetes by deceasing oxidative stress and pancreace b-celldamage. (arulselvan, 2007). Another study showed that curry leaves powder at concenterations as low as 0.2% is a very effective inhibitor of primary and secondary products in raw ground and cooked goat meat patties and has potential as a natural antioxidant in raw and cooked meat systems(Das *et al*., 2011)

Leaves of *Murraya koenigii* (L.) Sprengel, Piper betle L. and Mentha spicata L. are primarily used for recreational dietary purpose in the Indian subcontinent (Dutta.*et al*, 2017). And Mentha spicata L. (family Lamiaceae) are three major edible flora, consumed for their flavouring properties as well as used in Indian traditional medicinal systems as anti-cancer, cardiotonic, anti-microbial, anti-dysenteric, anti-inflammatory, anti-diabeti anti-spasmodic, etc. (Khare, 2008). *M. koenigii* and *M. spicata* are used as flavouring agent as strewn over cooked and/or processed foods and are also occasionally chewed as a part of dried raw food supplement. Prior pharmacognostic evaluations have revealed diverse bioactivities of P. betle, M. koenigii and M. spicata such as anti-diabetic, anti-inflammatory, hepatoprotective, analgesic activities (Dwivedi and Tripathi, 2014; Handral, Pandith, and Shruthi, 2012; Mahboubi, 2017; Mogosan *et al*.,2017).

Plants have also been used as medicines for thousands of years all over the world. WHO estimates indicate that 80% of the population, mostly in developing countries still relies on plant-based medicines. India is a country with a vast reserve of natural resources and a rich history of traditional medicine(Shivananda ,2006).

There is a proportional increase in demand for herbal products both locally and internationally. The demand for herbal products is caused by population increase, poverty, increasing awareness of herbal products, high cost of modern medicine and limited access to trained doctors (Daniyan,2008).

Traditionally, the plant is used as a stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery; insect bites and also used to allay heat of body (Kirtikar 1993). Several natural products such as alkaloids, flavonoids, terpenoids, saponins and glycosides are isolated from medicinal plants and are being reported to possess anti-diabetic activities(Kim.,2013).

Table 3: List of Ethanobotanical uses of *Murraya koenigii*		
Plant Parts	**Ethanobotanical uses**	**References**
leaves	used traditionally as antiemetic, antidiarrhoeal, febrifuge and blood purifier. tonic , stomachic,antioxidant, antidiabetic, antibacterial, antihypertensive and cytotoxic . used to cure - bronchial respiratory difficulties. Used as flavoring agent in curries and chetneys.	Ajay *et al*.,2011.
Whole plat	stimulant, stomachic, febrifuge, analgesic and for the treatment of diarrhoea, dysentery; insect bites and also used to allay heat of body	Kirtikar and Basu.,1993.
Leaves	Used to cure pimples, athlete's foot, ringworm, itches, acne, boils and septic of wounds and burns used as flavouring soups, curries, fish, meat dishes, eggs dishes, used in soap and cosmetic industry for aromatherapy	Khosa and Prasad.,1972;Dasgupta *et al*.,2003;Fiebig *et al*.,1985.

Materials and Methods

MATERIALS AND METHODS

3.1 Collection of plant material:

Plant materials were collected at respective places as given in the table.1. Collected plants were authenticated by Botanical survey of India, Coimbatore, and Tamil nadu, India.

Table 4. List of plant materials taken for this study

S.No	Plant Name	Family	Place
1	*Murraya koenigii L.*	Rutaceae	Coimbatore
2	*Mentha arvensis L.*	Lamiaceae	Coimbatore
3	*Cinnamomum tamala* Nees.	Lauraceae	Kozhikode

Leaves were rinsed with distilled water. Afterwards, the samples were dried under shade, ground and sieved for extraction process.

3.2 Solvent extraction [Farombi *et.al,* 2003]

Powdered samples (10g each) were weighed and soaked separately in 50 ml methanol in a conical flask stoppered with rubber cork and kept in orbital shaker for 24 hours. It was then filtered off using sterile filter paper (Whatman No: 1) into a sterile conical flask

3.3 Physiochemical parameters

3.3.1 Determination of moisture content (AOAC, 1990)

A quantity of 5 g of sample was dried in the oven at 105˚C and moisture content was calculated as percentage.

$$\text{Moisture Content} = \frac{W - D}{W} \times 100$$

Where,

W = Wet weight

D = Dry weight

Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Genus	*Murraya*
Species	*Koenigii*

Plate – 1.

Classification	
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Lamiaceae
Genus	*Mentha*
Species	*Arvencis*

Plate -2

Plate 3

3.3.2 Determination of solubility percentage (Kokate, 1994)

Ethanol

- ✓ About 5 g of powdered material was weighed and macerated with 100 ml of 90% ethanol in a closed flask for 24 hours shaking frequently during the first 6 hours and kept undisturbed for 18 hours.
- ✓ Thereafter, it was filtered rapidly taking precautions against loss of the solvent.
- ✓ About 25 ml of the filtrate was evaporated to dryness in a tarred flat bottomed, swallowed dish dried at 105˚ C for 6 hrs and cooled in a desiccator and weighed.
- ✓ The content of extractable matter (% w/w) air-dried material was calculated as follows.

$$\text{soluble extractives (\% w/w)} = \frac{\text{(Weight of residue) x 5 x 100}}{\text{Weight of the sample}}$$

3.4 Phytochemical Screenings

The extracts obtained in the successive extraction process of the four leaves extract were subjected to various preliminary Phytochemical screening for the identification of phytoconstituents present.

3.4.1 Test for Carbohydrates

Molisch's test:

To a small amount of the extract few drops of Molisch's reagent was added followed by the addition of conc. H_2SO_4 along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

3.4.2 Test for Reducing Sugar

Benedict's test

Extract (2 ml) were treated with 2 ml of Benedict's reagent and heated in a water bath for 3 minutes. Presence of green, red or yellow precipitate indicates the presence of reducing sugar.

3.4.3 Test for Proteins

Millon's test:

Extract (3 ml) was mixed with 5 ml of millon's reagent. White precipitate is formed. On warming precipitate turn's brick red or the precipitate dissolves giving red colored solution.

Biuret test:

To extract (3 ml) few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.

3.4.4 Test for Xanthoprotein

To 2 ml of extract, a few drops of concentrate nitric acid and 1 ml of ammonia were added. Appearance of a red precipitate indicates the presence of xanthoprotein.

3.4.5 Vitamin C

DNPH test:

Sample was treated with Dinitrophenyl hydrazine and sulphuric acid. Formation of yellow precipitate indicates the presence of vitamin C**.**

3.4.6 Test for Alkaloids

Mayer's test:

Sample (2 ml) was treated with few drops of Mayer's reagent. Appearance of white precipitate indicated the presence of alkaloids.

Wagner's test:

Sample (2 ml) was mixed with few drops of Wagner's reagent. Appearance of reddish brown precipitate indicated the presence of alkaloids.

Hager's test:

Sample solution and few drops of Hager's reagent were added. Appearance of yellow precipitate indicated the presence of alkaloids.

3.4.7 Tests for Flavonoids

Shinoda test:

Sample extract was treated with 5 ml of 95% ethanol, few drops of concentrated Hydrochloric acid and 0.5 g of magnesium turnings were also added. Pink colour was observed. Addition of increasing amount of sodium hydroxide to the residue shown yellow coloration, this decolorized after addition of acid indicates the presence of flavones.

Flavanones:

Sample extract (1 ml) was taken and 10 % of sodium hydroxide was added. Yellow to orange colour formation indicates the presence of flavanones.

Alkaline test:

Sample extract (1 ml) was treated with few drops of sodium hydroxide. Yellow colour is formed which turns to be colourless after adding a few drops of diluted acid.

3.4.8 Test for Glycosides:

Legal's test:

Sample extract was mixed with few drops of pyridine and 2 drops of 2 % sodium nitroprusside was added. To the reaction mixture 0.5 ml of 20 % sodium hydroxide was added. Appearance of pink to red color indicated the presence of glycosides.

Bromine water test

Sample (1 ml) was treated with 3 drops of bromine water and the formation of yellow precipitate indicates the presence of glycosides.

3.4.9 Test for cardiac Glycosides

Keller-killani test:

Five ml of each extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under layered with 1 ml of conc. sulphuric acid, a brown ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

3.4.10 Test for anthroquinone

Borntragor's test

Extract (0.5 ml) was added with 5-10 ml of dilute hydrochloric acid and boiled on water bath for 10 minutes. Solution was filtered and filtrate was extracted with benzene and mixed with ammonia solution. Red color was obtained in ammonia layer that indicated the presence of anthraquinone glycosides.

3.4.11 Test for Terpenoids

Extracts were treated with $CHCl_3$ (0.5 ml) and 1ml of Conc. H_2SO_4. Formation of reddish brown precipitate shows the presence of terpenoids.

3.4.12 Test for Diterpenes:

Extracts (3 ml) are treated with a few drops of copper acetate solution. Formation of emerald green colour indicates presence of diterpenes.

3.4.13 Test for Triterpenoids

Extract (5 ml) was dissolved in chloroform (2 mL) and then acetic anhydride (1 mL) was added to it. Concentrated sulphuric acid (1 mL) was added to the solution. Formation of reddish violet colour shows the presence of triterpenoids.

3.4.14 Test for Saponin

Foam test:

To 1 ml of the extract 5 ml distilled water was added and shaken vigorously. Formation of foam indicated presence of saponins.

3.4.15 Test for phenols

Extract (2 ml) was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour. To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium Ferro cyanide were added. Formation of blue or green color showed the presence of polyphenols.

Extract (2 ml) was treated with 3ml of 10 % lead acetate. Formation of precipitate indicates the presence of phenols.

3.4.16 Test for Tannins (Trease and Evans, 1989)

To 1ml of extract solution, 4 ml of water and 1-2 drops of 10 % ferric chloride solution was added. Blue colour indicates gallic tannins and green black catecholic tannins.

3.4.17 Test for Phlobatanins:

0.5 g extract was dissolved in distilled water and filtered. The filtrate was boiled with 2M HCl solution. Formation of red precipitate showed the presence of phlobatanins.

3.4.18 Test for Quinones

To 1ml of test solution Alcoholic KOH solution was added separately. Quinones were indicated by colour ranging from red to blue.

3.4.19 Test for Sterols

Extract (2 ml) was treated with 2 ml of trichloroacetic acid. On heating the colour changes from red to violet. This indicates the presence of sterols.

3.4.20 Test for Phytosterols

Salkowski's test:

0.2g of the extract was mixed with 2 ml of chloroform (CHCl3) and concentrated 6M H2SO4 (3ml) was carefully added forming a layer. A reddish brown coloration of the interface indicated the presence of phytosterols.

3.4.21 Test for Oxalate

1 ml of extract was mixed with 1 ml of dil. Sulphuric acid and dil. acetic acid and boiled for 3 minutes. 1ml ferrous sulphate was added. Yellow precipitate indicated the presence of oxalate

3.4.22 Test for Anthocyanin

NaOH test:

1 ml of extract was treated with 2ml NaOH. Blue green colour formation indicates the presence of anthocyanin.

3.4.23 Test for Resin

5 ml of distilled water was added to the 3 ml of the methanol extract for turbidity, which indicates the presence of resins in the plant sample.

3.4.24 Test for Coumarins

The aqueous leaf extract (5 ml) was evaporated to dryness in a water bath. Distilled water (3 ml) was added and the mixture heated on a water bath to boil and the mixture cooled under running water. The solution (0.5 ml, 10%) was added. Both test tubes were observed under Ultra Violet light and presence of coumarins was indicated by (blue or green) fluorescence in test tube containing ammonia solution.

3.4.25 Test for Catechin

Match stick was dipped in plant extract, dried and then moistened with concentrated HCl. Warm near flame, a red or pink wood is produced which shows the presence of catechin.

Data collected

The change of colour was observed when the test reagent was added to the prepared sample for the phytochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test.

3.4.2 Quantitative test

The biochemical parameters analyzed were

1. Carbohydrates
2. Proteins

3.4.2.1 Biochemical parameters

3.4.2.1.1 Total Carbohydrates: (Hedge and Hofreiter, 1962)

Principle

Carbohydrates are first hydrolysed into simple sugars using dilute hydrochloric acid. In hot acidic medium, glucose is dehydrated to hydroxyl methyl furfural. This compound forms with anthrone, a green coloured product with an absorption maximum at 630nm.

Materials

- 2.5N HCl
- Anthrone reagent: Dissolve 200 mg anthrone in 100 ml of ice cold 95 % H_2SO_4 prepared fresh before use.

Standard glucose (Stock): Dissolved 100 mg in 100 ml of water.

Working standard – 10 ml of a stock solution was diluted to 100 ml distilled water.

Procedure

- About 100 mg of the sample was taken in a boiling tube and it was hydrolysed by keeping it in a boiling water bath for three hours with 5 ml of 2.5N HCl and cooled to room temperature.
- Then it was neutralized with solid sodium carbonate until the effervescence created.
- The volume was made up to 100 ml and centrifuged.
- The supernatant was collected and 0.5 and 1 ml aliquots were taken for analysis.
- The standard was prepared by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard and '0' served as blank.
- The volume was made up to 1 ml in all the tubes including the sample test tubes by adding distilled water.
- Then, 4 ml of anthrone reagent was added and heated for eight minutes in a boiling water bath.
- Then it was cooled rapidly and the green colour developed was read at 630 nm.
- A standard graph was drawn by plotting concentration of the standard on the X-axis versus absorbance on the Y-axis.
- From the graph, the amount of carbohydrates present in the sample tube was calculated.

Calculation

Amount of carbohydrates present in 100 mg of the sample is calculated by

$$\frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

3.4.2.1.2 Estimation of protein (Lowry *et al*., 1951)

Principle

The blue colour developed by phosphomolybdic phosphotungstic components in the Folin-Ciocalteau reagent by the amino acids tyrosine and tryptophan present in the protein plus the colour developed by the biuret reaction of the protein with the alkaline cupric tartarate are measured in the Lowry's method.

Materials:

- Reagent A - 2 % sodium carbonate in 0.1 sodium hydroxide
- Reagent B - 0.5 % copper sulphate ($CuSO_4.5H_2O$) in 1% potassium sodium tartarate.
- Reagent C - Alkaline copper solution: Mixed 50 ml of A and 1 ml of B prior to use.
- Reagent D - Folin-Ciocalteau Reagent.

Stock standard: 50 mg of bovine serum albumin (Fraction V) was weighed and dissolved in distilled water and the volume was made up to 50 ml in a standard flask.

Working standard: About 10ml of the stock solution was diluted to 50 ml with distilled water in a standard flask. One ml of this solution contain 200 µg proteins.

Procedure

Extraction of protein from sample

Extraction is usually carried out with buffers used for the enzyme assay. About 50 mg of the weighed sample was ground well with pestle and mortar in 5- 10 ml of the buffered centrifuged. The sample was used for protein estimation.

Estimation of protein

- About 0.2, 0.4, 0.6, 0.8, and 1 ml of working standard were pipette into a series of test tubes and 0.1 ml and 0.2 ml of the sample extract in two other test tubes.
- The volume was made up to 1 ml in all test tubes. A tube with 1 ml of water severed as the blank.
- About 5 ml of reagent C was added to each tube including the blank, mixed well and allowed to stand for 10 minutes.

- Then 0.5 ml of reagent D was added. Mixed well and incubated at room temperature in the dark for 30 min. Blue colour developed was read at 660 nm.
- A standard graph was drawn and the amount of protein present in the sample was calculated.

Calculation

The amount of protein present in the sample was expressed in

$$\text{mg/g or 100g} = \frac{\text{mg of protein}}{\text{volume of the test standard}} \times \text{concentration of the standard}$$

3.4.2.2 Phytochemical Parameters

- Phenol
- Polyphenol
- Flavonoid
- Tannin

3.4.2.2.1 Estimation of Total Phenol content (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which can be estimated spectrophotometrically at 650 nm.

Materials

- Ethanol (80 %)
- Folin-ciocalteau reagent
- Na_2CO_3 (20 %)

Stock standard: Gallic acid (100µg/ml in water)

Working standard: Dilute 10 times of stock

Procedure

- Grind 0.5 g of the sample with a pestle and mortar in 10 times volume of 80 % ethanol.
- Centrifuge the homogenate at 10,000 rpm for 20 minutes. Re-extraction is done and the supernatants were pooled and evaporated to dryness.
- Dissolve the residue in a known volume of distilled water (5 ml).
- Pipette out different aliquots (0.2 to 2 ml) into test tubes.
- Make up the volume in each tube to 3 ml with distilled water.
- Add 0.5ml of Folin-Ciocalteau reagent.
- After 3 minutes, add 2 ml of 20 percent Na_2CO_3 solution to each tube.
- Mix thoroughly, place the tube in boiling water for exactly 1 minutes, cool and measure the absorbance at 650nm against a reagent blank.
- Prepare a standard curve using different concentrations of gallic acid.

Calculation

From the standard curve the concentration of phenols in the sample were observed and express as GAE mg of phenols/g of material.

3.4.2.2.2 Determination of total polyphenol (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin - Ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrophotometer at 650nm.

Reagents

- Diluted Folin - Ciocalteau reagent (1:10 dilution)
- 20% Sodium carbonate
- Ethanol

Stock solution: 100 mg of catechol was made up with 100 ml distilled water

Working standard: 10 ml of stock standard was diluted to 100 ml. 1.0 ml of this contains 100 µg of catechol.

Procedure

- ✓ A working standard of 0.5 – 2.5 ml catechol solution corresponding to 50 – 250 µg of catechol were pipetted out into a series of test tubes including samples and standard.

- ✓ The volume was made up to 2.5 ml with water. To all the tubes added 0.5 ml of diluted Folin – Ciocalteau reagent.
- ✓ After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly.
- ✓ The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank.

Calculation

The results were expressed as mg / g tissue.

3.4.2.2.3 Determination of total flavonoid content (Grubesic *et al*., 2005)

Principle

The content of flavonols was determined by using rutin as a reference compound. This method was based on the formation of complex with maximum absorption at 440 nm.

Reagents:

1. Aluminium chloride solution (20 mg/ml)
2. Sodium acetate (30 mg/ml)

Procedure

- ➢ About 1 ml of each extract was mixed with 1 ml aluminium chloride and 3 ml sodium acetate.
- ➢ After 2.5 hrs the samples were read at 440 nm absorbance.
- ➢ The absorption of standard rutin solution in methanol was measured under the same conditions.
- ➢ A duplicate was carried out for all the determinations.

Calculation:

The amount of flavonoids in plant extracts in rutin equivalents was calculated by the following formula.

$$X = (A - m_0) / (A_0 - m)$$

Where,

X = flavonoid content (µg/ml) in rutin equivalents

A = absorption of plant extraction solution,

A_0 = absorption of standard rutin solution,

m = weight of plant extract (µg)

m_0 = weight of rutin in the solution (µg)

3.4.2.2.4 Estimation of Tannin (Folin and Ciocalteau Method, 1927)

Principle

The principle behind this method is reduction of Phosphotungstomolybdic acid in alkaline solution to produce coloured complex.

Materials

1. Folin- Ciocalteu reagent
2. Sodium carbonate
3. Tannic acid

Procedure

- To 0.1 ml of the sample extract 7.5 ml of distilled water and 0.5 ml of Folin-Ciocalteu reagent, 1 ml of 35% sodium carbonate solution were added and diluted to 10 ml with distilled water.
- The mixture was shaken well, kept at room temperature for 30 min and was measured at 725 nm.
- Blank was prepared with water instead of the sample.
- A set of standard solutions of Tannic acid is treated in the same manner as described earlier and read against a blank.

Calculation

The results of Tannin are expressed in terms of Tannic acid in mg/g tissue TAE of extract.

3.4.3. Antioxidants assays

3.4.3.1 TLC screening for Phytochemical analysis and antioxidant activity

Qualitative screening of the constituents in each of the crude extracts of the selected three species for antioxidant activity was screened by thin layer chromatography (TLC) analysis. For about 10µL of each sample was loaded on the TLC plates. The TLC chromatograms were developed in the following solvent systems.

1. Ethyl acetate/ methanol/water (EMW) 10:1.35:1
2. Chloroform/ethyl acetate/formic acid (CEF) 10:8:2

For detection of chemical compounds and antioxidants in the extracts respectively, two spray reagents were separately used.

1. Vanillin in sulphuric acid
2. DPPH 0.2% in NaOH

3.4.3.2 **Enzymatic assays**

The enzymatic antioxidant analysed in this present study were Polyphenol oxidase and Peroxidase.

3.4.3.2.1. Polyphenol Oxidase (PPO) (Esterbauer *et al*., 1977)

Principle

Polyphenol oxidase was copper protein of wide occurrence in nature, which catalyses the aerobic oxidation of certain phenolic substrate to quinines, which are auto oxidized to dark brown pigments generally known as melanins. The polyphenol oxidase (PPO) comprises catechol oxidase and lactase.

Reagents

- Tris-HCl (50 mM, pH 7.2).
- Sorbitol (0.4 M).
- Sodium chloride (10 mM).
- Phosphate buffer (0.1 M, pH 6.5).
- Catechol (0.01 M).

Procedure

- ✓ Into a cuvette, 0.2 ml of the sample extract was added to the reaction mixture containing, 2.5 ml of phosphate buffer and 0.3 ml of catechol solution.
- ✓ The change in the absorbance was recorded every 30 sec up to 5 minute.

Calculation

One unit is equal to the changes in absorbance at 495nm/minute

The activity of PPO can be calculated using the formula

Enzyme units in the sample = $K \times (\Delta A/\text{minute})$

Where,

K for catechol oxidase = 0.272

K for lactase = 0.242

3.4.3.2.2. Peroxidase (Reddy *et al*., 1995)

Principle

In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts $H_2O_2$to H_2O and O_2. The oxidation of pyrogallol or dianisidine to a coloured product called purpurogalli can be followed spectrophotometrically at 430nm.

Reagents

- Pyrogallol : 0.05 M in 0.1M phosphate buffer (pH 6.5)
- H_2O_2: 1% in 0.1M phosphate buffer, pH 6.5

Procedure

- ✓ About 3 ml of pyrogallol solution, 0.1ml of the extract was added and the spectrophotometer was adjusted to read zero at 430 nm.
- ✓ To the test cuvette, 0.5ml of H_2O_2 was added and mixed.
- ✓ The change in absorbance was recorded every 30 seconds up to 3 minutes in a spectrophotometer.

Calculation

One unit of peroxidase is defined as the change in absorbance at 430 nm/minute.

3.4.3.3. Non-enzymatic assays

3.4.3.3.1. Ascorbic acid (Roe and Keuther, 1943)

Principle

Ascorbate is converted into dehydroascorbate on treatment with activated charcoal, which reacts with 2, 4-dinitrophenyl hydrazine to form osazones. These osazones produce an orange coloured solution when dissolved in sulphuric acid, whose absorbance can be measured spectrophotometrically at 540nm.

Reagents

- TCA (4%)
- 2,4-dinitrophenyl hydrazine reagent (2%) in 9N H2SO4
- Thiourea (10%)
- Sulphuric acid (85%)
- Ascorbic acid (100mg of Ascorbic acid in 100ml of 4% TCA)

Procedure

- ✓ Ascorbic acid solution of 1 ml were taken and added with 0.1 ml of plant extract.
- ✓ The reaction mixture was made up to 2.0 ml with 4% TCA.

- ✓ To this, 0.5 ml of DNPH reagent was added followed by 2 drops of 10% thiourea solution.
- ✓ The tubes were incubated at 37˚C for 3 h.
- ✓ The osazone formed was dissolved by the addition of 2.5 ml of 85% sulphuric acid.
- ✓ DNPH reagent and thiourea were added to the blank after the addition of sulphuric acid.
- ✓ After cooling the tubes, the absorbance was read spectrophotometrically at 540 nm.

Calculation

The concentration of ascorbate in the sample was calculated and expressed in terms of mg/g tissue.

3.4.3.3.2 Total polyphenol (Malick and Singh, 1980)

Principle

Phenols react with phosphomolybdic acid in Folin - ciocalteau reagent in alkaline medium and produce blue coloured complex (molybdenum blue), which is read in a spectrophotometer at 650nm.

Reagents

- ➢ Diluted Folin - Ciocalteau reagent (1:10 dilution)
- ➢ 20% Sodium carbonate
- ➢ Ethanol

Stock solution : 100 mg of catechol was made up with 100ml distilled water

Working standard: 10ml of stock standard was diluted to 100ml. 1.0ml of this contains 100µg of catechol.

Procedure

- ✓ A working standard of 0.5 – 2.5ml catechol solution corresponding to 50 – 250µg of catechol were pipetted out into a series of test tubes including samples and standard.
- ✓ The volume was made up to 2.5ml with water. To all the tubes added 0.5ml of diluted Folin – Ciocalteau reagent.
- ✓ After 3 minutes, 2 ml of 20% Na_2CO_3 solution was added to each tube and mixed thoroughly.
- ✓ The tubes were placed in a boiling water bath for exactly one minute. Cooled and measured at 650nm against a reagent blank.

Calculation

The results were expressed as mg / g.

3.4.4 Free radical scavenging activity

3.4.4.1. DPPH radical scavenging activity (Mensor *et al.*,2001)

Principle

DPPH radical reacts with an antioxidant compound that can donate hydrogen, and gets reduced. DPPH, when acted upon by an antioxidant, is converted into diphenylpicryl hydrazine. This can be identified by the conversion of purple to light yellow colour.

Reagents

1. DPPH – 2, 2-diphenyl-2-picryl hydrazyl hydrate (0.3mM in methanol) ***(0.1mM=39.4 mg in 1000 ml)***
2. Methanol

Procedure

The extracts (20µl) were added to 0.5ml of methanolic solution of DPPH and 0.48ml of methanol. The mixture was allowed to react at room temperature for 30 minutes. Methanol served as the blank and DPPH in methanol, without the extracts, served as the positive control. After 30 minutes of incubation, the discoloration of the purple color was measured at 518nm in a spectrophotometer.

Calculation

The radical scavenging activity was calculated as follows

$$\text{Scavenging activity \%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3.4.3.2 Ferric Reducing Antioxidant Power (FRAP) Assay (Pulido et al., 2000)

Principle

Ferric reducing ability of plasma (FRAP) assay is based on the principle of reduction of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+}-TPTZ) by

the antioxidants of a sample at low pH. The end product (Fe^{2+}-TPTZ) has blue color with absorption maximum at 593 nm

Procedure

900µL of FRAP reagent, prepared freshly and warmed at 37 ℃, was mixed with 90 µL of distilled water and 30 µL of test sample (Benzie and Strain, 1996). Readings were taken at 593 nm, for every 15s, the reaction monitored for up to 30 min.

Calculation

The radical scavenging activity was calculated as follows

$$\text{Scavenging activity \%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3.4.3.3 Reducing power assay (Oyaizu, 1986)

Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe_3+) to form potassium ferrocyanide (Fe_2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

$$\text{Potassium ferricyanide} + \text{Ferric chloride} \xrightarrow{\text{Antioxidant}} \text{Potassium ferrocyanide} + \text{ferrous chloride}$$

Reagents

1. Potassium ferricyanide (1%)
2. Phosphate buffer (0.2 M, pH 6.6)
3. Trichloro acetic acid (10%)
4. Ferric chloride (0.1%)
5. Ascorbic acid (1%)

Procedure

0.5 ml of the plant extracts were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). This mixture was kept at 50ºC in water bath for 20 minutes. After cooling, 2.5 ml of 10% trichloro acetic acid was added and centrifuged at 3000 rpm for 10 minutes whenever necessary. The upper layer of solution (2.5 ml) was mixed with distilled

water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm.

Calculation

Reducing power was measured by different extract.

The radical scavenging activity was calculated as follows

$$\text{Scavenging activity \%} = \frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100$$

3.4.3.4 Metal chelating activity (Dinis *et al.*, 1994)

Chemicals used

1. 2 mM of $FeCl_2.4H_2O$
2. 5 mM of ferrozine
3. Gallic acid

Procedure

Aliquots (1 ml) of the plant extracts dissolved in the same solvents at concentrations of 1, 2.5 and 5 mg/ml were separately added to 2.8 ml of distilled water, followed by mixing with 50 µl of 2 mM $FeCl_2.4H_2O$ and 150 µl of 5 mM ferrozine. All the above without plant extract served as control. The mixtures were then shaken vigorously and left standing at room temperature for 10 min. Absorbance levels of the solutions were measured using a spectrophotometer at 562 nm. All tests and analyses were run in triplicate and averaged.

Calculation

Inhibition of ferrozine – Fe_2+ complex formation was calculated using the formula given below:

$$\%\text{Inhibition} = [1 - (A_{sample}/A_{Control})] \times 100$$

Where $A_{Control}$ is the absorbance of the control and A_{sample} is the absorbance in the presence of the plant extracts or standards.

Results and Discussion

4. Result and Discussion

Considering the beneficial effects of spices in treating diseases, methanolic extracts of these commonly used spices i.e. Murraya koenigii, cinnamomum tamala and mentha *arvensis* were evaluated for their phytocontituent and comparative antioxidant and free radical scavenging activity. Methanol, a polar solvent, extracts more polar components together with the non-polar constituents of the plant (Liu *et al*., 2007). Experiments were performed using methanolic extracts because of its relevance with traditional usage, especially in Indian Ayurvedic medicinal system (Khare, 2008).polar-solvents are known better for extracting bioactive constituents (Harborne, 1998).

4.1 Physiochemical Parameters

4.1.1 Moisture content

Present investigation showed maximum moisture content (3.96 %) in Cinnamomum tamala when compared with *M. arvensis* and *M. koenigii*. The moisture in *C. tamala* was found to be 23.42 % by Iagara *et al*., (2016). Work done by Jain *et al*., (2017) showed that *Murraya koenigii* contains 61.77- 66.2% of moisture content, whereas work done by Pande *et al*., (2009) revealed 15 per cent of moisture content.

4.1.2 Solubility test

Solubility test of the four methanolic extracts varied from 2.25 % to 4.21 %, where the maximum and minimum solubility was found in *C. tamala* and *M. arvensis* respectively. *M. koennigii* exhibited 1.35-1.82% of alcohol soluble extractive (Jain *et al*., 2017) and 3 per cent (Pande *et al*., 2009).

Table:4. Physiochemical parameters of three plants

Physiochemical parameters	Moisture content (%)	Methanol Solubility (%)
Cinnamomum tamala	4.21	3.96
Murraya koenigii	3.35	3.85
Mentha arvensis	2.25	3.75

4.2 Preliminary Qualitative tests

4.2.1 Phytochemical Screening

Preliminary phytochemical screening of the various methanolic leaf extracts of *C. tamala*, *M. koenigii* and *M. arvensis* revealed the presence and absence of various phytoconstituents present in them (Table 5).

Table: 5 Phytochemical investigation of methanolic extract of three plants

S.No	Name of the Phytochemicals		Name of the test	Methanol Extract		
				C. tamla	*M. koengii*	*M. arvensis*
1	Carbohydrates		Molisch's	+	+	+
2	Proteins		Millon's test	+	+	+
			Biuret test	-	+	+
			Tannic acid test	-	-	-
3	Vitamin C		DNPH test	+	+	+
4	Alkaloids		Mayer's	+	+	+
			Wagner's	-	+	-
			Hager's test	+	+	+
5	Flavonoids	Flavones	Shinodia test	+	-	-
		Flavanones	NaOH test	+	+	-
		Alkaline test		-	+	-
6	Glycosides		Bromine water test	+	+	-
			Legal's test	+	+	+
7	Cardiac glycosides		Keller-killani test	+	+	+
8	Anthraquinone		Borntragors test	+	-	-
9	Terpenoids		Salkowki's test	+	+	+
10	Diterpenes		Copper acetate test	+	+	+
11	Triterpenoids			+	+	+
12	Saponins			-	+	+
13	Phenols		Ferric chloride test	+	+	+
14	Tannin	Gallic tannin		+	+	+
15	Phlobatanins			+	-	+
16	Quinones			+	-	+
17	Sterols			+	-	-
18	Phytosterols		Liberman Burchard	+	+	+
19	Oxalate			+	+	+
20	Anthocyanin			+	-	-

Key: + = Presence - = Absence

The primary metabolites, carbohydrate and protein and the secondary metabolites like alkaloids, Flavonoids, glycosides, ascorbic acid, terepenoides, phenols, tannin and phytosterol were present in all plants investigated.

Cinnamomum tamala showed absence of saponin. *C. tamala* has been studied for its phytochemical constituents by different researchers. Results obtained by Prakash *et al*. (2014) in phytochemical analysis of the hexane, methanol, acetone and chloroform extracts of the *C. tamala* leaves showed presence of alkaloid and tannins but didn't spot glycosides. Similarly, Sukumar (2014) and his co-workers reported polyphenoles, flavonoids, tannins, alkaloids and saponins. Methanolic leaf extract of *C. tamala* indicated strong presence of alkaloids, terpenoids flavanoids, tannins, phenols and reducing sugars while saponins and Steroids were absent in the tested extracts (Rahman *et al*., 2013 and Hassan *et al*., 2016). Preliminary phytochemical analysis done by Nagaraja *et al* ,(2016) in *C. tamala* using petroleum ether ,Chloroform ,ethenolic and aqueous revealed the majority of compounds of 9 out of 11 where found using ehanolic solvent. The ethenolic extract showed absence of steroid and gums (Nagarguna *et al*., 2016).

Murraya koenigii is very rich source of organic compounds with different chemical composition such as alkaloids, flavonoids carbohydrates, and sterol is present in the plant extract prepared in solvents such as petroleum ether, ethyl acetate, chloroform, ethanol and water (Prabhu & Tamilanban., 2012; Sathaye *et al*., 2011; Nagappan *et al*., 2012; Gupta & Sharma., 2010; Bandyopadhyaya *et al*., 2002). Alkaloids, carbohydrates, tannins and flavonoids, saponins are present and steroids absent in acetone extract of Murraya koenigii (Arun *et al*., 2017). Phytochemical screening investigation revealed the presence of alkaloid, saponin in the work carried out by Pande *et al*., (2009)

The preliminary phytochemical screening of *M. arvensis* methenolic leaf extract showed the presence of carbohydrates, protein, vitamin C, Alkaloides, glycosides, terepenoides, phenol, tannin, quinones, saponin, oxalate and phytosterols, while falvonoides, antheaquinone, sterols and anthocyanin were not detected. The phytochemical analysis carried out in methanolic extract of mentha *arvensis* by Rathishkumar *et al*., (2012) revealed the presence of Cardiac glycosides, Flavonoides, Steroids and tannin while, alkaloides, Glycosides, and saponins were not detected. Work done by Kowti Rajesh *et al*., (2013) in ethanolic extract of *M. arvensis* revealed the presence of alkaloids, carbohydrates, glycosides, flavonoids, triterpenoids and tannins. Qualitative phytochemical analysis of the crude methanolic mint leaf extract done by Pramila *et al*., (2012) and Anindya Bose *et al*., (2012) showed the presence of tannins and flavanoids and absence of glycosides, saponins, alkaloids and anthroquinone.

4.2.2 Thin Layer Chromatography for phytochemicals

TLC were carried out on the methanolic extract of *C. tamala*, *M. koennigii* and *M. arvensis* using two solvents (Table 6)

Table: 5.a TLC Screening of various methanolic extracts of three plants

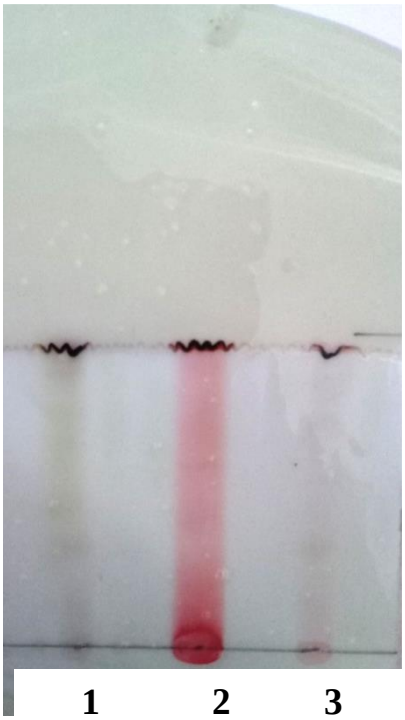
Solvent System Used	Detection Reagent	Observation	Inference	Methanolic Extract		
				1	2	3
Ethyl acetate/ methanol/water (EMW) 10:1.35:1	Vanillin in sulphuric acid	Green/ Red/ Pink	Bitter Principle	+	+	+
Chloroform/ethyl acetate/formic acid (CEF) 10:8:2		Green/ Red/ Pink	Steroid / terpinoids	+	+	+

1 – *C. tamala*; 2- *M. koenigii;* 3- *M. arvensis*

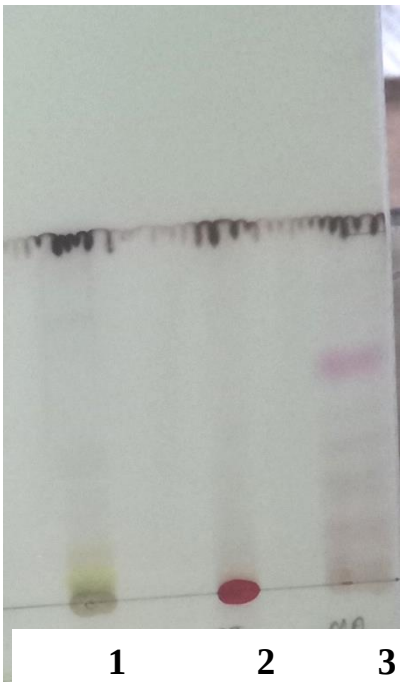
Sandeep *et al*., (2010) used petroleum ether extract, chloroform extract and ethanol extract of *M. arvensis* to study the qualitative antioxidant test using TLC and found eight spots in Benzene: Ethanol (19:1), nine spots in Chloroform: Methanol (19:1), six spots in Toluene: Ethyl acetate (93:7) using iodine vapour as a viewing medium. Pande *et al*., (2009) in various extracts of *M. koenigii* did Phytochemical screening using Ethyl acetate: Methanol: Water (75.5: 13.5:10) and toluene: Ethyl acetate (93:7).

Plate 5. Phytochemical Quantitative analysis using TLC

Ethyl acetate/ Methanol/ Water



a. Chloroform/ Ethyl acetate/ Formic acid



1 – *Murraya koenigii*; 2 – *Cinnamomum tamala*; 3 – *Mentha arvensis*

4.3 Quantitative test

4.3.1 Biochemical parameters

4.3.1.1 Total Carbohydrates

Carbohydrate was found to be maximum and minimum in *Murraya koenigii* (160.48 mg/100 g) and *Cinnamomum tamala* (115.90 mg/100g) respectively. Previous study done by Igara *et al*., (2016) in *C. tamala* showed that the plant leaves contain a substantial amount of carbohydrate 39.44 %. This shows that the leaf is a good source of carbohydrate (Igara *et al*., 2016).

4.3.1.2 Total Protein

The proteins in the leaf can make fair contributions to protein diet as proteins are involved in formation of hormones, enzymes and structural membranes (Igara *et al*., 2016). Protein quantity of *Murraya koenigii* was found to be maximum (1078 µg/100mg) when compared with *M. arvensis* and *C. tamala*. *M. arvensis* was found to be having minimum protein content (444.17 µg/100mg). Our result were par with the result obtained by Igara *et al* (2016) who found the protein content in leaf di etyl ether extract of *M. koenigii* was 8.38 mg/100g.

4.3.2 Plant Physiochemical parameters

4.3.2.1 Total Flavonoids

Flavonoid was found to be maximum in *M. arvensis* (214.53*)* and minimum in *M. koenigii* (118.13) Previous studies showed that the total flavonoid content was found to be 89.12 ± 0.02, 153.78 ± 0.04 and 136.38 ± 0.04 mg/mL quercetin equivalent/ 100 mg plant, respectively (Dutta *et al*., 2017). The total Flavonoid content (mg/g) in Rutin equivalent was found to be 213.33 in methanolic root extract of *Mentha arvensis* (Akbar *et al*.,2014)

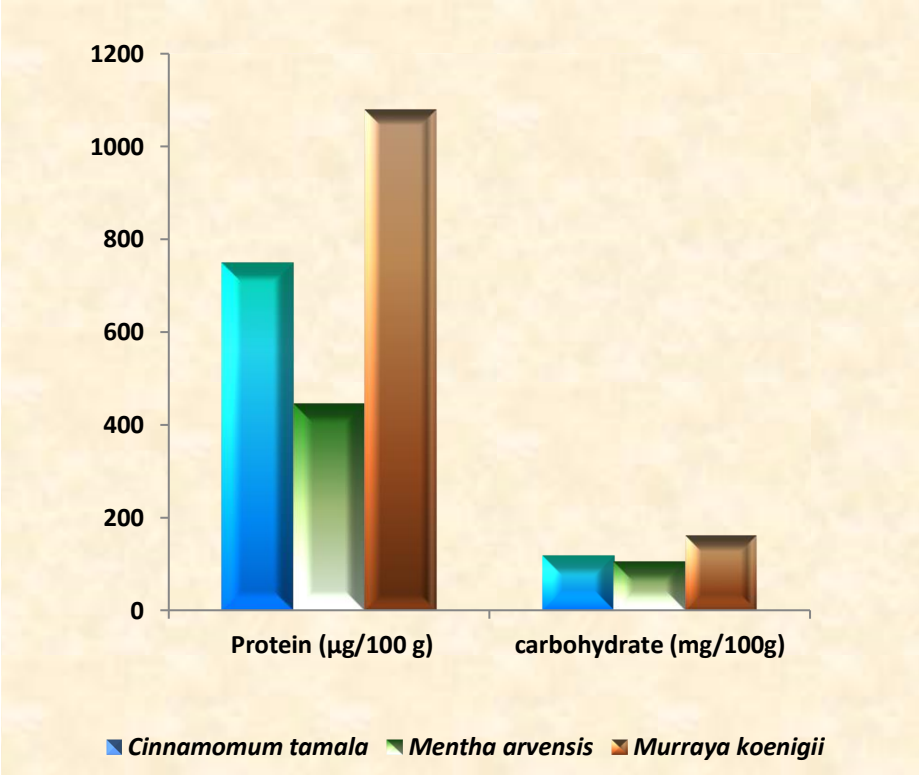
It is reported to possess antioxiant, antibacterial, antifungallarvicidal, anticarcinogenic, hypoglycemic, anti-lipid peroxidative, hypolipidemic and antihypertensive activity (Iyer & uma., 2008). The content of flavonoides compounds in quercetin equivalent was found to be 45.76mg/g of in ethanolic leaf extract of *M. koenigii*. (Victoriya and Manimekalai. 2016). our result were par with the result obtained by Dandapat *et al*.,2015 who found the total poly phenol flavonoid content in methanol and aqueous extract of *C. tamala* was 1.00 g/100g). Flavonoids

Table: 6 Quantitative test for Biochemical Parameters – Carbohydrates and Proteins

Plant name	Carbohydrate (mg/100g)	Protein (µg/100g)
Cinnamomum tamla	115.90 ± 26.88	750.28 ± 1.27
Mentha arvensis	103.19 ± 0.19	444.17 ± 0.84
Murraya koengii	160.48 ± 13.18	1078 ± 2.09
SEd	14.1119	1.2205
CD(<0.5)	34.5322	2.9867

alues are mean ± SD of three triplicates

Fig: 1. Comparision of Carbohydrate and Protein in methanolic extract of *C. tamala*, *M. koenigii* and *M. arvensis*



have strong anti-oxidant and anti-inflammatory properties. They have ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals (Allan and Miller., 1996).

4.3.2.2 Total Phenolic Content:

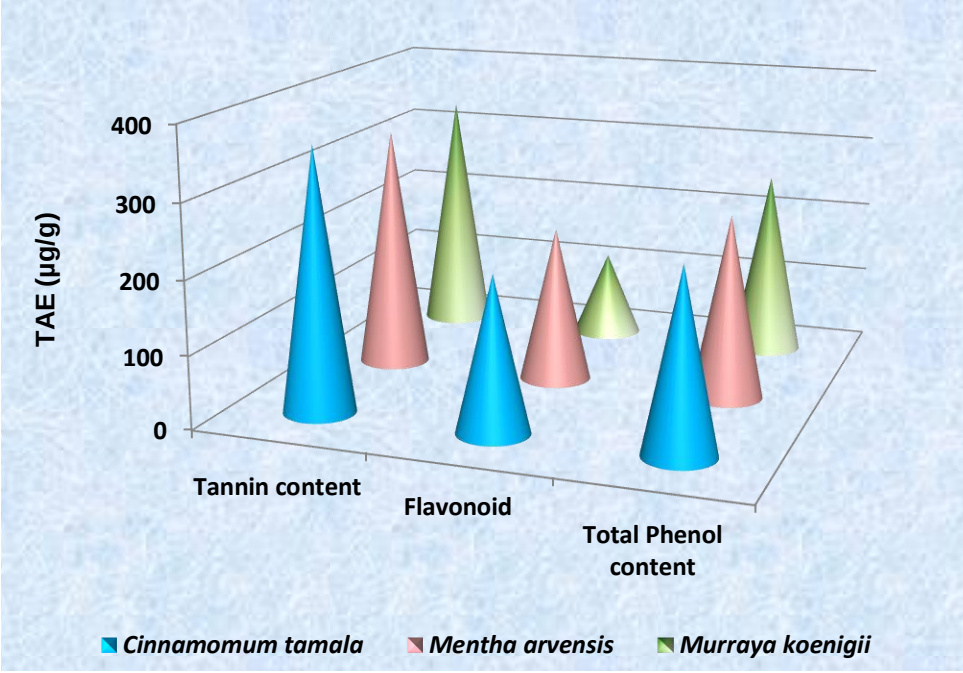
Phenols are very important plant constituents; they show high scavenging ability of free radicals due to their hydroxyl group. Therefore, the phenolic content of plants may contribute directly to their antioxidant action (Tosun *et al*., 2009). Total flavonoids and total phenolic contents were determined as quercetin equivalents in micrograms per gram of extract (QE/gm of extract), while total phenolic contents were calculated as gallic acid equivalents in micrograms per gram of extract (GAE/gm of extract). Results from the quantitative determination of total phenolics and flavonoids of the methanolic extracts of the different spices were summarized in table. Total phenol content was found to be maximum in *Murraya koenigii* (255.53 µg GAE/ml*)* and minimum in *Cinnamomum tamala* (248.20 µg GAE/ml). Previous studies reported that total phenolics content extracted better in methanol compared to ethanol and water. This is because methanol inhibits polyphenol oxidase which is responsible for the oxidation of phenolics content and its reduced amount. Another reason for choosing methanol over ethanol and water is its ability to evaporate easily (Pérez *et al*., 2007). Our result were far with the result obtained by Dutta *et al*.,2017 who found The total phenolic content in methenolic leaf extract of M. koenigii, was about be 63.86 ± 0.03, mg gallic acid equivalent/100 mg plant extract. Rahman *et al*., (2013), observed that the Total phenolic content in methanolic leaves extract of *C. tamala* was about 276 gallic acid equivalent/100 g. The content of phenolic compounds (mg/g) in Gallic acid equivalent was found to be 489.31 mg/g in methanolic root extract of *Mentha arvensis* L (Akbar *et al*., 2014). Urquiaga and Leighton (2000) reported that phenols and phenolics have anti-tumour and anti- oxidant effects. They also have anti-inflammatory and anti-carcinogenic. Properties and play roles in scavenging H2O2 by donating electrons to the peroxide thus neutralizing them to water (Mikail *et al*., 2013). Sasidharan and Menon, (2011) sdudied the total phenolic content in various combinations of extracts in M. koennigii, among them maximum (501.4 ± 4.6 mg/g GAE) was found in EtOH—water mixture extract (AT) and lowest in chloroform extract (BT) (140 ± 3.4 mg/g GAE).

Table: 7.Quantitative test for the phytochemical – Flavonoid, Phenol and Tannin

Plant name	Flavonoid (µg QE Eq)	Total phenol content (µg GAE Eq)	Total tannin content (µg GAE Eq)
Cinnamomum tamala	212.60 ± 2.00	248.20 ± 0.53	361.80 ± 0.72
Mentha arvensis	214.53 ± 2.10	253.47 ± 2.19	335.00 ± 0.53
Murraya koengii	118.13 ± 1.17	255.53 ± 2.30	334.40 ± 1.44
SEd CD(<0.5)	1.4747 3.6087	1.5193 3.7177	0.8000 1.9576

Values are mean ± SD of three triplicates

Fig. 2 Comparision of Total Flavonoid, Total Phenol and Total Tannin in methanolic extract of *C. tamala*, *M. koennigii* and *M. arvensis*



4.3.2.3 Total Tannin

Total tannin content activity was found to be maximum in *C. tamala* and minimum in *M. koenigii* with a range of 361.80 TAE/g and 334.40 TAE / g respectively. The content of Tannins compounds in catechin equivalent was found to be 73.95mg/g of in ethanolic leaf extract of *M. koenigii.* (Victoriya and Manimekalai., 2016). Our result were par with the result obtained by Igara *et al* (2016) who found the tannin content in leaf dietyl ether extract of *M. koenigii* was about 0.86 mg/100g .

4.4 Antioxidant

Lack of antioxidants in organism, promotes the oxidative stress due to the presence of free radicals, which in turn causes a variety of pathological conditions. Antioxidants, which are an integral part of biologically active substances, are of great interest. They can reduce mutagenic influence, regulating the oxidation process of free radicals (Trevisan *et al*., 2017). Ascorbic acid was chosen as the reference antioxidant for the test spices and herbs have been extensively studied in different countries because of high antioxidant activity in certain spices and their beneficial effect on human health.

4.4.1 Qualitative study using TLC

In the TLC-based qualitative antioxidant assay using DPPH assay, extract showed the yellow colour on a purple background on the TLC plate. Simillar results were observed by Biswas *et al*., (2014) in ethanolic extract of *M. arvensis*. It led to the quantitative antioxidant activity.

4.4.2 Enzymatic Antioxidant

4.4.2.1 Polyphenol Oxidase

Polyphenol oxidase is a copper enzyme that is responsible for the enzymatic browning reactions of fruit and vegetable source. Polyphenol Poly phenol oxidase activity was found to be maximum in *Murraya koenigii* (40.67 U/g*)* and minimum in *Cinnamomum tamala* (30.50 U/g).

.

Plate 5: Qualitative Antioxidant assay using DPPH in TLC

Ethyl acetate/ Methanol/ Water

1 2 3 4

1 – Ascorbic acid

2 - *Murraya koenigii*; 3 – *Cinnamomum tamala*; 4 – *Mentha arvensis*

Table: 8 Levels of various antioxidant enzymes in *C. tamala*, *M. koenigii* and *M. arvensis*

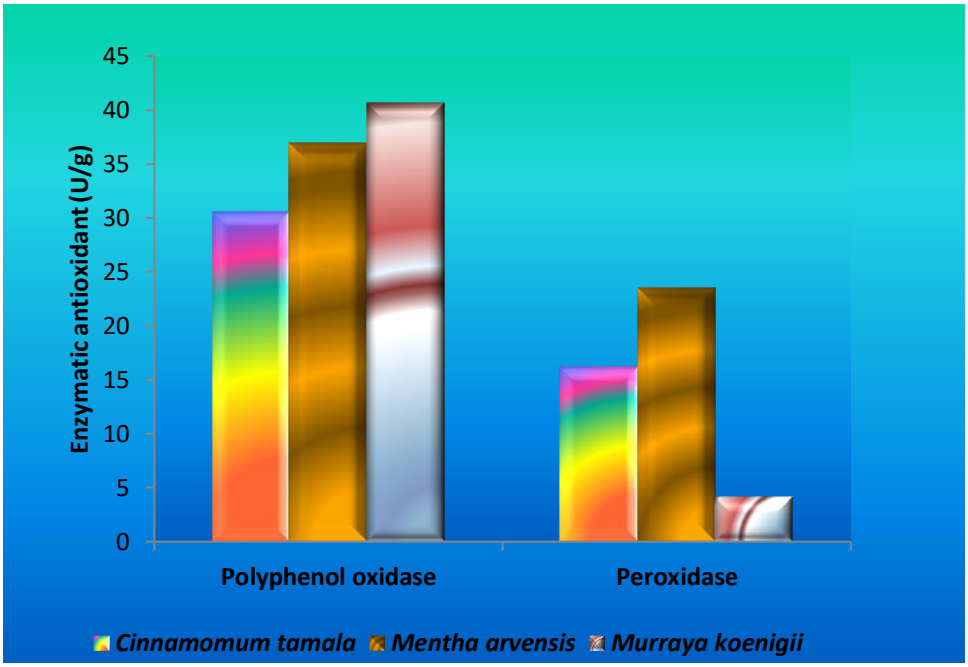
Plant name	Polyphenol oxidase (U*/g)	Peroxidase ($U^{\#}$/g)
Cinnamomum tamala	30.50±0.80	16.17±1.26
Mentha arvensis	37.00±0.50	23.50±1.14
Murraya koenigii	40.67±1.10	4.10±1.15
SEd	0.5915	0.9665
CD(<0.5)	**1.3639**	2.3650

Values are mean ± SD of three triplicates

*1 Unit – Activity of catechol oxidase which transforms 1 U/mol of dihydro-phenol to quinine/min.

#1 unit – U/g

Fig 3. Comparision of Enzymatic Antioxidants in *C. tamala*, *M. koenigii* and *M. arvensis*



4.4.2.2 Peroxidase

The peroxidases are enzymes whose primary function is to oxidize hydrogen donors at the expense of peroxides. They are highly specific for hydrogen peroxide, but they accept a wide range of hydrogen donors, including polyphenols.

Peroxidise activity was found to be maximum in *Mentha arvensis* (23.50 U/g) and minimum in *Murraya koenigii* (4.10 U/g)The antioxidant analysis conducted by Akbar *et al.*, (2014) was found to be the hydrogen peroxide scavenging activity of methanol extract at 500 µg/ml was found to be 61.39% and for ascorbic acid at same concentration was 96.43%.

4.4.3 Non-Enzymatic Antioxidants

4.4.3.1 Ascorbic Acid

Ascorbic acid was found to be more in *Mentha arvensis* and low in *Cinnamomum tamala* with a range of 5.30µg/g and 3.00 µg/g respectively. Our results were par with the result obtained by Igara *et al.*, (2010) who found the ascorbic acid content in diethyl ether extract of *M. koenigii* leaf was 0.004 mg/100g

4.4.3.2
Total Polyphenol

Plant antioxidants have generally phenolic moiety. Phenolic compounds can easily donate electrons to reactive radicals because of the resonance stability of phenoxy radical and thus retard radical chainreactions (ozgen *et al*., 2010).

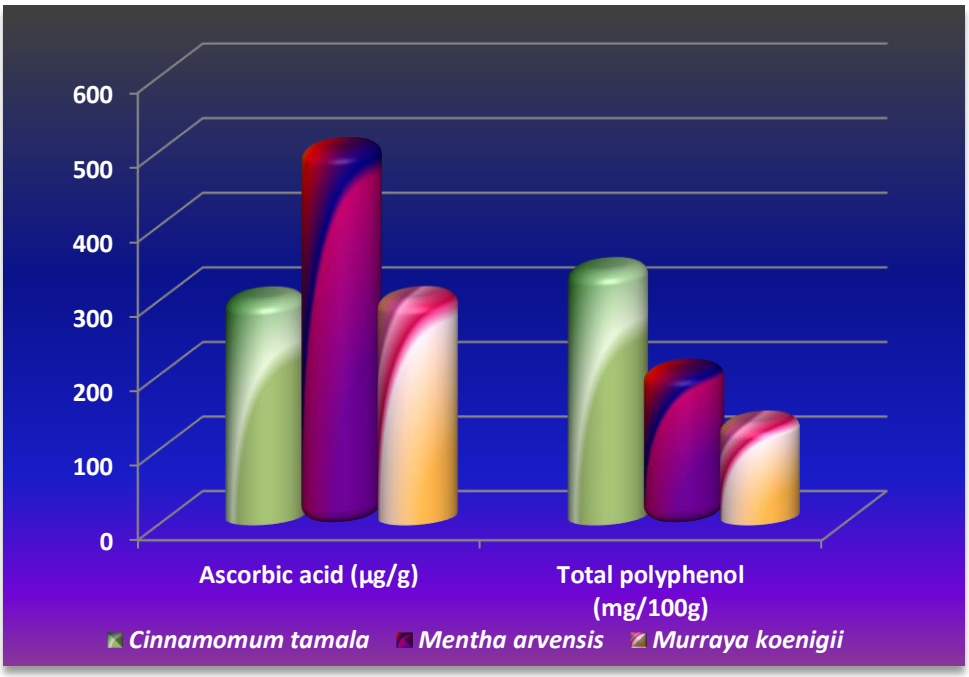
Total poly phenol was found to be maximum in *Cinnamomum tamala* (339.30 µg/g) and minimum in *Murraya koenigii* (40.67 µg/g). Our result were par with the result obtained by Dandapat *et al*., 2015 who found the total poly phenol content in methanol and aqueous extract of *C. tamala* was g/100g).

Table: 9. Levels of Non-Enzymatic enzymatic activity in *C. tamala, M. koennigii* and *M. arvensis*

Plant name	Ascorbic acid (µg/g)	Total polyphenol (mg/100g)
Cinnamomum tamala	3.00 ± 0.08	339.30±0.66
Mentha arvensis	5.30 ± 0.39	203.03±0.85
Murraya koenigii	3.36 ± 0.36	133.47±1.00
SEd CD(<0.5)	0.2547 0.6233	0.6293 1.6940

Values are mean ± SD of three triplicates

Fig 4 Comparision of Total Non-enzymatic antioxidant Ascorbic acid and Total Polyphenol in *C. tamala, M. koenigii* and *M. arvensis*



4.5 Free Radical Scavenging activity

4.5.1 DPPH Scavengigng activity

The DPPH assay is based on the ability of the antioxidants present in the sample to decolorize DPPH free radical by virtue of their scavenging activities. Ascorbic acid was chosen as the reference antioxidant for this test. The DPPH method is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors, and to evaluate antioxidant capacity. DPPH contain odd electrons, which is responsible for the absorbance at 517nm. Antioxidants donate an electron to DPPH and decolorize it, which can be be quantitatively measured from the changes in absorbance (Ebrahimzadeh *et al*., 1997).

The parameter IC50 (efficient concentration value), is used for the interpretation of the results from the DPPH method and is defined as the concentration of substrate that causes 50% loss of the DPPH activity (color) **(**Aju *et al*., 2017).

The DDPH scavenging activity of methanol extract of *C. tamala*, *M. koenigii* and *M. arvensis* was concentration dependant (increasing from 10 µg/ml - 50 µg/ml) and it was able to inhibit the formation of DPPH radicals.

The antioxidant activity of methanol extract increased in a dose dependent manner with IC_{50} value of 28.23, 34.24, 26.44 and 25.49 in *C. tamala*, *M. koenigii* *M. arvensis* and L-Ascorbic acid respectively.

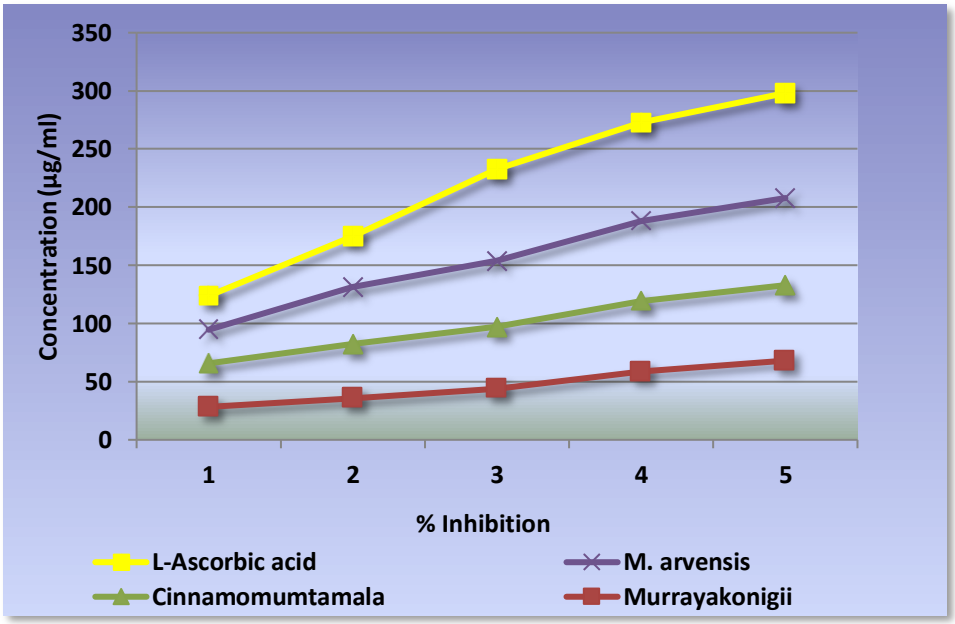
According to the study of Aju *et al*., 2017, hydroalcoholic extract of *M. koenigii* leaves exhibits greater scavenging activity compared to all other extracts. Comparing all the IC_{50} valus of all the three plants in methanol extract, it seems *M. koenigii* had a good value of 34.24 while *M. arvensis* showed minimum IC_{50} value (25.49).

Sumit *et al*., (2009) conducted antioxidant analysis in Acetone, alcohol and aqueous extracts of *M. koenigii* leaves have shown potent DPPH radical scavenging activity with an IC_{50} value of 4.72µg/ml,4.10µg/ml and 4.46µg/ml. IC_{50} value of ascorbic acid was found to be 2.69 µg/ml.

Table: 10. DPPH Scavenging activity of *C. tamala*, *M. koenigii* and *M. arvensis*

CONC (µg/ml)	*C. tamala*	*M. koenigii*	*M. arvensis*	L-Ascorbic acid
10	37.39±0.71	28.30±0.90	28.92±1.00	29.11±0.90
20	46.63±0.50	35.61±0.85	48.91±0.71	43.77±0.80
30	53.30±0.90	43.92±0.90	56.70±0.55	78.49±0.44
40	61.03±0.95	58.48±0.60	68.68±0.90	84.42±0.75
50	64.88±0.68	68.06±0.85	74.87±0.81	90.25±0.71
Sed	**0.6242**	**0.6769**	**0.6610**	**0.6139**
CD(p<0.05)	**1.3909**	**1.5083**	**1.4728**	**1.3679**

Fig Comparision of DPPH scavenging activity in *C. tamala*, *M. koennigii* and *M. arvensis*



4.5.2 Reducing Power assay

The reducing properties are generally associated with the presence of reductones (4). Reducing power measures the ability of the extract to donate electron to Fe(III) and it is evaluated by the transformation of Fe3+/ferricyanide complex to ferrous ions (Fe2+) in the presence of the sample extracts (Gulcin *et al*., 2003). In this assay, the yellow colour of the test solution changes to green depending on the reducing power of test specimen.

IC_{50} values of the extracts also calculated from the graph of absorbance at 700 nm against extracts concentration and the results have been shown in Table 15. The lower the IC50 value, the higher will be the reducing power.

The reducing power assay of methanol extract of *C. tamala*, *M. koennigii* and *M. arvensis* was concentration dependent (increasing from 10 µg/ml – 50 µg/ml).The antioxidant activity of ethanol extract increased in a dose dependent manner with IC_{50} value of 31.28, 27.61, 36.81 and 19.12 in *C. tamala*, *M. koenigii*, *M. arvensis* and L-ascorbic acid respectively.

Comparing IC_{50} values of all the three plants extracts, it seems *M. arvensis* had a good value of 36.81in methanol extract, while *M. koennigii* showed minimum IC_{50} value.

According to Dar *et al*., 2013 the hydroalcohol extract of *M. koenigii* showed good reducing power than aqueous extract when compared with standard ascorbic acid.

Dar *et al*., (2014) conducted phytochemical and antioxidant analysis in hydroalcoholic extract of *M. arvensis* was revealed that the reducing power shown by aqueous extract was 0.75 and 1.48 by hydro alcohol extract at concentration of 0.25 mg/ml as compared to 2.69 shown by standard ascorbic at same concentration.

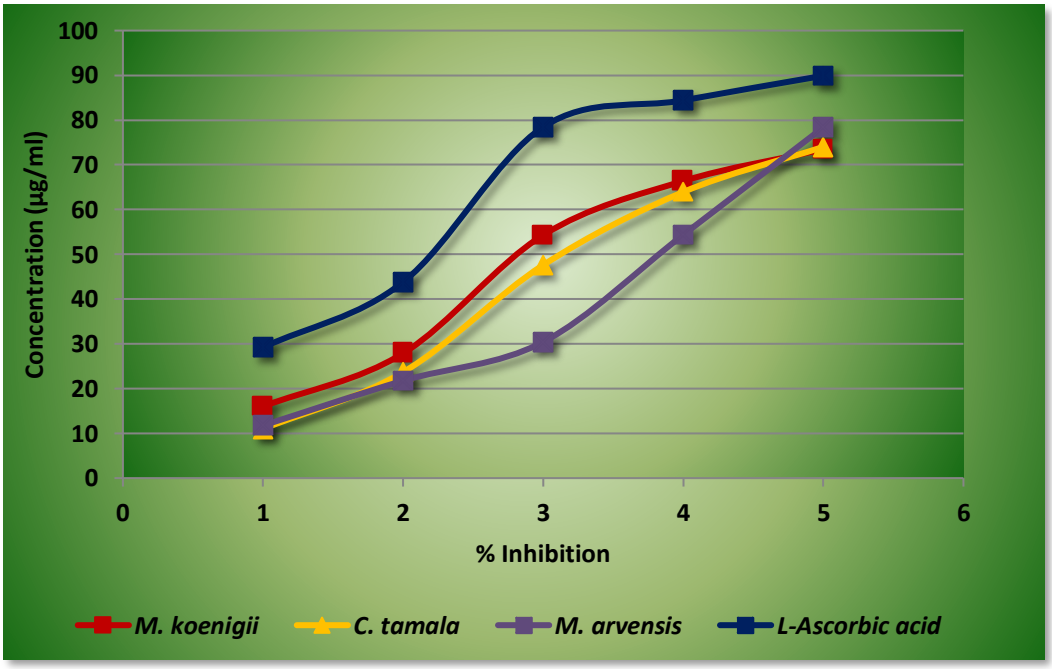
Phytochemical and antioxidant analysis conducted by Akbar *et al.*, (2014) revealed that the reducing power shown by methanolic root extract of *M. arvensis* was 1.98 at concentration of 0.25mg/ml as compared to 2.69 shown by standard ascorbic acid at same concentration.

Table: 11. Reducing power assay of *C. tamala*, *M. koenigii* and *M. arvensis*

Conc (µg/ml)	*C. tamala*	*M. koenigii*	*M. arvensis*	L-Ascorbic acid
10	10.98±0.76	16.00±0.72	11.75±0.94	29.21±0.70
20	23.67±0.66	27.88±0.82	21.76±0.79	43.80±0.69
30	47.60±0.56	54.31±0.65	30.42±0.73	78.46±0.86
40	63.93±0.89	66.38 ±0.77	54.36±0.67	84.42±0.68
50	74.01±0.95	73.46±0.81	78.38±0.58	89.91±0.41
SEd	**0.6331**	**0.6179**	**0.6130**	**0.5565**
CD (p<0.05)	**1.4106**	**1.3768**	**1.3659**	**1.2400**

Values are mean of three triplicates

Fig 6. Comparision of Reducing power assay in *C. tamala*, *M. koenigii* and *M. arvensis*



4.5.3 Metal Chelating Activity

The production of the hydroxyl radical is a key initial step to producing other harmful radicals which should be avoided (Prasad *et al*., 1989). Ferrozine has a tendency to form red-coloured complexes quantitatively with ferrous ion, but in the presence of other iron chelating agents, the complex formation is disrupted resulting in a decrease in the red colour of the complex (Rajauria *et al*., 2010).

The metel chelating ability of the hydroalcoholic and aqueous extracts of *Mentha arvensis* was measured by the formation of ferrous ion ferrizine complex. Ferrozine react with ferrous ions forming a red coloured complex which selectively absorbant 562 nm (Yamaguchi *et al*., 2000).

The ability of chelating agent to form σ bond with a metal, may act as effective as secondary antioxidants, because they reduce the redox potential thereby stabilizing the oxidized form of the metal ion (Duh *et al*.,1999).

The metal chelating activity of methanol extract of *C. tamala, M. koenigii and M. arvensis* was concentration dependent (increasing from 10µg/ml – 50 µg/ml). The free radical scavenging activity of methanol extract increased in a dose dependent manner with IC_{50} value of 31.28, 27.61, 36.81 and 19.12 in *C. tamala*, *M. koenigii*, *M. arvensis* and L-ascorbic acid respectively.

Comparing all the IC_{50} values of all the three plants, it seems *M. arvensis* had a good value of 36.81in methanol extract, while *M. koenigii* showed minimum IC_{50} value of 27.61.

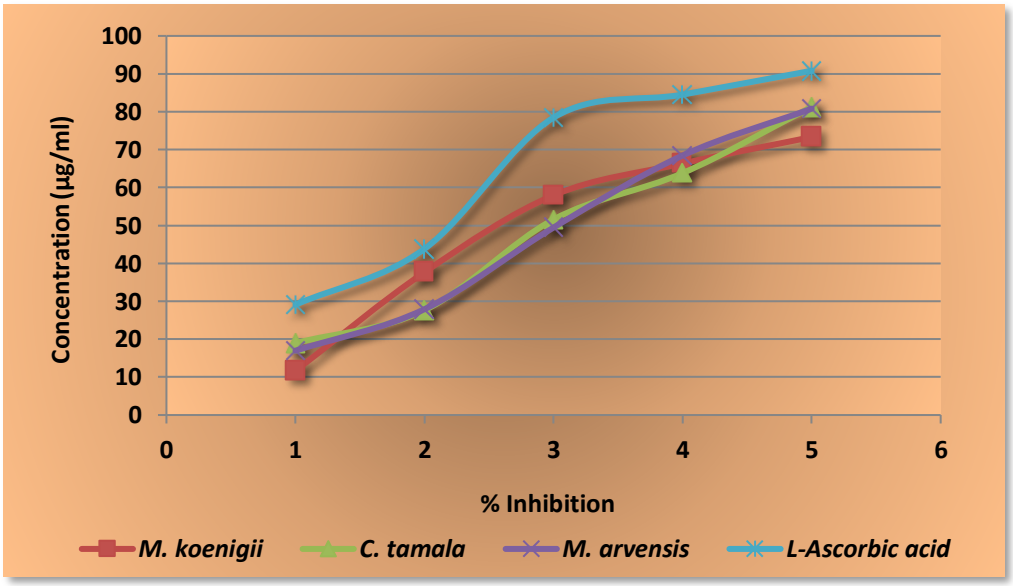
Dar *et al*., (2014) reported that the percentage of metal chelating capacity at the concentration of 500 µg /ml was 46.1 and 65 for aqueous and hydroalcohol extracts of root of *M. arvensis* respectively. The percentage inhibition for standared ascorbic acid was 98.8 at same concentration.

Table: 12. Metal chelating activity of *C. tamala*, *M. koenigii* and *M. arvensis*

Conc (µg/ml)	*C. tamala*	*M. koenigii*	*M. arvensis*	L-Ascorbic acid
10	18.98±0.88	11.68±0.83	16.96±0.77	29.11±0.58
20	27.64±0.56	37.67±0.65	27.91±0.67	43.84±0.78
30	51.58±0.83	57.96±0.69	49.45±0.79	78.46±0.58
40	63.81±0.71	66.35±0.69	68.46±0.71	84.52±0.95
50	81.28±0.85	73.32±0.56	80.80±0.69	90.80±0.60
SEd	**0.6327**	**0.5636**	**0.5945**	**0.5833**
CD (p<0.05)	**1.4098**	**1.2557**	**1.3246**	**1.2996**

Values are mean of three triplicates

Fig 7. Comparison of Metal chelating activity in *Cinnamomum tamala, Murraya koenigii* and *Mentha arvensis*



The stem bark and leaves methanol extract shows good activity in reducing Fe^{+3} to ferrous ion. Reductones in an extract exhibit their antioxidant activities through the action of breaking the free radical chain by donating a hydrogen atom to convert radicals into stable and non-harmful products (Singh and Rajini., 2004)

4.5.4 FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP assay, is presented as a novel method for assessing "antioxidant power." Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex to form. This is based on the reduction power of all types of leaves extract from Fe^{3+} to Fe^{2+}.

The FRAP scavenging activity of methanol extract of ***C. tamala***, *M. koenigii* and *M. arvensis* was concentration dependent (increasing from 10µg/ml-50µg/ml). The antioxidant activity of methanol extract incraesed in a dose dependent manner with IC_{50} value 31.05, 26.44, 28.89 and 19.12 in *C. tamala*, *M. koenigii*, *M. arvensis* and L-ascorbic acid respectively.

Comparing all the IC50 values of all the three plants it seems, *C. tamala* had a good value of 31.05,while *M. koenigii* exhibit minimum value(26.44).

Ishtiaque *et al*., (2015) conducted a phytochemical and antioxidental analysis on crude mathanolic leaves extract have shown 40-50µg /100µl anti oxidant potential.

Table:13. FRAP assay of *C. tamala*, *M. koennigii* and *M. arvensis*

Conc (µg/ml)	*C. tamala*	*M. koenigii*	*M. arvensis*	L-Ascorbic acid
10	20.12 ± 0.70	18.27 ± 0.80	16.44 ± 0.73	29.10 ± 0.81
20	30.30 ± 0.64	32.62 ± 0.60	22.40 ± 0.92	43.67 ± 0.84
30	42.42 ± 0.80	56.70 ± 0.55	51.59 ± 0.75	78.52 ± 0.74
40	64.34 ± 0.75	73.53 ± 0.55	75.83 ± 0.78	84.50 ± 0.93
50	88.23 ± 0.58	81.25 ± 0.80	83.16 ± 0.67	90.12 ± 0.84
SEd	0.5695	0.5477	0.6318	0.6806
CD (P<0.05)	1.2689	1.2204	1.4076	1.5164

Fig 8. Comparision of FRAP assay in *C. tamala*, *M. koenigii* and *M. arvensis*

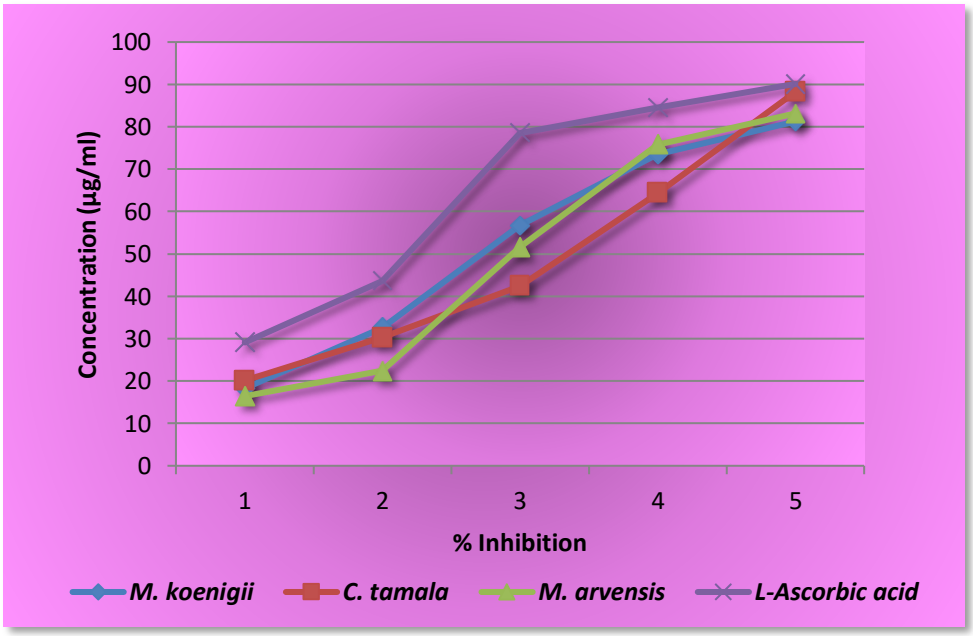
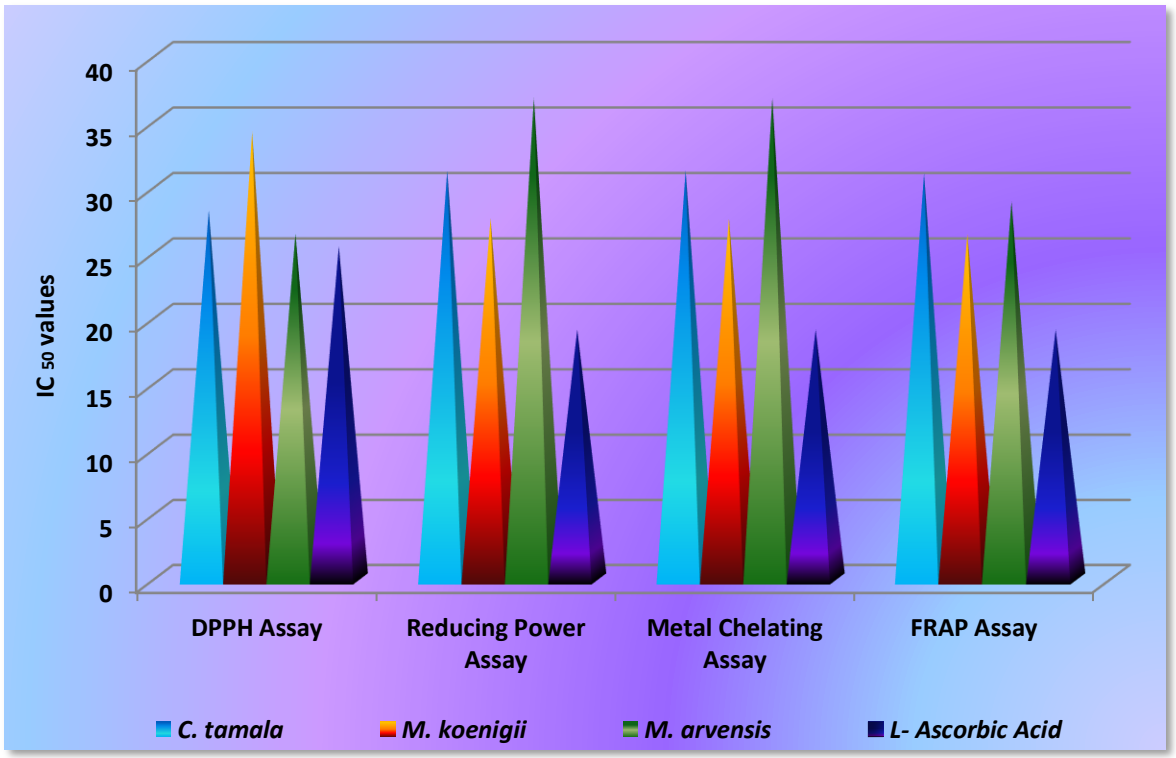


Table:14. IC $_{50}$ values of various methanolic leaf extracts and standards

Plant Extracts / Standards	IC $_{50}$ (µg/ml)			
	DPPH Assay	**Reducing Power Assay**	**Metal Chelating Assay**	**FRAP Assay**
Cinnamomum tamala	28.23	31.28	31.28	31.05
Murraya koenigii	34.24	27.61	27.61	26.44
Mentha arvensis	26.44	36.81	36.81	28.89
L- Ascorbic Acid	25.49	19.12	19.12	19.12

Fig: 9. Graphical representation of IC$_{50}$ values of various free radical scavenging capacity of three plants.



Summary and Conclusion

Summary and Conclusion

Phytochemical screening is of paramount importance in identifying new source of therapeutically and industrially valuable compound having medicinal significance, to make the best and judicious use of available natural wealth.

The solubility % was good in all the samples. The primary metabolites, carbohydrate and protein and the secondary metabolites like alkaloids, Flavonoids, glycosides, ascorbic acid, terepenoides, phenols, tannin and phytosterol were present in all plants investigated. TLC screening also revealed the presence of Red, green and pink spots in Vanillin sulphuric acid detector which reveals the presence of steroids and terpenoids. It is obvious that constituents like tannins, reducing sugars and proteins, which are present in the extracts, may be liable for antioxidant activity.

C. tamala was found to have higher carbohydrates, while *M. koenigii* was found to have more protein. The phytochemical parameters which have a direct link to antioxidant properties of a plant like flavonoid, tannin and phenol were also estimated. In flavonoid, phenol and tannin the highest concentration in µg equivalent to Gallic acid were found in *M. arvensis*, *M. koenigii* and *C. tamala*.

Enzymatic antioxidants in *M. koenigii* and *M. arvensis* proved to be the best among the three plants in polyphenol oxidase and peroxidase test, where it is expressed in units/g. non-enzymatic antioxidants, where also found to show good results in ascorbic acid and total polyphenol.

Free radical scavenger activity of methanolic extract of *Cinnamomum tamala*, *Murraya koenigii* and *Mentha arvensis* were evaluated. Here ascorbic acid was used as standard whose IC_{50} value was 19.12 µg/ml.

Among, the three studied species *Mentha arvensis* showed higher DPPH activity having the IC_{50} value of 26.44 µg/ml, which was nearer to the standard ascorbic acid on the other hand methanolic extract of *Murraya koenigii* exhibited the lowest antioxidant activity

with IC50 value 34.24 µg/ml. the IC 50 values obtained from DPPH assay, Reducing power assay, metal chelating assay, FRAP assay revealed that *M. koenigii* had values nearer to the standard when compared with other samples.

M. koenigii have shown potential as sources of natural antioxidants, further studies need to be directed to isolate and characterise antioxidant active compounds from the extracts which could be responsible for the high antioxidant activities.

Bibliography

References

Adebajo, A.C., Olayiwola, G., Verspohl, E.J., Iwalewa, E.O., Omisore, N.O.A., and D. Bergenthal. 2004. Evaluation of the ethnomedical claims of *Murraya koenigii*. *Pharm Biol.* 42(8):610-620.

Ahuja, I., Kissen, R. And A.M. Bones. 2012. Phytoalexins in defense against pathogens. *Trends Plant Sci.* 2012, *17*, 73–90.

Aju, B.Y., Rajalakshmi, R. And S.Mini. 2017. Evaluation of Antioxidant activity of *Murraya koenigii* (L.) spreng using different in vitro methods.

Andrade, K.S. and S.R.S. Ferreira. 2013. Antioxidant activity of black pepper (*piper nigrum l.*) Oil obtained by supercritical co2. III Iberoamerican Conference on Supercritical Fluids Cartagena de Indias (Colombia).

Anuradha, S., Lakshmi Devi, S.,and Kamappan.2007. Evaluation of in vivo antioxidant activity of Indian bay leaf, *Cinnamomum tamala* (Buch.-Ham.) T.Nees and Eberm using rat brain synaptosomes as model system. Indian Journal of Experimental Biology.

Arun, T.,Omprakash, S.,Saravanan P,Tadele, A,Abawa G,*et al*.2017.Evaluation of preliminary phytochemical constituents and Antibacterial activity of edible plants against urinary trac infection Causing Bacteria in Children.J Clin Micribiol Biochem Technol 3(2): 024-030.

Azzouz, M.A. and L.B.Bullerman. 1982. Comparitive antimycotic Effects of Selected herbs, species, plant components and commercial antifungal agents.Journal of food protection. 45(14): 1298-1301.

Bandyopadhyaya, S., K.C, Roy,M., Roy, Pal, B.C.and Bhandra R, *et al*. 2002. Herbal Composition Of Blend Of Active Components Prepared Form *Murraya koenigii* And Piper betle Useful For Blocking of 5- Lipooxygenase Activity, US Pat Appl Publ, USA, p.1- 86.

Baselga-Escudero, L., Souza-Mello, V.,Pascual-Serrano, A., Rachid, T., Voci, A.Demori. and I. Grasselli, E.2017.Beneficial effects of the Mediterranean spices and aromas on non-alcoholic fatty liver disease.Trends Food Sci. Technol., 61, 141–159.

Bi, X. Lim .and J. Henry., C.J.2017. Spices in the management of diabetes mellitus. Food Chem. 2017, 217, 281–293.

Broadhurst C.L, Polansky, M.M. and Anderson R.A.2000. Insulin-like activity of culinary and medicinal plant aqueous extracts *in vitro*. J. Agric. Food Chem. 48: 849–852.

CALIXTO.,J.B.Twenty-five years of research on medicinal plants in Latin America: personal review. Journal Ethnofarmocology. V.100, p.131-134,2005.

Chakrabarty ,M., Nath, A., Khasnobis S., Chakrabarty ,M., Konda ,Y. and Harigaya, Y *et al*. Carbazole alkaloids from *Murraya koenigii*. Phytochemistry 1997; 46(4):751-755.

Chanda N., Shukla R, Zambre A, Mekapothula S, Kulkarni RR, Katti K.2011. An effective strategy for thesynthesis of biocompatible gold nanoparticles using cinnamon phytochemicals for phantom CT imaging and photoacoustic detection of cancerous cells.Pub med. 28(2): 279-91.

Chang, S.T, Cheng, S.S.2002. Antitermitic activity of leaf essential oils and components from Cinnamomum osmophleum. J Agric Food Chem 50: 1389-1392.

Charles, D.J.2013. Antioxidant Properties of Spices, Herbs and Other Sources; Springer: New York, NY, USA,; p. 612.

Choi, I.S. and Cha, H.S.2014 Physicochemical and antioxidant properties of black garlic. Molecules 2014,19, 16811–16823.

Chomchalow, N. 1996. Spice production in Asia-An overview.IBC's asia spce market'96 conference, Singapore,27-28,May 1996.

Chopra,R.N. and Chopra, I.C.1994. Indigenous drugs of India, (2nd Ed.) Calcultta, India:Academic Publishers, 196.

Dandapat, S., Kumar ,M. and Sinha, M.P.2014.Therapeutic efficacy of *Cinnamomum tamala* (Buch.-Ham.) and *Aegle marmelos* (L.) leaf. Balneo Research Journal 5: 113-122.

Daniyan,S.Y. and Muhammad H.B.2008.Evaluation of the antimicrobial activities and phytochemical properties of extracts of *Tamaridus indica* against some diseases causing bacteria. African Journal of Biotechnology; 7 14 : 2451-2453.

Dar, M.H., Masoodi. and M. A. Mir.2014.phytochemical screening and antioxidant potential of root of *M.arvencis.* L from Kashmir region.M.A. International Journal Of Pharmaceutical Sciences And Research (*IJPSR), 2014; Vol. 5(4): 1572-1580* .

Devi ,S.L, Kannappan S. and Anuradha, C.V.2007. Eval*uation of in-vitro antioxidant activity of* Indian bay Leaf, *Cinnamomum tamala* using rat brain synaptosomes as model system. *Indian Journal of Experimental biology*. Vol 45:778-784.

Dhar, U., Manjkhola, S., Joshi, M., Bhatt, A., Bisht, A. K., and Joshi, M. 2002. Current status and future strategy for development of medicinal plants sector in Uttaranchal, India. *Current Science*, *83*(8), 956-964..

Dorman, H. J., M. Kosar, K. Kahlos, Y. Holm and R. Hiltunen. 2003. Antioxidant Prosperities and composition of aqueous extracts from Mentha species, hybrids, varieties and cultivars. J. Agri. and Food Chem., 51:4563–4569.

Dutta,s.,Dey.P.,k.Soha,M.R,Sankar,I.,Sarkar,R.,mardi,J.A.,Barman,J.,Sen,A.,and T.K.Chaudhai.2017.Differential interaction with O2 and N2 free-radicals, phytochemical fingerprinting and molecular docking reveals potent antioxidant activities of three major recreational foods of the Indian subcontinent,Journal of functional foods,39:112-122.

Fabri R.L, Nogueira, M.S., Braga,F.G. and Coimbra, E.S.2009. Scio E. Mitracarpus frigidus aerial parts exhibited potent antimicrobial, antileishmanial and antioxidant effects. Biores. Technol. 100: 428–433.

Gambhire Manoj Nilkant., Archana Ramesh Juvekar., Shaijesh Shirish Wankhede. 2009.Anti inflammatory activity of aqueous extract of *Cinnamomum tamala* leaves by *in vivo* and *in vitro* methods Journal of Pharmacy Research, 2(9),1521-1524.

Gandhi, N., Pillai S. and Patel P. Efficacy of pulverized *Punica granatum* (Lythraceae) and *Murraya koennigii* (Rutaceae) leaves against stored grain pest *Tribolium castaneum* Coleoptera: Tenebrionidae). International Journal of Agricultural Biology 2010; 12(4):616-620.

Ganesh CJ, Manjeshwar SB.2002. Influence of the leaf extract of Mentha arvensis Linn.(mint) on the survival of Mice exposed to different doses of Gamma Radiation. Strhlenther Onkol., ,178; 91-8.

Gill, S., Panthari, P. And H.Kharkwal. 2015. Phytochemical investigation of High altitude medicinal plants *Cinnamomum tamala* (Buch-Ham) Nees and Eberm and Rhododendron arboretum Smith.American Journal of phytomedicine and clinical therapeuitics.3 (6): 512-528.

Goyal P, Chauhan, A. and Kaushik, P .2009. Laboratory evaluation of crude extracts of *Cinnamomum tamala* for potential antibacterial activity. Electronic Journal of Biology 5: 75-79.

Gulcin I, Oktay ,M., Kirecci. And E,Kufreviolu I.2003. Screening of antioxidant and antimicrobial activities of anise (pimpella anisum L.) seed extracts.Food chem..83:371-382

Gupta, Rahul , Kumar Gaurav Bajpai1., Samta Johri, A.M. Saxena An Overview Of Indian Novel Traditional Medicinal Plants With Antidiabetic Potentials *Afr. J. Trad. Cam* (2008) 5 (1): 1 –1.

Gupta,S.,George M, Singhal M, Sharma GN, Grag V.2010.Leaves extract of *Murraya koenigii* Linn for anti-inflammatory and analgesic activity in animal models. J Adv Pharm Technol Res 1(1): 68-77.

Gupta,S., And Prakash ,J.2009**.** Studies on Indian green leafy vegetables for their antioxidant activity. Plant Foods Hum Nutr 64(1): 39-45.

Gupta, V. and Sharma, M.2010. Protective effect of *Murraya koenigii* on lipid peroxide formation in isolated rat liver homogenate. Int J Pharma Bio Sci 1(3): 1-6.

Gurdip Singh a, Sumitra Maurya., M.P. deLampasona , Cesar A.N. and Catalan.2007**.** "A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils,oleoresins their constituents" Food and Chemical Toxicology 45 :1650–1661.

Hannah, Vasanthi ,R. and Parameswari, R .P .2010.Indian Spices for Healthy Heart - An Overview. *Cur Card Rev,* 2010; 6: 274-279.

Hannah,Vasanthy ,R. And R p. Parameshwari. 2010.Indian spices for healthy art-An overview. *Cur Card* Rev. 6:274-279.

Hassan W., Kazmi S.N.Z., Noreen H., Riaz A. And Zaman B.2016. Antimicrobial Activity of *Cinnamomum tamala* Leaves. J Nutr Disorders Ther 6: 190. doi:10.4172/2161-0509.1000190.

Hussain, A, Virmani O.P, Popil S.P, Mishra L.N and Gupta ,A.K.2008**.** Dictionary of Indian Medicinal Plants, CIMAP Lucknow,; Vol. 7(3)2008, 425-428 .

Iyer,D, Uma, D.P.2008. Phyto-pharmacology of *Murraya* . Pharmacognosy Reviews 2008; 2: 180-184.

Iyer,UM, Mani, U.V.1990.Studies on the effect of curry leaves supplementation (*Murraya koenigii*) on lipid profile, glycated proteins and amino acids in non-insulin dependent diabetic patients. Plant Foods for Human Nutrition 1990; 40(4):275-282.

Jain, V, Momin M, Laddha K.2012. *Murraya koenigii*: An Updated Review. International Journal of Ayurvedic and Herbal Medicine 2(4): 607-627.

Jayanthi, P. Thamaraiselvi P. 2012.Preliminary studies on phytochemicals and antimicrobial activity of solvent nextracts of Eichhornia crassipes (Mart.) Solms. *Asian Journal of Plant Science and Research*, 2012, 2(2):115-122.

Joshi,B.S., Kamat .V.N and Gawd D.H.1970. Structures of Girinimbine, Mahanimbine, Isomahanimbine, Koenimbidine And Murrayacine. Tetrahedron 26(6): 1475-1482.

Kanjanapothi, D., Smitasiri, Y., Pathong, A., Taesotikul, T., Rathanapanone and V. Postcoital.1981**.** antifertility effect of Mentha arvensis. Contraception., 1981, 24;559-567.

Khare, C.P. Encyclopedia of Indian Medicinal plants, 2004. Springerverlag Berlin Heidol, 309-10.

Khosa, R.L and Prasad, S .1974. Pharmacognosy of roots of *Murraya koenigii* and Murraya paniculata. J Res Indian Med 9(3): 105.

Khuntia, T. and , Panda D.S.2011. Evaluation of antibacterial, antifungal and anthelmintic activity of *Murraya koennigii* Spreng. Pharma Sci Monit 2011; 2(2):105-110.

Kiritikar, K.R. and Basu B.D.1982.Indian medicinal Plants, 1998. (2nd Ed.) Allahabad, India: Lalit Mohan Basu.

Kirtikar,K. R., And Basu, B. D.1993.Indian Medicinal Plants. Vol 1, 2nd edition.Bishen Singh Mahendra Pal Sigh, India: 472-474.

Kowti Rajesh, A.H.M., Vishwanath Swamy,. Shivakumar.S., Inamdar,Vedamurthy Joshi , Abdul Nasir.2014**.** kurnool4.Hepatoprotective And Antioxidant Activity Of Ethanol Extract Of Mentha arvensis Leaves Against Carbon Tetrachloride Induced Hepatic Damage In Rats. Journal of Applied Pharmaceutical Science 4 (03); 2014: 050-057.

Kumar, V.S, Sharma. A., Tiwari R and Kumar S. 1999.*Murraya koenigii* (curry leaf): a review. Journal of Medicinal and Aromatic Plant Science 1999; 21(4):1139-1141.

Mandal S, Nayak A, Kar M, Banerjee SK, Das. and Upadhyay SN *et al*. 2010.Antidiarrhoeal activity of carbazole alkaloids from *Murraya koenigii* Spreng (Rutaceae) seeds. Fitoterapia; 8(1):72-74.

McCormick. The History of Spices. Available online: http://www.mccormickscienceinstitute.com/ resources/history-of-spices (accessed on 30 May 2017).

Mhaskar, K.S, Blatter ,E., Caius J.F., Kirtikar and Basu's Illustrated Indian Medicinal Plants, Vol. I. XI. Edn 3, Indian Medical Science Delhi, India 2000, 86-96.

Middleton E, Kandaswami C.1993. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer.619-652.

Mohamed, D.A. and l-okbi S.Y. 2004.In vivo evaluation of antioxidant and anti-inflammatory activity of different extracts of date fruits in adjuvant arthritis. Polish J. of food and nutr. Sci., 13/54(4): 397–402.

Mohamed ,D.A. and l-okbi S.Y.2004 In vivo evaluation of antioxidant and anti-inflammatory activity of different extracts of date fruits in adjuvant arthritis. Polish J. of food and nutr. Sci., 13/54(4): 397–402.

Msaada,K, Jemia ,M.B., Salem N, BachrouchO, SritiJ, Tammar S *et al*.2013. Antioxidant activity of methanolic extracts from three coriander (*Coriandrum sativum L*.) fruit varieties. Arabian J. of Chem., 2013,12(011).

Muthumani P, Venkatraman , S, Ramseshu K.V, Meera ,R, Devi P, Kameswari B. 2009.Pharmacological studies of anticancer, anti inflammatory activities of *Murraya koenigii* (Linn) Spreng in experimental animals. J Pharm Sci Res 2009;1:137-41.

Nagappan T, Segaran T.C, Wahid M..EA, Ramasamy P. And Vairappan CS.2012.Efficacy of Carbazole Alkaloids, Essential Oil and Extract of *Murraya koenigii* in Enhancing Subcutaneous Wound Healing in Rats. Molecules 17(2): 14449-14463.

Narasimha N.S, Paradkar M.V, Chitguppi VP, Kelkar SL.1975.Alkaloids of *Murraya koenigii*: Structures Of Mahanimbine, Koenimbine, (-) Mahanine, Koenine, Koenigine, Koenidine And (+)- Isomahanimbine. Indian Jour Chem 13(10): 993-999.

Nayak, A, Banerji, J., Banergy, A, Mandal S. 2010.Review on chemistry and pharmacologyof *Murraya koenigii* Spreng (Rutaceae). J Chem Pharm Res;2:286-99.

Ningappaa,M.B., Dhananjayaa,B.L., Dineshaa,R.,Harshaa,R.and Srinivas L. 2010.Potent antibacterial property of APC protein from curry leaves (*Murraya koenigii* L. Food Chemistry; 118(3):747-750.

Nutan, M.T.H., Hasnat, A. and Rashid, M.A.1998.Antibacterial and cytotoxic activities of *Murraya koenigii*. Fitoterapia. 1998; 69(2):173-175.

Okuda ,T., Kimura, Y., Yoshida ,T., Hatano ,T., Okuda, H. And Arichi S. 1983. Studies on the activities of tannins and related compounds from medicinal plants and drugs. Chem. Pharm. Bull. 31: 1625–1631.

Parekh, J. and Chanda S, *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae African Journal of Microbiology Research Vol. 1 (6) pp.092-099, November 2007.

Parrota, J.A.2001. Healing Plants of Peninsular India. CASI Publication, USA, pp. 639.

Parthasarathy, V.A., Chempakam, B. and Zachariah, T.J.2008. Chemistry of Spices; CABI: Oxfordshire, UK.

Patra, K., Jana, K., Mandal, D.P. and Bhattacharjee, S.2016. Evaluation of the antioxidant activity of extracts and active principles of commonly consumed Indian spices. J. Environ. Pathol. Toxicol. Oncol, 35, 299–315.

Pérez, M.B., Calderón, N.L., and Croci, C.A.2007. ―Radiationinduced enhancement of antioxidant activity in extracts of rosemary (Rosmarinusofficinalis L.)‖, Food Chemistry,Volume 104, pp. 585-592, Argentina, 2007.

Peter, K.V. 2001.Handbook of Herbs and Spices; Woodhead Publishing Limited and CRC Press LLS: Cambridge, UK, 2001; Volume 1.

Peter, K.V.2004. Handbook of Herbs and Spices;Woodhead Publishing Limited: Cambridge, UK, 2004; Volume 2.

Ponnusamy, S., Ravindran, R., Zinjarde, S., Bhargava, S. and Ravi Kumar, A.2010. Evaluation of traditional Indian antidiabetic medicinal plants for human pancreatic amylase inhibitory effect in vitro. Evid Based Complement Alternat Med Epub.

Prabhu, K. A. and Tamilanban. T.2012. Investigation of anti-diabetic activity of stem of *Murraya koenigii*. International Journal of Research in Pharmacology and Pharmacotherapeutics 1(2): 165-168.

Prajapati, N.D., Purohit S.S., Sharma A.K. and Kumar, T.2003.A handbook of Medicinal Plants. Agrobios India 1: 401.

Prakash .2014. Phytochemical analysis of *Cinnamomum tamala* of leaves extract. Indian Streams Research Journal 4: 1-3.

Prakash, V., Natarajan CP.1974. Studies on Curry Leaf. Food Sci and Technol 11(6): 284-286.

Prasad, M.R., Engleman R.M., jones RM and Das DK.1989. Effect of oxyradicals onoxymyoglobin, Deoxygenation, haem removal and iron release. Biochem. J.263:731-736.

Preethi, R., Devanathan., V.V. and Loganathan, M.2010, ―Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens‖, Advances in Biological Research, Volume 4, No. 2, pp. 122-125, India, 2010.

Purohit, S.,S, Sharma.,A.K, Prajapati, N.D. and Kumar ,T.2009. A handbook of medicinal plants: a complete source book. Edn 2, Jodhpur: Agrobios (India) 2009:352-353.

Purthi, J.S.1976**.** Spices and Condiments. New Delhi, India: National Book Trust, 1976.

Rajauria, G, Jaiswal AK,Ghannam N.A and Gupta. S.2010.Effect of hydrochemical processing on colour antioxidant and free radical scavenging capacities of edible Irish brown seaweeds. Int.j.Food sci.Technol.45:2485-2493.

Rana, V.S, Juyal J.P, Rashmi. and Blazquez M.A. 2004.Chemical constituents of the volatile oil of *Murraya koennigii* leaves. International Journal of Aromatherapy 2004; 14(1):23-25.

Rao, B.R.R., Rajput, D.K and Mallavarapu, G.R.2011. Chemical diversity in curry leaf (*Murraya koenigii*) essential oil. Food Chem :126(3):989-994.

Rao, Chandana., Venkateswara ., Madhavan Vijayakumar., K. Sairam and Vikas Kumar.2008. Antidiarrhoeal activity of the standardised extract of *Cinnamomum tamala* in experimental rats. Journal of Natural Medicines, 2008.*ent Science*, 83(2002): 956-963

Saafi-Ben Salah, E.B., El Arem, A., Louedi, M., Saoudi, M., Elfeki, A., Zakhama, A., Najjar, M.F., Hammami, M., and Achour, L.,2012. ―Antioxidant-Rich Date Palm Fruit Extract inhIbits Oxidative Stress and Nephrotoxicity Induced by Dimethoate in Rat‖ Journal of Physiology and Biochemistry, Volume 68, pp. 47-58, Tunisia.

Saeed, N., Khan, M.R. and Shabbir, M. Antioxidant activity, total phenolic and total flavonoid contents of whole extracts *Torilis leptophylla* L. BMC Compl.and Alt. Med., 2012, 12:221.

Samah, N.A.; Mahmood, M.R. Muhamad.2014**. S**. The role of nanotechnology application in antioxidant from herbs and spices for improving health and nutrition: A review. Selangor Sci. Technol. Rev., 1, 17–23.

Sanjive Kumar.2017.,Antioxidant Properties Of Common Spices Used In Indian Kithens.Indian Journal of Science,8(1):121-124,2017.

Sasidharan,I .and A.N. Menon. 2011. Effects of temperature and solvent on antioxidant properties of curry leaf (*Murraya koenigii* L.).Journal of food science and Technology.48 (3): 366-370.

Sathaye, S., Bagul Y, Gupta S, Kaur H and Redkar R.2011. Experimental and Toxicologic Pathology : Official Journal of the Gesellschaft fur Toxikologische Pathologie. Experimental and Toxicologic Pathology 63(6): 587-591.

Serafini, M. and Peluso, I.2016 Functional foods for health: The interrelated antioxidant and anti-inflammatory roleof fruits, vegetables, herbs, spices and cocoa in humans. Curr. Pharm. Des. 2016, 22, 6701–6715.

Shan ,B., Cai ,Y.Z., Sun ,M. and Corke, H.2005. Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. J. Agric. Food Chem., 2005, 53 (20): 7749–7759.

Sharma, N. and Jacob. D.2001. Antifertility investigation and toxicological screening of the petroleum ether extract of the leaves of Mentha arvensis L. in male albino mice. J Ethnopharmacol., 2001, 75(1); 5-12.

Sharma, N. and Jacob,D.2005. Assessment of reversible contraceptive efficacy of menthanol extract of Mentha arvensis L. leaves in male albino mice. J Ethnopharmacol., 2002 , 80(1); 9-13.plants. J Ethanopharmacol., 97; 305-311.

Shivananda Nayak.2006. Influence of Ethanol Extract of *Vinca rosea* on Wound Healing in Diabetic Rats. Online Journal of Biological Sciences; 6 2 : 51-55.

Simic ,A., Sokovic M.D, Ristic M, *et al*.2004. The chemical composition of some Lauraceae essential oils and their antifungal activities. *Phytother Res.* 18: 713-770.

Singh, V., Gupta A.K, Singh., S.P and Kumar A .2012.Direct analysis in real time by mass spectrometric technique for determining the variation in metabolite profiles of *Cinnamomum tamala*Nees and Eberm genotypes. The Scientific World Journal 2012: 549265.

Singhal, P. Singla, N., Sakhare, D. and Sharma, K.A. A.2017.comparative evaluation of in vitro antioxidant activity of some commonly used spices of northern India. Nat. Prod. J., 7, 131–136.

Sola A.V.2014. Indian medicinal plants, 1995. Orient Longaman Private Ltd. (vol 4), 15.

Srinivasan, K. Antioxidant potential of spices and their active constituents (on line). Crit. Rev. Food Sci. Nutr., 54, 352–372.

Srivastava, B., Sagar.A and Dubey ,N.K .2011.Evaluation of *Cinnamomum tamala* oil and its phenylpropanoideugenol for their antifungal and antiaflatoxigenic activity. Food Analytical Methods 4: 347-356.

Srivastava, A., Shereen, R.H., and Shivanandappa, T.2006.,―Antioxidant activity of the roots of Decalepishamiltonii (Wight andArn.)‖, LWT-Food Science and Technology, Volume 39, No. 10, pp. 1059–1065, India.

Sumit Gupta., Padma, M., Paarakh. and Usha Gavani.2009. Isolation of Phytoconstituents from the 184 leaves of *Murraya koenigii* Linn. *Journal of PharmacyResearch 2009, 2(8),1313-1314.*

Surh, Y.J.2006. Chemopreventive Phenolic Compounds in Common Spices; Taylor and Francis: New York, NY, USA.

Tamokou, J.D., Chouna, J.R., Fischer-Fodor, E., Chereches, G., and Barbos, O.2013. ―Anticancer and antimicrobial activities of some antioxidant-rich cameroonian medicinal plants‖, Plos One, Volume 8, No.2, pp. 1-14, Romania, 2013.

Vardapetyan,H.,Tiraatsuyan,S.,Hovhannisyan,A.,Rukhkyan, M.and Hovhannisyan, D.2013. ―Phytochemical Composition and Biological activity of Laurusnobilis L. Leaves collected from two regions of South Caucasus‖, Journal of Experimental Biology and Agricultural Sciences, Volume 1, No. 2, pp. 45-51, Armenia, 2013.

Wealth of India: Dictionary of Indian raw materials and industrial products- Raw material series. Publication and Information Directorate.CSIR, New Delhi, 1950, vol. 2, pp. 178-179

Xie, J.T., Chang WT.,Wang ,C.Z., Mehendale,S.R., Li, J., Ambihaipahar. R *et al*.2006. Curry leaf *Murraya koenigii* Spreng. reduces blood cholesterol and glucose levels in ob/ob mice. The American Journal of Chinese Medicine; 34:279-284.

Yadav,P., Dubey,N.K, Joshi,V.K., Chansouria. and J.P.N, Yadav P.1999.Antidermatophytic activity of essential oil of Cinnamomum. Journal of Medicinal and Aromatic Plant Sciences 21: 347-351.

Yesiloglu, Y., Audin. and H. Kilic, I.2013. In vitro antioxidant activity of various extracts of ginger seed. Asian J. Chem., 25, 3573–3578.

Yesiloglu, Y.Audin., H.and Kilic, I.2013. In vitro antioxidant activity of various extracts of ginger seed. Asian J. Chem. 2013, 25, 3573–3578.

Zarai ,Z, Boujelbene,E., Salem N.B. and Gargouri Sayari, Y.A.2012**.** Antioxidant and antimicrobial activities of various solvent extracts piperine and piperic acid from *Piper nigrum*.LWT Food Science and Technology.