Comparative Studies on Antimicrobial Activity of *Coriandrum sativum* L. and *Eryngiumfoetidum* L.

BY

PINKY RAIHING

(14PBO009)

A Dissertation Submitted to the

Avinashilingam Institute for Home Science and Higher Education for Women,

Coimbatore -641 043

In Partial Fulfillment of the Requirement for the

DEGREE OF MASTER OF SCIENCE IN BOTANY

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A. Vijayalakshmi 29/4/16

Signature of the Head of Department

29/4/16

Signature of Supervisor

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CONTENT

CONTENT

List of Tables

List of Figures

List of Plates

INTRODUCTION

Chapter I

INTRODUCTION

Plant has been the important source of medicine used from prehistoric time. Medicinal plants are harvested from wild plant which has the tendency to cure and give relief to our human body. Due to its natural origin and less side effect, the growth of herbal medicine has been increasing in developing countries. Herbal drugs play an important role in the treatment of diseases. Numerous medicinal plants and their formulations are used for various disorders in ethno-medicinal practices as well as traditional systems of medicines in India. Since, pre-historic days attempts have been made to find out suitable drugs from natural sources for treatment of different diseases. The rational approach on the experience of folk medicines provides a valuable approach in the search for the development of new and useful therapeutic agents (Chandira & Jaykar, 2013).

According to World Health Organization (WHO), about 80 percent of the world's population relies on plants for their primary health care and some 35,000 to 70,000 species has been used as medicaments, a figure corresponding to 14-28% of the 250,000 plants species estimated to occur around the world and equivalent to 35-70% of all species used world-wide. In today's global market, more than 50 major drugs are originated from tropical plants. The medicinal plants are extensively utilized throughout the world in two distinct areas of health management, such as, traditional system of medicine and modern system of medicine. Traditional system of medicine deals with the use of local or folk or tribal streams and Indian system of medicines like Ayurveda, Siddha, Unanni etc.

Plants are the storehouse of natural products which differ widely in their structures, biological properties and mechanism of action. Natural products have been a major source of new drugs (Vourelaa *et al.*, 2004).

A wide range of medicinal plant parts are used to extract raw drugs and they possess varied medicinal properties. Plants used for traditional medicine contains a wide range of substances that can be used to treat chronic as well as infectious diseases (Anusha *et al.*, 2015).

There are about 250,000 species of higher plant found around the world of which, only 17% has been investigated to have medical potential to cure disease and many are yet to discover its medicinal properties. China and North America have about 35,000 species of flowering plant. However, traditional Chinese used only 5000 species for medicine but North America used 2564 species of medicinal plant. Nowadays, most of the people depend mostly on medicinal plant because of its fewer side effects.

Asia is considered as a land of medicinal plant because most of the Asian countries such as China, India and Nepal have major production of medicinal plant. Medicinal plants have played a significant role in various ancient traditional systems of medication such as the Ayurvedic and Unanic systems of India, the Chinese traditional medicine, and their derivatives in many Asian countries. International and National trade in alternative medicines including herbal products is increasing rapidly. Significant quantities of herbal products are now imported by countries in Asia, Europe and North America. The estimated global trade in medicinal and aromatic plants was over US$60 billion in 2000 and is expected to reach 5 trillion by 2050. China and India are the world's leading exporters of medicinal and aromatic plant materials (Kumar, 2003).

India is one of the oldest and most diverse cultural traditions related to the use of medicinal plant, which dates back several thousand years to the Rig-Veda (3700 B.C.), the collection of Hindu sacred verses. India has a rich culture of medicinal herbs and spices, which includes about more than 2000 species and has a vast geographical area with high potential abilities for Ayurvedic, Unani, Siddha traditional medicines but only very few have been studied chemically and pharmacologically for their potential medicinal value (Gupta *et al.*, 2005; Sandhu and Heinrich, 2005). People living in rural areas from their personal experience know that these traditional remedies are valuable source of natural products to maintain human health, but they may not understand the science behind these medicines, but knew that some medicinal plants are highly effective only when used at therapeutic doses (Van Wyk *et al.,* 2000). Thus, many medicinal plants have been used to cure disease which may be low in cost and easily available in the surrounding. The different plant parts

used include root, stem, flower, fruit, twigs exudates and modified plant organs (Satish and Mahesh, 2008).

In the family *Umbelliferae* (*Apiaceae*) 117 cultivated spiecies excluding ornamentals have been recorded now, primarily used as medicinal plants (41%) (Mohammad *et al*., 2009).

Eryngium foetidum and its phytochemicals

Eryngium foetidum L**.** belongs to the Class Magnoliopsida, Order Apiales and Family *Apiaceae*, is a biennial herb that is used extensively as medicinal plant in most tropical regions. *Eryngium foetidum* L., spiny coriander is also known as 'Culantro' is an aromatic plant, grown as a leafy vegetable (Saikia and Shadeque, 1996). The plant is slender, evergreen, branched perennial with the height and width of 24 inches, 2-10 inches long leaves having spiny margin and a tap root system. The botanical genus name *Eryngium* is derived from the Greek sea holly, *Eryngium vulgare*, and its specific name comes from the Latin *foetidum* meaning stink or bad odor; its smell is sometimes equated to a crushed bed bug (Rahman, 2007). The genus contains flavonoids, namely kaempferol and quercetin glycosides. Its aromatic essential oil consists of about 40 compounds and is important to the perfumery, flavor, and pharmaceutical industries. As a food flavoring minor spice, it is a substitute for coriander (Martin, 2004).

It is found in Central America, Thailand, West Indies, West Africa and South East Asia. The plant produces a well branched cluster of flower heads in spikes forming the characteristic umbel inflorescence on a long stalk arising from the centre of the leaf rosette .The calyx is green while the corolla is creamy white in colour (Ramcharan, 1999). This leafy vegetable species is rare and is grown in certain regions of India. Large-scale propagation is a pre requisite to meet the pharmaceutical needs, and also for effective conservation of this rare medicinal plant (Martin, 2006).

The general chemical constituents of *Eryngium foetidum* L. are alkaloids, flavanoids, glycosides, reducing sugar, phytosterol, saponins, steroids, tannins, proteins, carbohydrates, phenol, triterpenoids, amino acids, vitamin C, anthocyanin, quinines and coumarins. The plant is reportedly rich in calcium, iron, carotene, and

riboflavin and its harvested leaves are widely used as a food flavoring and seasoning herb and many other foods. As a food flavoring minor spice, its pungent unique aroma gives the characteristic flavor to the dishes in which it is incorporated and this is responsible for its increasing demand among ethnic populations.

Eryngium is also widely used in herbal medicines and reportedly beneficial in the treatment of a number of ailments (Wong, 1976). *Eryngium foetidum* is used for the treatment of burns, epileptic fits, asthma, fevers, malaria, snake bites, stomach ache, diarrhea, infertility complications, hypertension, constipation, ear ache and worms. The root, an important part of the herb, is aphrodisiac, nervine tonic, expectorant, diuretic, diaphoretic, and stomachic. Its medicinal value includes its use as a tea for flu, diabetes, constipation, and fevers. One of its most popular uses is in chutneys as an appetite stimulant (Ramacharan, 1999). It is used mainly as a seasoning herb in the preparation of a range of foods, including vegetable and meat dishes, chutneys, preserves, sauces, and snacks.

Coriandrum sativum **and its phytochemicals**

Coriandum sativum L. belongs to the Class Aterid, Order Apiales and Family *Apiaceae*, also known as cilantro and also commonly known as Dhanya, is an aromatic herbaceous plant. The root system is fibrous root and delicate in nature. The stem is erect and branching and the leaves are flat, small, sessile and commonly green in colour. The green stem is edible and tender. The flower is small with white or pinkish purple in colour. The plant is cultivated for its aromatic leaves and seeds. This herb grows extensively in India and Mediterranean regions. It is cultivated in all state of India and also in Europe, North Africa, South America, Thailand, Malaysia and China. The chemical constituent present in *Coriandrum sativum* L. are alkaloids, flavanoids, glycosides, reducing sugar, saponins, steroids, tannins, proteins, carbohydrates, phenol, triterpenoids, amino acids, vitamin C, anthocyanin, quinines, coumarins, terpenoids, phlobatannins and iridoids. This herb is rich in iron, calcium, phosphorus, carotene, thiamine, niacin, Vitamin C and riboflavin.

Coriander possesses many medicinal uses from its olden days. Coriander is recognized as one of the most important spices in the world and is of great significance in international trade (Small, 1997). Today, the herb is cultivated

worldwide for fruit, as a spice or production of essential oil and fatty acid. The stem, leaves and fruits due to its pungent aromatic odour, is commonly used as a condiment or spice in the Mediterranean area (Shriti *et al.*, 2009). The leaves of coriander act as stimulant or tonic which is good for the stomach, for cooling effect on the mind and for inducing sleep. Fresh juice of the leaves, mixed with sugar, is given and also used in biliousness, intestinal irritations, heartburn, thirst and nausea (Kirtikar & Basu, 1999). The regular use of coriander water can help in lowering the blood cholesterol levels.

Coriandrum sativum seeds are used to check the problem of excessive menstrual flow, and for relieving fever during typhoid. Coriander is used in disorders of digestive, respiratory and urinary system, as it is diuretic, diaphoretic, carminatives and stimulant. Coriander is given to those that have loss of appetite, convulsion and insomnia. Externally, powdered green coriander alleviates burning sensation and pain in diseases like inflammation caused by pitta, erysipelas and lymphadenopathy. Decoction of green coriander is useful in stomatitis. In epistaxis, nasal drops of green coriander act as a haemostat and thus stop bleeding. In conjuctivitis, either juice or decoction of green coriander is put in eyes. The paste of dry coriander is used in headache (Pandey, 2010).

Dry coriander is helpful in relieving chronic cases of dysentery, diarrhoea as well as acidity. Chutney made with green chillies, coconut, ginger, black grapes and dry coriander is an ancient remedy used for relieving problem of abdominal pain caused due to indigestion. The plants of coriander are used in sauces, soups and curries. The oil of the herb is used in medicines and the dried coriander is an important additive in curry powders. This herb is also used to pickle spices, seasonings and sausages. *Coriandrum sativum* has a great anti-inflamatory and wound healing property because of the presence of terpenes and flavanoids are present in the plant.

Plate I

***Coriandrum sativum* L. – Habit**

Plate II

Eryngium foetidum L. – Habit

The curative properties in medicinal plant are due to the presence of various secondary metabolites and thus many researches have been done on these plants which has antidiabetic, antihypertensive, antioxidant, antimicrobial, anticancer and anti-inflammatory properties. Secondary metabolites present in the plant organs have ecological functions, so that they protect against herbivorous and microbes. Secondary metabolites usually occur in complex mixtures that also differ among plant organs. Innumerable biologically active compounds that are found in plants (Samy *et al*., 1999) possess antibacterial properties (Samy and Ignacimuthu, 1998). Plant produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents (Balandrin *et al.,* 1985).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999).

These plants have been used in traditional medicine since time immemorial. Although related work has been carried out in *Corinadrum sativum* but *Eryngium foetidum* have not been exposed to exhaustive research work, the present study thus focuses on the following objectives:

- To compare the phytochemical composition of the two plants *Eryngium foetidum* L. and *Coriandrum sativum* L.
- To evaluate the antimicrobial activity of *Eryngium foetidum* L. and *Coriandrum sativum* L. plant extracts.

REVIEW OF LITERATURE

Chapter II

REVIEW OF LITERATURE

Phytochemical Studies of Plant Extract:

Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. However, a sustained supply of the source material often becomes difficult due to the factors like environmental changes, cultural practices, diverse geographical distribution, labour cost, and selection of the superior plant stock and over exploitation by pharmaceutical industry (Joy *et al*., 1998).

The modulation of diseased states by using medicinal plant products as a possible therapeutic measure has become a subject of active scientific investigations in the recent years. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non allopathic systems (Devasagayam *et al*., 2007). The ready availability and economy of plants as direct therapeutic agents make plants more attractive when compared to modern medicine (Agbor *et al*., 2007).

Various medical plants have been used for years in daily life to treat disease all over the world. According to a study performed by the WHO based on publications on pharmacopoeias and medical plants in 91 countries, the number of medicinal plants is nearly 20,000. Plants are the essential and integral part in complementary and alternative medicine and due to this they develop the ability for the formation of secondary metabolites like proteins, flavonoids, alkaloids, steroids and phenolic substances which are inturn used to restore health and heal many diseases (Chakraborthy, 2009).

Phytochemical study of *Apiaceae* family

The knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Fransworth, 1966). Most drugs of plant origin used by medical practitioners are in the form of extract of the whole plant material or part of it. Some of the effects elaborated by extract of

plants used in traditional medicine include antiviral, antitumor, antimicrobial, insecticide and central nervous system effect (Sofowora, 1982). The chemical constituents may be therapeutically active or inactive. The ones which are active are called active constituents and the inactive ones are called inert chemical constituents (Iyengar, 1995).

Secondary metabolites are bioactive compounds produced in specific plant cells in meagre amounts, which find diverse application in the field of medicine and food industries. Their role in plant is ambiguous. Nevertheless, these high value low volume products owing to their complex structures with many chiral centers are not only an impossible task to synthesize artificial but not economically feasible. This has put pressure on the plants growing in sites or cultivated use sites depleting the natural resources at an alarming rate rendering many plants on the verge of extinction. The production of secondary metabolites during a certain period of life cycle is triggered by genetic factors that are strongly influenced by the environment. Therefore, random exploitation of plants without due regards to the presence of metabolites during the time of collecting, not only exhibits fertility of the effort but has tremendous impact in reducing the population (Rajendra and D'Souza, 1998).

Plants synthesize thousands of primary and secondary compounds that have a dizzying array of chemical structures. Tens of thousands of secondary plant compounds have been isolated and identified in over 20-30% of higher plants. The levels of secondary metabolites are affected by many factors, including pathogens and climate changes. Plant secondary metabolites have been successfully manipulated by researchers to develop useful agrochemicals and pharmaceutical agents (Williams *et al*., 2003).

Males *et al*., (2003) in their investigation reported that *Crithmum maritimum* L. an Apiaceae member showed the presence of flavonoids, phenols and tannins in the aerial parts of the plant. Sigaroodi *et al*., (2006) in their study phytochemical analysis of *Ferulogo Bernardii* Tomk & M.Pimen isolated from the hexane extract of the aerial parts of *Ferulago bernardii* (*Apiaceae*) four coumarins , pranstschimgin 1, oxpeucedanin 2, psoralen 3 and umbelliferone 4;one steroid,ß –sitosterol 5 and a normal alkane, nonacosane 6. In recent years, secondary plant metabolites (phytochemicals), previous with unknown pharmacological activities have been

extensively investigated as a source of medicinal agents (Parekh and Chanda, 2007). Razavi *et al*., (2008) studied on the phytochemical of the aerial parts of *Prangos uloptera (Apiacea)*, an endemic Iranian species of the genus *Prangos*, and reported that five coumarins, xanthotoxin, prangenin, scopoletin, deltoin and prangolarin were obtained.

Chemical investigations on the *E. dichotomum* have led to the isolation of stigmasterol and stigmasterol-3-*O*-β-D-glucoside. The isolation and the structure determination of a cyclohexenone-*O*-glycoside**,** Naringenine 7- *O*-α-LRhamnopyranosyl-(1→2)-*O*-β-D-glucopyranoside, β-D-fructofuranose**,** 2-*O*-methyl-α- Dfructofuranose**,** Sucrose and D-mannitol, from its aerial parts is described by Nacef *et al*.,(2008). Adegoke *et al*., (2009) found alkaloids, saponins, tannins, flavonoids, anthraquinones in varying concentrations were analysed in the methanolic leaf extracts of *Lasienthra africanum*. Akkal *et al*., (2013) during their phytochemical investigation of the aerial parts of *Foeniculum vulgare Mill* isolated of two compounds from chloroform extract: graveolone 1 and 1-p-Menthene-3,6-diol 2 and two compounds from butanolic extract: Hexane- 1,2,3,4,5,6-hexol (L-Iditol) 3 and Isorhamnetin-3-O- β-glucoside 4.

Phytochemical studies of *Coriandrum sativum*

Hirvi *et al.,* (1985), investigated the oil content of coriander fruit cultivated at two different localities in Finland during 1983 and 1984. They found the oil content vary from 0.34 to 1.49 ml/100 g in the samples of the two consecutive years. Gil *et al.,* (2002), compared the essential oil composition of coriander fruits from plants growing in Argentina and Europe and reported that, the variation in the oil composition was related to the relative proportion of the constituents and not to the presence or absence of a particular component.

According to Ishikawa *et al.*, (2003) from a portion of the methanol extract of coriander fruit 33 compounds were isolated and identified. According to Melo *et al*., (2005) the phytochemical studies of *Coriandrum sativum* leaf extract showed the presence of flavanoids, linolool, camphor, isocoumarines, coumarines, coriandrones, caffeic acid, protocatechinic acid, acid glycitin. Padma (2009) in her study isolated 5 bioactive compounds such as coriandrinonediol, coriandrin,

dihydrocoriandrin and coriandrone A & B from *Coriandrum sativum*. According to Pathak *et al*., (2011) phytochemical screening of *Coriandrum sativum* showed the presence of carbohydrates, reducing sugars, glycosides, triterpenes, proteins and essential oil.

Kousar *et al*., (2011) carried out the phytochemical analysis of *Coriandrum sativum* leaf extract and the results revealed the presence of flavanoids of 5%, glycosides of 16%, alkaloids of 0.20%, sterols of 11.13% and tannins of 3.4% and the absence of anthroquinones and saponins.

Sindhu *et al*., (2012) during their phytochemical analysis of six plants (*Anethum graveolens, Piper betel, Coriandrum sativum, Cinnamomum zeylanicum*, wheat grass and jack fruit) leaves reported the presence of cardiac glycosides and terpenoids in all the 6 samples, alkaloids in all the 3 samples except *A.graveolens, C.sativum*, wheat grass, tannins in 4 samples except *C.sativum* and *P.betel*, flavanoids in 3 samples, *A.graveolens, C.sativum* and wheat grass, saponins in 4 samples except *A.graveolens* and wheat grass and anthroquinones in jack fruit, *C.zeylanicum* and *P.betel*. Their study also revealed the presence of highest amount of β-carotene in jack fruit and wheat grass (1.544 and 1.584mg/100mg extract) followed by *C.sativum* (0.912mg/100mg extract).

Nirmala *et al*., (2013) during their investigation on phytochemical and biological investigation of *Coriandrum sativum* leaves, revealed the presence of alkaloids, carbohydrate, glycosides, saponins, flavanoid and tannin. Shalini and Smitapadma (2013) conducted research on antioxidant activity of *Coriandrum sativum* and the preliminary phytochemical analysis of crude leaf powder revealed the presence of carbohydrates, proteins, phenolic compounds, tannins, flavanoids and terpenes.

Dharmalingam and Nazni (2013) investigated on Phytochemical evaluation of *Coriandrum* L flowers and the preliminary phytochemical analysis of the crude methanolic extract showed the presence of alkaloids, flavanoids, steroids, reducing sugar and glycosides, saponins and tannins and anthroquinine. They also isolated 30 bioactive phytochemical constituents, among them, the major constituents were benzofuran,2,3-dihydro (15.4) hexadecanoic acid, methyl ester (10.32%) 2,4a-epioxy-3,4,5,6,7,8,-hexahydro-2,5,5,8a-tetramethyl-2h-1-benzofuran (9.35%), 2-methoxy-4-

vinylphenol (8.8%), 2,3,5,6-tetrafluroanisole (8.62%) 2,6-dimethyl-3-aminobenzoquinone (6.81%) and dodecanoic acid (5.00%).

Rajeswari *et al.,* (2013) investigated the phytochemicals and medicare potential of ethyl acetate fraction of methanolic extract of *Coriandrum sativum* seeds. The preliminary phytochemical analysis revealed the presence of carbohydrate derivatives, phenolic compounds, tannins, flavonoids, steroids, terpenoids, saponins, glycosides and alkaloids. Total phenolic compounds, total flavanoids and tannins were found to be very high in n-butanol extract sample of 5.76±1.07mg/100 g(GAE), 3.30±0.09 mg/100g (RE) and 0.80±0.08mg/100g(CE) respectively.

According to Nithya and Sumalatha, (2014) the preliminary phytochemical analysis of *Coriandrum sativum* leaf extract found to contain tannins, alkaloids and steroids. Zohreh *et al.,* (2014) investigated the phytochemical and phytopharmacological activity of *Coriandrum sativum*. The results of their study showed the presence of essential oils and flavanoids, fatty acids and sterols.

Sasi Kumar *et al.,* 2014 screened the ethanol extraction of roots of the fresh *Coriandrum Sativum* for the presence of various phytochemicals by standard procedures. The results indicated that the fresh roots contain alkaloids, flavonoids, terpenoids, sterols, carbohydrates, saponins and phenolic compounds as phytochemical constituents.

Mohan *et al.,* (2015) studied on the effect of ethanolic extract of Coriandrum sativum L. on tacrine induced orofacial dyskinesis and indicated the presence of flavanoids (quercetin 3-glucoronide), linalool, camphor, geranylacetate, geraniol, isocoumarins and coriandrones. They also characterized caffeic acid, protocatechinic acid, and glycitin the major polyphenolics of coriander. The flavonoids content and phenolic content of the seeds were reported to be 12.6 quercetin equivalents/g and 12.2 gallic acid equivalents/g.

Al–Marzoqi *et al.,* (2015) in their study isolated and identified seven bioactive phytochemical compounds from the methanolic extract of *Coriandrum sativum*. They were 1, 6-octadien-3-ol,3,7 –dimethyl, 2- aminobenzoate, bicycle(2.2.1), heptan-2-one,1,7,7-trimethyl., geranyl vinyl ether, 9,10-secocholesta-5,7,10(19)-triene-3,24,25-triol., ascorbic acid 2,6-dihexadecanoate and 7aH-cyclopenta(a) cyclopropa(f) cycloundecene. Nithya (2015) studied the phytochemical constituents of *Coriandrum*

sativum leaves and reported all aqueous, methanol, acetone and chloroform extracts showed the presence of alkaloids, amino acids, flavanoids, glycosides, phytosterols, saponins, steroids and triterpenoids. Among them more phytochemical compounds were observed in aqueous extraction when compared with other extracts.

Padma *et al*., (2015) indicated that in 10 µl of petroleum ether extract of dried fruit and fresh leaves of *Coriandrum sativum*, there are 9, 10 and 5 spots respectively. Out of 9 components for soxhlation dried fruit with Rf values 0.57, 0.73 and 0.84 were found to be predominant as the percentage area was found to be 9.25 %, 15.07% and 24.40%. Out of 10 components for maceration dried fruit with Rf values 0.17 and 0.78 were found to be predominant as the percentage area was found to be 5.06 % and 23.95 %. Out of 5 components for maceration fresh leaves with Rf values 0.25, 0.34 and 0.82 were found to be predominant as the percentage area was found to be 33.51 %, 21.58 % and 5.63%. And that in 10 µl of benzene extract of dried fruit and fresh leaves of *C.sativum*, there are 9, 7 and 10 spots respectively. Out of 9 components for soxhlation dried fruit with Rf values 0.24, 0.51 and 0.81 were found to be predominant as the percentage area was found to be 15.76 %, 7.52% and 6.58%. Out of 7 components for maceration dried fruit with Rf values 0.23, 0.33, 0.40, 0.51, 0.7 and 0.79 were found to be predominant as the percentage area was found to be 7.97%,5.89 %,5.26 %,10.43 %,12.86 % and 48.32 %. Out of 10 components for maceration fresh leaves with Rf values 0.23, 0.5, and 0.73 were found to be predominant as the percentage area was found to be 10.75 %, 6.18 % and 13.76 %.

Phytochemical study of *Eryngium foetidum*

Garcia *et al*., (1999), observed that the essential oil can be obtained from the root of *Eryngium foetidum* and it is dominated mainly by unsaturated alicyclic or aromatic aldehydes (2,3,6-trimethylbenzaldehyd 40%, , 2-formyl-1,1,5-trimethyl cyclohexa-2,4-dien-6-ol 20%,) and the essential oil from the seeds contains sesquiterpenoids (carotol 20%, ß-farnesene 10%), phenylpropanoids (anethole) and monoterpenes (a-pinene) were found, but no aldehydes reported.

Anam (2002) in their study isolated tritetrapenoids saponin from the aerial part (stem and leaves) of *Eryngium foetidum.* Kartal *et al*., (2005) in their study on isolation of Two new triterpene saponins from *Eryngium campestre* reported that the

presence of flavonoids and monoterpene glycosides of the cyclohexanone type coumarin derivatives from the aerial parts and small amounts of saponins in roots. According to Paul *et al*., (2006) the phytochemical analysis of *Eryngium* showed the presence of flavanoids, essential oils, coumarins and rosmarinic acid derivatives. Suciu *et al.,* 2006 during their comparative phytochemical study on *Eryngium* sp. from Romania recorded the total flavonoids (expressed as rutin) ranged of 0.32 – 0.56% in dried aerial parts of *Eryngium planum*, *E. campestre* and *E. maritimum.*

Chowdhury *et al*., (2007), isolated the essential oil from leaves of *E. foetidum* by hydrodistillation method, and Sixty there compounds have been identified with 2-dodecenal (E) (37.4 %), dodecanoic acid (10.7 %), trans-2-dodecanoic acid (9.7 %), 2-tridecenal(E) (6.7 %), duraldehyde (5.1 %) and tetradecanal (4.4 %) as the major constituents. Other major constituents of above 1 % were 2-undecenal (1.7 %), 7-octadecenal (3.7 %), capric acid (1.9 %), caryophyllene oxide (1.2 %), capraldehyde (1.2 %), durylic acid (2.3 %), α-durenol (2 %) and limonene (2 %).

Zhang *et al*., (2008) during their investigation reported that the plant *Eryngium yuccifolium* showed the presence of 12 poly hydroxylated glycosides namely erygioside A-L, kaemferol, caffeic acid and saniculasaponin III. Warda *et al*., (2009) in their study reported that *Eryngium illicifolium* showed the presence of flavonoids and saponins. According to Nebija *et al.,* (2009) the results of their study on chemical characterization and antioxidant activity of *Eryngium campestre* L., Apiaceae from Kosovo revealed the total flavonoid percentage in *Eryngii herba* ranged from 0.12 - 0.14%) expressed as total quercetin.

Aly (2010) investigated on Biosynthesis of phenolic compounds and water soluble vitamins in Culantro (*Eryngium foetidum* L.) plantlets as affected by low doses of gamma irradiation" and observed the presence of total phenolic content of 8.91±0.01 mg/g, coumarin of 5.61±0.11 mg/g, caffeic acid 8.62±0.06 mg/g, salicyclic acid of 3.53±0.17 mg/g and benzoic acid of 6.60±0.11 mg/g in the leaf extract of *Eryngium foetidum.* Banout *et al*., (2010), employed hydrodistillation and reported that (E)-2- dodecenal being the main constituent of the *Eryngium* essential oil and averaging 61.8–62.2%, followed by n-dodecanal (10.9–15.5%), (E)-2- tetradecenal (6.7–7.6%) and 1-tetradecene (3.6–5.7%). Wang *et al*., (2012) during their investigation on phytochemical constituents and pharmacological activities of

Eryngium found that the leaf extract contain phytochemical like terpenoids, triterpenoids, saponins, flavanoids, coumarins, polyacetylenes and steroids.

Chandira and Jaykar, (2012) investigated on the phytochemical constituents on aqueous extract of *Eryngium foetidum* and found to contain the presence of carbohydrates, glycosides, proteins, amino acids, saponins, phytosterols, flavanoids, gums and mucilage, phenolic compounds and tannins. Shavandi *et al*., (2012), in their study on *Eryngium foetidum* L. *Coriandrum Sativum* and *Persicaria Odorata* L. revealed that *Eryngium foetidum* leaf extract is an excellent source of vitamin A, B_2, B_1 and C.

Shivandi *et al*., (2012) in their study revealed that fresh leaves of *Eryngium foetidum* consist of 86–88% moisture, 3.3% protein, 0.6% fat, 6.5% carbohydrate, 1.7% ash, 0.06% phosphorus and 0.02% iron. He also reported that leaves showed an excellent source of vitamin A (10,460 I.U./100 g), B2 (60 mg %), B1 (0.8 mg %), and C (150–200 mg %) on a dry weight basis and also consist of 0.1–0.95% volatile oil, 27.7% crude fiber, 1.23% calcium, and 25 ppm boron. Bhavana *et al*., (2013) carried out investigation on the quantitative determination of secondary compounds and vitamins C of *Eryngium foetidum* collected from Andaman, Darjeeling and Karnataka. Among the 3 sample plant extracts, total phenol content of 41.61±0.29 mg pyrogallol/g was significantly high in Darjeeling, total flavanoids of 34.36±0.19 mg QE/g, total tannin content of 45.03±0.23 GAE/g and vitamin C of 86.31±0.21 mg ascorbic acid/g were reported in Andaman.

Chandira and Jaykar (2013) evaluated the formulation of herbal tablets from bark extracts of *Albizia odoratissima*, leaf extract of *Eryngium foetidum* and tuberous leaf extracts of *Ipomea digitata*. The results of the study revealed the presence of carbohydrates, glycosides, proteins and amino acids, saponins, phytosterols, flavanoids, gum, mucilages, phenolic compounds and tannins. Okon *et al*., (2013) analysed phytochemical constituents of *Eryngium foetidum* and revealed the presence of saponins, flavanoids, alkaloids, tannins anthroquinones, gardiac glycosides and terpenes. Rajagopal *et al*., (2015) reported the presence of secondary compounds like flavanoids, tannins, saponin and several terpanoids in *Eryngiun foetidum* L. leaves.

Antimicrobial Activity of *Apiaceae* family

Bacterial membranes can be affected by certain saponins, resulting in a significant loss of vital activity especially in some gram positive genera like *Bacillus sp.* (Naidu, 2000).

Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The synergistic effect from the association of antibiotic with plant extracts against resistant bacteria leads to new choices for the treatment of infectious diseases. This effect enables the use of the respective antibiotic when it is no longer effective by itself during therapeutic treatment (Nascimento *et al.*, 2000).

Many efforts have been made to discover new antimicrobial compounds from various sources such as micro-organisms, animals and plants. One such resource is folk medicines. Systematic screening of these may result in the discovery of novel effective compounds (Tomoko *et al.*, 2002).

Plant lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al*., 2006).

Brkovic *et al*., (2006) investigated on Antibacterial activity of some plants from family *Apiaceae* in relation to selected phytopathogenic bacteria and reported that an extracts of *Torilis anthriscus* as well as *Daucus carota*, *Aegopodium podararia*, *Heracleum sphondylium* and *Pimpinella saxifraga* have significant antibacterial activities so potentially they might be used as biological pesticides in the control of some phytopathogenic bacteria although further researches are also needed.

Causes of many plant diseases are phytopathogenic bacteria which greatly determine the quality of plant production. Quick and effective management of plant diseases and microbial contamination is generally achieved by the use of synthetic pesticides. The application of synthetic pesticides can develop the appearance of

resistant strains and can cause environmental hazards - accumulation in food chain, high and acute toxicity, long degradation period and extension of their power to destroy both useful and harmful pests. Therefore great attention is dedicated to producing of new antibacterial agents that are used in the control of pathogenic microorganisms without environmental effect (Brkovic *et al*., 2006). Phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections (Parekh and Chanda, 2007).

Gurinder and Daljit., (2009) conducted antibacterial activity on *Anethum graveolens* Linn., *Foeniculum vulgare* Mill. and *Trachyspermum ammi* L. using aqueous and organic seed extracts. They reported that hot water and acetone seed extracts showed considerably good antibacterial activity minimum inhibitory concentration ranged from 20–80 mg/ ml and 5–15 mg/ml respectively against all the bacteria except *Klebsiella pneumoniae* and one strain of *Pseudomonas aeruginosa*.

For the treatment of emerging microbial infections, continuous efforts on searching for novel antimicrobial agent are still of utmost demand. Substantial attention has been focussed on medicinal plants, largely on the basis of their ethno-medicinal uses. It is evident from various reports that success of finding new antimicrobial agents from plants selected using ethnomedicianl approaches is much higher than that of plants selected randomly (Svetaz *et al*., 2010).

Amirhossein and Mehrdad, (2010) conducted a study on *Ferula* oils belonging to the family *Apiaceae* and indicated the presence of anti-fungal, anti-bacterial and anti-viral activities. The bacteriostatic and fungistatic properties of these essential oils may be associated to the high content of α-pinene and β-pinene or polysulfides that are present in these oils and for which strong anti-microbial activities.

Singh *et al.,* (2012) conducted a study on the antibacterial properties of the essential oils obtained from the seeds of *Seseli indicum*, of Apiaceae family. Essential oils were investigated for activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* using a punched-hole method and found that essential oils inhibited all bacteria at both, low and high concentration. Abdoune *et al*., (2013) evaluated the antimicrobial activity of solvent extracts of different part of *Daucus crinitus* Desf. and found remarkable antimicrobial effect in organic extracts of stems

and seeds (MIC = 0.31-0.83 mg/ml on *S. aureus*, *B. cereus*, and *C. albicans*) containing many phytochemical components such as coumarins, flavonoids, reducing sugars, steroids, tannins, and terpenes.

Bagdassarian *et al.,* (2013) carried out on a comparative investigation on antibacterial activity of seeds of *Apiaceae* family, such as: Fennel (*Foeniculum vulgare*), Dill (*Anethum graveolens*), Anise (*Pimpinella anisum*), Caraway (*Carum carvi*) and Coriander (*Coriandrum sativum*) and reported that methanol extract of seed did not have any antimicrobial activities. Sood *et al.,* (2014) carried out investigation on phytochemicals and antimicrobial activity against standard microbial strains on aqueous extract of four member of *Apiaceae* i.e *Hydrocotyle javanica* Thunb., *Hydrocotyle rotundifolia* Roxb., *Eryngium foetidum* L. and *Centella asiatica* L and found that *B. subtilis* exhibited resistance to all the four plant.

Jelena *et al*., (2015) investigated antioxidant and antimicrobial potential of methanol and ethyl-acetate extracts from dried aerial parts, inflorescences and fruits of *Opopanax hispidus* of the family *Apiaceae*. The antimicrobial activities revealed that most susceptible were *Listeria monocytogenes* and *Escherichia coli* on ethyl-acetate extracts from fruits and inflorescence, respectively. Golfakhrabadi *et al*., (2016) during their study on phytochemical analysis, antimicrobial, antioxidant activities and total phenols of *Ferulago carduchorum* in two vegetative stages (flower and fruit) reported that the best antioxidant activity was determined in flower crude extract (IC50=0.44mg/mL) and the flower ethyl acetate fraction (FLE) showed better antimicrobial and antifungal activities than other fractions. So, FLE was selected for phytochemical investigations, resulting in isolation of a flavonoid (hesperetin) which showed that the antimicrobial and antioxidant effects during the flowering were obviously more than the fruit season.

Antimicrobial Activity of *Coriandrum sativum*

Mckeegan *et al.,* 2002 observed and reported that coriander extract has less antimicrobial effect on gram negative bacteria which can be probably due to the presence of cell wall polysaccharides, preventing active compounds from reaching to cytoplasmic membrane of these bacteria.

Vijayan *et al*., (2003) investigated the antibacterial activity of water and acetone extracts of black pepper, coriander, fenugreek and asafoetida against six clinically important bacteria namely *Escherechia coli, Klebsiella* sp., *Staphylococcus aureus, Bacillus* sp., *Salmonella typhi* and *Salmonella paratyphi* A. The preparations of the plant materials were active against most of the tested organisms, except the water extract of black pepper, which was active against *Bacillus* sp. only. The control discs produced no discernible zone of inhibition. *E. coli* was found to be susceptible to extracts, except the water extract of black pepper. The acetone extract of asafoetida and coriander gave a zone of inhibition of 10.5 mm and other extracts were between 8 and 10 mm. The acetone extract of coriander also was active against *S. typhi* and all extract showed activity against *S. aureus.*

Figueiredo *et al*., (2003) during their investigation on Antimicrobial Activity of the Essential Oils of Some Spice Herbs. They reported that the essential oil from *Anethum graveolens* showed antimicrobial activity against *Staphylococcus aureus* (inhibitory zone=18 mm), *Salmonella* sp. (=11 mm) and *E. coli* (10 mm) *Cuminum cyminum* was found to be effective against *E. coli, P. aeruginosa* and *Salmonella* sp. and their inhibitory zones were 18, 10 and 23 mm, respectively and *Coriandrum sativum* oil was active only against *Salmonella* sp. (18 mm) and *Foeniculum vulgare* inhibited only *E. coli* (9 mm).

Claudiu and Maria, (2009) in their study reported antimicrobial activity of diethyl ether extract of coriander seed on bacterial and fungal cultures such as *Pseudomonas aeruginosa, Mycobacterium smegmatis, Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Salmonella typhimurium, Enterococcus faecalis, Micrococcus luteus and Candida albicans* and observed inhibitory activity on *M. smegmatis, K. pneumoniae, S. aureus, E. coli, E. faecalis, M. luteus and C. albicans,* of which *S. aureus* was found to be the most sensitive strain, the least inhibitory effect was found on *E. coli*.

Uma *et al*., (2009) carried out a study on the preliminary phytochemical study and *in vitro* antimicrobial activity of *Coriander sativum* (*Apiaceae*) against some pathogens isolated from patients with infectious diarrhea using various solvent extract like aqueous, methanol, chloroform, petroleum ether and hexane. Antimicrobial activities were screened against Enterotoxigenic *E.coli,* Enteropathogenic *E.coli,,*

Salmonella typhimurium, Salmonella entertidis, Shigella dysentriae, Shigella flexineri, Candida albicans, Candida tropicalis and *Candida krusei* and the results of antimicrobial activity revealed that methanol extract of the plant exhibit good activity compared to chloroform and aqueous extracts to *E.coli, Salmonella* sp and *Shigella* sp. Petroleum ether and hexane extracts did not show any activity. None of extracts exhibited antifungal activity.

Dash *et al.,* (2011) indicated the use of methanol and acetone extract of two spice Fenugreek (*Trigonella foenum* L) and coriander (*Coriandrum sativum* L). Crude extract of the spices with methanol and acetone were screened for antibacterial activities against four Gram negative pathogenic bacteria -*Pseudomonas* spp., *Escherichia coli, Shigella dysentiriae* and *Salmonella typhi*. Methanol extract of Fenugreek and Coriander revealed an elevated antimicrobial activity against *Pseudomonas* spp. whereas acetone extract of spices exhibited highest activity against *Escherichia coli*. Acetone extract of Fenugreek and Coriander showed no activity against *Salmonella typhi*. Methanol extract of Fenugreek were found sensitive to *Pseudomonas spp., E. coli, Shigella dysentiriae* and *Salmonella typhi.*

Xin *et al*., (2012) studied the antimicrobial activity of dried coriander extracted by petroleum ether, 95% ethanol, and water and reported that significant antimicrobial activity was observed only in water extract and the minimum inhibition concentration (MIC) was below 10% of original extract. Joglekar *et al*., (2012) investigated the methanolic extracts of leaves of *Aegle marmelos, Coriandrum sativum* and *Trigonella foenum greacum* for antibacterial activity against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and observed that *A. marmelos* has antibacterial activity against *klebsiella pneumoniae* which showed 6 mm of zone of inhibition followed by *C. sativum* with 3.5 mm of zone of inhibition against *Staphylococcus aureus* at 500 µg/ml concentration.

Zardini *et al.,* (2012) analysed antibacterial and antifungal activity of crude extracts from seeds of *Coriandrum sativum* and revealed that the antimicrobial effects of the plant extract showed the greatest effect on *Staphylococcus aureus* resistant strain and also observed an effective germicidal effects on *Klebsiella pneumonia, Pseudomonas aeruginosa* as well as *Penicillium lilacinum* and *Asperjilus niger*.

Tiwari *et al*., (2013) studied the ethanolic and aqueous extract of three medicinal plants i.e. *Coriandrum sativum, Ocimum tenuiflorum* and *Phyllanthus emblica* for their antimicrobial activities against four bacterial strains such as *Pseudomonas aeruginosa, Bacillus amiloliquifaciens, Staphyllococcus aureus* and *Escherichia coli*. The results revealed that *Coriandrum sativum* showed very low resistance towards the bacterial strains, but gave good result against *Pseudomonas aeruginosa*. *Ocimum tenuiflorum* gave good results for *Escherichia coli* and *Staphylococcus aureus.* Ethanolic extract of *Phyllanthus emblica* indicated maximum resistance for *Pseudomonas aeruginosa* and *Escherichia coli.* The results showed that *Ocimum tenuiflorum* and *Phyllanthus emblica* indicated maximum activity against *P. aeruginosa* with 20±0.32 and 25±0.25 mm zone of inhibition respectively and also exhibited potent antimicrobial action against all bacterial isolates tested.

Dawood *et al.*, (2013), reported that the crude extract of *Phyllantus emblica* fruit exhibited strong activity against standard cultures of all studied bacteria (*B. cereus, S. aureus, P. aeruginosa* and *E. coli*) and *Coriandrum sativum* was effective only against *Bacillus cereus*. Chaudary *et al.* (2014) observed the antimicrobial activity by using methanol and acetone extract of *Coriandrum sativum* against bacterial strains, *Staphylococcus aureus* (MTCC-737), *Streptococcus thermophillus* (MTCC-5461), *Bacillus subtilis* (MTCC-121), *Proteus vulgaris* (MTCC- 426), *Pseudomonas aeuroginosa* (MTCC-429), *E. coli* (MTCC-1303), *Serratia marcescens* (SRM) (MTCC-8708), *Klebsiella pneumoniae* (MTCC-109), Methicillin-resistant *Staph. Aureus* (MTCC-84), *Salmonella typhimurium* (MTCC-1254), *Staphylococcus epidermis* (MCC-10623), *Lactobacillus acidophilus* (MTCC-447), *Micrococcus luteus* (MTCC-1538), *Vibrio cholera* (MTCC-3906), *Candida albicans* (MTCC-227) and *Saccharomyces cerevisiae* (MTCC-170). The results indicated that acetone extract was found to exhibit better inhibitory effect against bacteria and fungi than methanolic extract. However, none of the extracts were effective against *K. pneumonia* and *S. cerevisiae*.

Sasi Kumar *et al.,* (2014) conducted the preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Coriandrum sativum* L. roots (Coriander) and revealed ethanol extract and fractionates of fresh roots of *Coriandrum sativum* showed a significant and remarkable activity against

Staphylococcusaureus (15mm), Bacillus cereus(19mm), Salmonella typhi (11mm), Klebsiella (20 mm), when compared to standard.

Sasi Kumar *et al.,* (2014) carried out investigation on fresh root of *Coriandrum sativum* using ethanol and its antimicrobial activity using four types of bacteria such as *Staphylococcus aureus, Bacillus cereus, Salmonella typhi, Klebsiella* and one fungus *Candida*. The result showed a significant and remarkable activity against *Staphylococcus aureus, Bacillus cereus, Salmonella typhi, Klebsiella,* when compared to standard. Debjani *et al.,* (2015) observed the antimicrobial activity of *Coriandrum sativum* and *Laurus nobilis* against *E.coli* and *Bacillus* species and reported that aqueous extracts of coriander and Bay leaves were more effective against *Bacillus* species than *E.coli* because they depicted higher zones of inhibition against *Bacillus* species . In comparison to both spices, Bay leaves showed a wider zone of inhibition against both the bacteria compared to coriander and its results suggested that these spices have inhibitory effects against bacteria and can be used for various purposes in the field of medication for the welfare of the mankind. These spices can be used for the treatment of infectious diseases and also help in the development of new drugs, thus providing an effective source of herbal medication and also indicated the presence of medicinal properties.

Farah *et al.,* (2015) investigated the antioxidant and antimicrobial activities of the ethanolic extract of both seeds and green parts of parsley (*Petroselinum erispum*) and coriander (*Coriandrum sativum*). The antimicrobial activity, results showed that the extract of parsley seeds has the highest reduction percent in growth of all the examined microorganisms than the extract of coriander seeds which revealed a reduction percent of 44% in the growth of *S.typhi* followed by *Mucor sp.* (28%), *Emericella nidulais* (27%), and *Candida tropicals* (25%) and also the extract of coriander green parts had the lowest effect among the four extracts on the growth of the microorganisms.

Antimicrobial Activity of *Eryngium foetidum*

According to Durmaz *et al.,* (2006) the methanol, ethanol, *n*-hexane and water extracts of *Allium vineale* (*Liliaceae*), *Chaerophyllum macropodum* (*Apiaceae*) and *Prangos ferulacea* (*Apiaceae*) have antibacterial activity against a panel of Gram

positive and Gram negative bacteria. The methanol extracts of *Allium vineale* showed higher antibacterial activity against *Bacillus subtilis*. Bergaoui *et al*., (2007) reported that the volatile extracts from *Opuntia macrordasys* leaves, rich in hexadecanoic acid and (E) -3– Butyldiene pthalide when tested against *Alternaria solani, Fusarium solani* and *Rhizoctina solani,* the strongest inhibition against *Altenaria solani.*

The spread of multidrug-resistant strains of fungus and the reduced number of drugs available makes it necessary to discover new classes of antifungals and compounds that inhibit these resistant mechanisms. This has led to a search for therapeutic alternatives, particularly among medicinal plants and compounds isolated from them used for their empirically antifungal properties (Abad *et al*., 2007). In a work by Warda *et al.,* (2009) the aqueous and methanolic extracts of four plant such as *Eryngium ilicifolium, Marrubium vulgare, Thymus pallidus* and *Lavandula stoechas* against *Streptococcus pneumoniae* were studied and a significant activity was observed with methanol extract of three plants such as *M. vulgare, T. pallidus* and *L. stoechas.*

Marčetić *et al.,* (2013) investigated the chemical composition, antimicrobial and antioxidant activity of *Eryngium palmatum*, an endemic plant species from the Balkan Peninsula. Their study regarding the antimicrobial activity by broth microdilution showed that the methanol and chloroform extracts of aerial parts and roots exerted a significant effect (MIC 3.5–15.6 μg mL−1) against tested gram-positive and gram-negative bacteria.

Kaushal and Yadav (2014) in their investigation reported that aqueous extract of *Eryngium foetidum* was found to exhibit the highest extractive value (330.33 ± 1.53 mg/g ADPM), antimicrobial activity (PI= 0.88), antioxidant activity (IC50=196.36 ± 0.01 μg/mL) and total phenolic content (3.11 ± 0.02 mg GAE/g extract). They also revealed that *E. foetidum* is a potential source of antimicrobial and antioxidant compounds and has the potential use for health benefits. According to Anusha *et al*., (2015) *E. foetidum* and *C. sativum* possess antibacterial activity and they are good natural sources of antioxidant compounds for use in food and pharmaceutical industry.

Lingaraju *et al.*, (2016) during their study on the antimicrobial activity of isolated leaf extracts of *Eryngium foetidum* L. performed with microorganisms like two Gram positive bacteria - *Bacillus subtilis, Staphylococcus aureus*, two Gram negative bacteria - *Escherichia coli, Pseudomonas aeruginosa* and a fungus *Candida albicans* by agar well diffusion method revealed that the ethylacetate extract of *E. foetidum* showed the strongest antimicrobial activity among the five extracts against the four bacterial strains and *C. albicans*. They also reported the inhibition of ethylacetate extract against *P.aeruginosa* (28mm) and *C. albicans* (18mm) was comparatively higher to that of the standard Gentamycin (18mm) and Nystatin (14mm) respectively.

MATERIALS AND METHOD

Chapter III

MATERIALS AND METHODS

The methodology of the study pertaining to "**Comparative Studies on Antimicrobial Activity of *Coriandrum sativum* L. and *Eryngium foetidum* L.**", are presented under the following headings.

1. **Collection and authentication of plant material**
2. **Preparation of extraction**
   - Aqueous extraction
   - Chloroform extraction
3. **Physiochemical parameters**
   - Determination of moisture content
   - Determination of solubility percentage
4. **Qualitative analysis of phytoconstituents**
5. **Quantitative estimation of phytoconstituents**
   - Estimation of Carbohydrate
   - Estimation of Protein
   - Estimation of Total Phenol
   - Estimation of Total Flavonoid
   - Estimation of Total Tannin
6. **Antimicrobial activity**
   - Antibacterial assay
   - Antifungal assay

1. Collection and authentication of plant material

The fresh leaves and root of *Eryngium foetidum* L. was collected from Shangshak Khullen, Ukhrul district, Manipur and *Coriandrum sativum* L. from Saibaba Colony, Coimbatore, Tamil Nadu. The plant specimens *Eryngium foetidum* L. were authenticated by Prof (Dr.) C. Murugan Scientist 'D'-In-Charge Botanical Survey of India, T.N.A.U campus, Coimbatore-641003, with voucher specimen no BSI/SRC/5/23/2016/Tech/183.

- **Preparation of samples**

The plant materials *Coriandrum foetidum* and *Eryngium foetidum* (L.) were washed carefully with water to remove dust and foreign materials. Then the washed leaves were dried in hot air oven at 45^0C for 7 days for the extraction process. After drying the samples (150 gm) were ground into a powder form using electric blender. The powdered samples were stored in glassware container until the time of extraction.

2. Extraction of plant samples

Extraction was done by two methods from the powdered plant material used in water and chloroform using soxhlet apparatus until the occurrence of decolourisation of the solvent. All solvent extracted fractions were evaporated to dryness to obtain residues. The extracts were stored at 4ºC in air tight containers.

- **Aqueous extraction (Farombi *et al.*, 2003)**

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

- **Chloroform extraction**

Powdered samples (10g each) were weighed and soaked in 100ml chloroform in a conical flask stoppered with rubber cork and left undisturbed for 24 hours. It was then filtered off using sterile filter paper (Whatman No: 1) into a sterile conical flask.

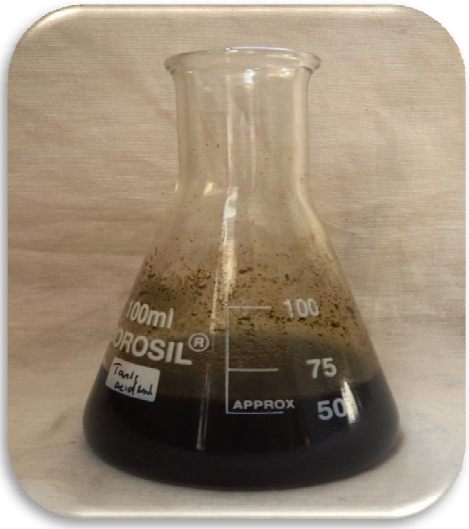
3. Physiochemical parameters

Plant powder samples of *Coriandrum sativum* and *Eryngium foetidum* leaves and root were subjected for determination of physicochemical parameters such as moisture content and solubility ash values, pH value in 1% and 10% solution, aqueous, and alcoholic extractive values were carried out according to the methods recommended by the World Health Organization

Plate III

***Coriandrum sativum* leaf extracts**

Aqueous



Chloroform

Plate IV

***Coriandrum sativum* root extracts**

Aqueous

Chloroform

Plate V

***Eryngium foetidum* leaf extracts**

Aqueous

Chloroform

Plate VI

***Eryngium foetidum* root extracts**

Aqueous

Chloroform

- **Determination of moisture content (AOAC, 1990)**

Each sample about 5g taken in pre-weighed petridish was weighed and dried in the oven at 105° C for eight hours. The petridishes were transferred immediately to desiccators, cooled and weighed. The loss in weight represented the moisture content of the samples. Moisture content was calculated in percentage

$$\text{Moisture Content (\%)} = \frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

- **Determination of solubility percentage (Kokate, 1994)**

- **Chloroform**

About 5gms of powdered material was weighed and macerated with 100 ml of 90% chloroform and shaken in a closed flask for the first 6 hours and kept undisturbed for 18 hours. It was then filtered rapidly taking precautions against loss of the solvent. The filtrate (25 ml) 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.

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

- **Water**

The procedure adopted for the solubility percentage of the plant powder in chloroform is used with water instead of alcohol to get the water solubility percentage.

4. Qualitative analysis of phytoconstituents

The stock solution was prepared from each of the crude extracts such as aqueous and chloroform extracts (100 mg); and was dissolved in 10 ml of its own mother solvents. The obtained stock solutions were subjected to preliminary phytochemical screening (Harborne, 1984).

❖ **Test for Amino acids:**

Ninhydrin Test:

To 3 ml of extract 3 drops of 5% ninhydrin was added and the mixture was heated in boiling water for 10 min. The development of blue colour indicates the presence of amino acid.

❖ **Test for Alkaloids:**

Wagner's Test:

A quantity of 0.2 g of the selected plant samples were added in test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then 5 ml of 2% HCl was poured in a test tube having the mixture of plant extract and hexane. The test tube with the mixture was heated and filtered and few drops of picric acid were poured in a mixture. Formation of yellow colour precipitate indicated the presence of alkaloids.

❖ **Test for Acidic compounds:**

To 1 ml of extract, sodium bicarbonate solution was added and observed for the production of effervescence.

❖ **Test for Anthroquinone:**

A plant extract of 0.1 ml is shaken well with 10 ml of benzene and filtered. 5ml of 10% ammonia is added to the filtrate. The mixture is shaken and the presence of pink, red or violet colour indicates the presence.

❖ **Test for Anthocyaines:**

2ml of extract is treated with 2 M NaOH and observed the formation of green colour indicates the presence of anthocyaines.

❖ **Test for Coumarins:**

Three ml of the diethyl ether extract was evaporated to dryness in a test tube and the residue was dissolved in hot distilled water. It was then, cooled and divided into two test portions, one was the reference. To the second non-reference test tube, 0.5 mL of 10 % NH_4OH was added. The occurrence of an intense/fluorescence under UV light (λmax= 365 nm) is a positive test for the presence of Coumarins and its derivatives.

❖ **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 and 1ml of conc. sulphuric acid. Formation of reddish brown color at the junction of two layers and the upper layer turned bluish green indicates the presence of glycoside.

❖ **Test for Carbohydrates:**

Molisch Test:

To 2ml of extract 2-3 drops of alpha naphthalene solution in alcohol was added, shaken for 2 min and 1 ml of concentrated sulphuric acid was added slowly from the sides of the test tube. A deep violet colour at the junction of two layers indicates the presence of carbohydrates.

❖ **Test for Essential oils:**

To 1ml extract, a few drops of Sudan III solution were added. A shining orange colour obtained showed the presence of oils.

❖ **Test for Flavonoids:**

Shinoda Test

A small quantity of extract was dissolved in 5 ml of ethanol and treated with few drops of concentrated hydrochloric acid 0.5 mg of magnesium turning and observed for the formation of pink color.

❖ **Test for Glycosides:**

Legal's Test:

To 2ml of Aqueous and chloroform sample extracts, 1 ml of pyridine and 1ml of sodium nitroprusside was added. Appearance of Pink to red colour indicated the presence of glycosides.

❖ **Test for Phenols:**

Lead acetate Test:

To 1ml of extract, lead acetate solution was added, and the precipitate formation indicated the presence of phenolic compounds.

❖ **Test for Phlobatannin:**

Deposition of a red precipitate when an aqueous extract of each plant sample was boiled with 1 % aqueous hydrochloric acid was taken as evidence for the Phlobatannin.

❖ **Test for Protein:**

Millon's Test:

To 3 ml of extract 5-6 drops of Million‟s reagent (Solution of mercury nitrate and nitrous acid) were added and observed for the formation of white precipitate when warmed turns to brick red or dissolves to red coloured solution.

❖ **Test for Quinone:**

To 1ml of extract alcoholic KOH solution was added. The appearance of red or blue colour indicates the presence of Quinones.

❖ **Test for Reducing Sugar**:

Fehling's Test:

Fehling's A and Fehling's B solutions, each 1ml were mixed and boiled, for 1ml and 2 ml of extracts were added heated in boiling water bath for 10 min, appearance of yellow and then brick red precipitate indicates the presence of reducing sugars

❖ **Test for Starch:**

To one ml of aqueous extract 10 ml of NaCl saturated solution was added. After heating, starch reagent was added. A blue-purplish colour is a positive test for the presence of starch.

❖ **Test for Steroids:**

Salkowski Test:

Conc.H_2SO_4 and chloroform were added to the aqueous and chloroform extract in a test tube. The appearance of wine red colour developed, was considered as positive test for presence of steroids.

❖ **Test for Sterols**

Hersche's Son's reaction:

To the residue, 2-3ml of Trichloroacetic acid was added and heated. Presence of red to violet colour on heating indicated the presence of sterols.

❖ **Test for Saponin:**

Foam Test:

The extract (2g) was shaken vigorously with 20 ml of water and observed for persistent foam, which indicates the presence of saponins.

❖ **Test for Tannin compounds:**

Ferric chloride Test:

To 3 ml of extract, 3 ml of 5% w/v ferric chloride solution was added. The blue – black colour indicates the presence of tannins and phenols.

❖ **Test for Terpenoids:**

Salkowaski Test:

To 2 ml of extract 5 drops of concentrated sulphuric acid was added, shaken and allowed to stand. Appearance of greenish blue colour indicates the presence of triterpenoids.

5. Quantitative estimation of phytoconstituents

The biochemical parameters analysed were Carbohydrates, Protein, Total Phenol, Total Flavonoid and Total Tannin

❖ **Estimation of Carbohydrate (Hedge and Hofreiter, 1962)**

Principle

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

Reagents

1. Glucose stock standard: 100 mg of glucose was dissolved in 100 ml of water in a standard flask.
2. Working standard: 10 ml of the stock was diluted to 100 ml. 1.0 ml of this solution contains 100µg of glucose.
3. Anthrone reagent: 0.2% anthrone was dissolved in ice cold concentrated sulphuric acid. Prepared fresh before use
4. 2.5 N HCl.

Procedure

Weighed 100mg of the sample into a boiling tube, hydrolysed by keeping it in a boiling water bath for three hours with 5.0 ml of 2.5 N HCl and cooled to room temperature. Neutralized it with solid sodium carbonate until the effervescence cease, made up the volume to 100 ml and centrifuged, collected the supernatant and take 0.2 to 1.0 ml for analysis. Prepared the standards by taking 0.2-1.0 ml of the working standards. 1.0 ml of water serves as a blank made up the volume to 1.0 ml in all the tubes with distilled water and then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly and read the green to dark green colour at 630 nm.

Calculation

A standard graph was drawn by taking the concentration of glucose on X axis and spectrophotometer reading on Y axis. From the graph the concentration of glucose in the sample was calculated.

❖ **Estimation of Protein (Lowry *et al.*, 1951)**

Principle:

The blue colour developed by the reduction of the 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 tartrate are measured in the Lowry's method.

Reagents:

i. Sodium carbonate in 0.1N sodium hydroxide (**Reagent A**).

ii. 0.5% Copper Sulphate ($CuSO_4.5H_2O$) IN 1% potassium sodium tartrate **(Reagent B).**

iii. Alkaline copper solution.: Mix 50ml of A and 1ml of B prior to use (**Reagent C)**

iv. Folin –ciocalteau reagent (**reagent D**)-reflux gently for 10 hours a mixture consisting of 100g Sodium tungstate ($Na_2WoO_4.2H_2O$), 25g Sodium molybdate ($Na_2MoO_4.2H_2O$), 700ml water, 50ml of 80% phosphoric acid, and 100ml of concentrated hydrochloric acid in a 1.5L flask. Add 150g lithium sulfate, 50ml water and a few drops of bromine water. Boil the mixture for 15 min without condenser to remove excess bromine. Cool, dilute to 1L and filter. The reagent should have no greenish 20%

v. **Protein Solution (Stock Standard):**

Weigh accurately 50mg of bovine serum albumin (**fraction V**) and dissolve in distilled water and make up to 50ml in a standard flask.

vi. **Working Standard Solution:**

Dilute 10ml of the stock solution to 50ml with distilled water in a standard flask. 1.0ml of this solution contains 200µg protein.

Extraction of protein from Sample:

Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind well with a pestle and mortar in 5-10mL of the buffer. Centrifuge and use the supernatant for protein estimation.

Estimation of Protein:

- Pipette out 0.2, 0.4, 0.6, 0.8 and 1.0ml of the working standard into a series of test tubes.
- Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes.
- Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0ml of water serves as the blank.
- Add 5.0 ml of reagent C to each tube including the blank. Mix well and allowed to standing for 10mins.
- Then add 0.5 ml of reagent D, Mix well and incubate at room temperature in the dark for 30min, blue colour is developed.
- Take the reading at 660nm.Draw a standard graph and calculate the amount of protein in the sample.

Calculation

The amount of protein present in the sample was expressed in

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

❖ **Estimation of Total Phenols (Mallick and Singh, 1980)**

Principle

Phenols react with phosphomolybdic acid in Folin-Ciocalteau reagent to produce a Blue-coloured complex in alkaline medium, which can be estimated spectrophotometrically at 650nm.

Reagents

1. Ethanol (80%)
2. Folin-Ciocalteau reagent (1N)
3. Sodium carbonate (20%)
4. Standard catechol solution (100µg/ml in water)

Procedure

The sample (0.5g) was homogenized in 10X volume of 80% ethanol. The homogenate was centrifuged at 10,000rpm for 20 minutes. The extraction was repeated with 80% ethanol. The supernatants were pooled and evaporated to dryness. The residue was then dissolved in a known volume of distilled water. Different aliquots were pipetted out and the volume in each tube was made up to 3.0ml with distilled water. Folin- Ciocalteau reagent (0.5ml) was added and the tubes were placed in a boiling water bath for exactly one minute. The tubes were cooled and the absorbance was read at 650nm in a spectrophotometer (Genesys 10-S, USA) against a reagent blank. Standard catechol solutions (0.2-1ml) corresponding to 2.0-10µg concentrations were also treated as above. The concentration of phenols is expressed as mg/g tissue.

Calculation

From the standard curve the concentration of phenols in the sample was determined and expressed as GAE mg of phenols/g of material.

❖ **Determination of Total Flavonoid content (Kim *et al.*, 2003)**

Principle

This method given was used for analysing total flavonoid content (TFC) employing rutin as a standard.

Procedure

- 1 ml extract (each of 100 µg/ml concentrations) was added to 4ml of H_2O, 300 µg of $AlCl_3$.
- The mixture was then incubated at room temperature for 5 min. After incubation, 2 ml of sodium hydroxide (1 M) was added.

- Then, final volume of solution was increased to 10 ml by further addition of distilled water .the absorbance of sample and blank were determined at 510 nm by UV-VIS spectrometer.
- The total flavonoid content was expressed in terms of mg rutin equivalents (RE) / g of dry sample.

❖ **Estimation of Total Tannin (Folin and Ciocalteau Method, 1927)**

Principle

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

Reagents used

1) Folin – Ciocalteau reagent
2) Sodium carbonate
3) Tannic acid

Procedure

The tannins were determined by Folin and Ciocalteau method. 0.1ml of the sample extract was added with 7.5ml of distilled water and 0.5ml of folin – ciocalteau reagent, 1ml of 35% sodium carbonate solution and diluted to 10ml with distilled water. The mixture was shaken well, kept at room temperature for 30min. an absorbance was measured at 725nm. 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 of extract.

6. Antimicrobial Activity

Test organisms

The test organisms selected for the study were gram positive *Staphylococcus aureus*, and gram negative bacteria *E.Coli* obtained from Department of Zoology, and fungi *Aspergillus flavus and Trichoderma harzianum* obtained from Department of

Botany, Avinashilingam University, Coimbatore. All the bacterial and fungal cultures were cultured in nutrient agar (Hi-media) and PDA medium for 24 and 72 hours respectively 37°C.

- **Antibacterial Assay**

Preparation of nutrient agar medium

The medium was prepared by dissolving 28 g of the commercially available nutrient Agar (HiMedia) in 1000ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121ºC for15 minutes. The autoclaved medium was mixed well and distributed on clean glass tubes and plugged with cotton for further studies.

Inoculum:

Culture of test organisms were used as inoculum, loop full of organism was taken from the slant and transferred to a flask containing sterilized nutrient broth. One litre of nutrient broth was prepared by dissolving 13 g of commercially available nutrient broth medium (HiMedia) in 1000ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure at (121ºC) for 15 minutes and allowed to grow at 37ºC. The 18 hr cultures were then plated on a nutrient agar plate to study the morphological character. The cultures from nutrient broth were centrifuged and suspension of cells was used for further studies.

- **Antifungal Assay**

Growth medium for fungal culture

Potato Dextrose Agar Medium (PDA) – (Riker and Riker, 1936).

- Peeled potato - 250 g
- Dextrose - 20 g
- Agar - 15 g
- Distilled water - 1000 ml
- pH - 6 to 7

Peeled potato was made into thin chips, boiled in 500 ml water and after extraction; dextrose was added into the extract. The agar was melted separately in other half of water and mixed with the above solution and the volume was made into 1000 ml. The medium was poured into sterile petriplates (15 ml / plate) and fungal cultures were inoculated in the centre of the petriplate.

- **Antimicrobial Assay**

The effect of leaf and root extracts of *Eryngium* and *Coriandrum* on the several bacterial and fungal strains was assayed by Agar well diffusion method and Disc diffusion method.

- **Agar-well diffusion Method (Bauer *et al*., 1996)**

Principle

The antimicrobials present in the plant material extract were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameter of zone of inhibition can be measured in the millimeters.

Procedure

Petriplates containing 20ml Nutrient Agar medium and PDA medium were seeded with 24 and 72 hrs culture of bacterial and fungal strains respectively, separately. Wells were cut and 20 µl of the leaf and root extracts (namely, aqueous and chloroform extracts) were added. Each bacterial and fungal suspension were spread over the surface of nutrient and PDA agar plates with cotton swab and four wells each of 6mm diameters was made on the agar. The wells were filled with 20 µl each of Chloroform and Water extracts along with proper control (Chloramphenicol) at the centre. The plates were incubated at 37^{o}C for 24 and 72 hours, respectively. The same procedure was repeated for other test organisms.

- **Disc diffusion Method:**

Principle

A filter-paper disc, impregnated with the plant extract to be tested, is placed on the surface of the agar. The extract diffuses from the filter paper into the agar.

The concentration of the extract will be highest next to the disc, and will decrease as distance from the disc increases. If the extract is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration.

Procedure

The *in vitro* antibacterial activity of sample solution was studied by disc diffusion method. Plates were prepared by pouring 20ml of sterile nutrient and PDA agar into the sterile petridishes and after solidification 0.25 ml of test strains were inoculated in the media separately. Sterile paper disc (Whatman no.1, 3.6mm diameter) impregnated with 20µl quantity of dimethyl sulfoxide solution (DMSO) of the plant extracts were air dried and placed on the agar plates and were incubated at 37°C for 24 and 72 hrs respectively. Control studies with Chloramphenicol discs (2mg/ml) and the solvent DMSO were done concurrently.

Statistical analysis

Standard error of means of all the replicates of each variable were computed using computer software: Microsoft exel data. The data were analysed using 3 way analysis of variance (ANOVA) followed by LSD method to delineate mean difference (Panse and Sukhatme, 1978).

RESULT AND DISCUSSION

Chapter IV

RESULTS AND DISCUSSION

The results and relevant discussions pertaining to *Coriandrum sativum L.* and *Eryngium foetidum L.* leaf and root were discussed under the following headings

1. Physiochemical analysis
2. Preliminary phytochemical screening
3. Quantitative phytochemical analysis
4. Antimicrobial activity

1. Physiochemical analysis

Table I
Moisture content of *Coriandrum sativum* and *Eryngium foetidum* leaves and root

S. No.	Plant	Leaf	Root
1.	*Coriandrum sativum*	87.67%	85.98%
2.	*Eryngium foetidum*	69.1%	62.06%

Moisture content

The moisture content of *Coriandrum sativum* was found to be higher in the leaf (87.67%) and root (85.98%) than the leaf (67.1%) and root (62.06%) of *Eryngium foetidum.*

The present result is in agreement with the findings of Garcia *et al.* (1997), Pathak *et al.* (2011) and Mohammad *et al.* (2012). They reported moisture content of 86-88% in the fresh leaves of *Eryngium foetidum.*

Table II

Solubility test of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Solubility (%)			
		Aqueous		Chloroform	
		Leaf	Root	Leaf	Root
1.	*Coriandrum sativum*	6.3	12.15	18.3	10.5
2.	*Eryngium foetidum*	7.9	2.4	7.3	4.2

The solubility tendency of chloroform leaf extracts was 18.3% and it was 12.15% in aqueous extracts of *Coriandrum sativum.* The least solubility percentage was observed in aqueous (2.4) and chloroform extracts of *Eryngium foetidum.*

2. Preliminary phytochemical screening

The results of the preliminary phytochemical (Qualitative) analysis revealed the presence of amino acid and carbohydrates in the aqueous and chloroform extracts of the leaf *Coriandrum sativum* and *Eryngium foetidum*.

Essential oil and starch were present in the aqueous leaf extract of *Coriandrum sativum* and *Eryngium foetidum*. Reducing sugar was present in all the three extract except the aqueous extract of *Eryngium foetidum*. Protein is present only in the aqueous extract of *Coriandrum sativum.* Acidic compounds are found to be absent in all the leaf extract of *Coriandrum sativum* and *Eryngium foetidum.*

The secondary metabolites like anthroquinone, anthrocyanin, coumarins, phenol, phlobatannins, quinines, sterols, saponins and terpenoids were present in aqueous leaf extract of *Coriandrum sativum* and *Eryngium foetidum.* Tannins and glycosides were present in aqueous and chloroform leaf extracts of *Coriandrum sativum* and *Eryngium foetidum.* Glycosides and steroids were present in the chloroform extracts of leaf of *Coriandrum sativum* and *Eryngium foetidum*. Alkaloids and flavonoids were found to be absent in *Coriandrum sativum* and *Eryngium foetidum* leaf extracts.

The qualitative phytochemical screening of aqueous and chloroform extracts showed the presence of carbohydrate and essential oil in the aqueous and chloroform extracts of root in *Coriandrum sativum* and *Eryngium foetidum.* Amino acid and acidic compounds were present in the aqueous root extract of *Coriandrum sativum* and *Eryngium foetidum.* Reducing sugar was found only in the aqueous extract of *Eryngium foetidum.* Acidic compound was present in the chloroform root extracts of *Coriandrum sativum* and *Eryngium foetidum.* Protein and starch were absent in the root extracts of both the plants.

Table III

Photochemical screening test of *Coriandrum sativum* and *Eryngium foetidum* Leaf

S. No	Phytochemical constituent	*Coriandrum sativum*		*Eryngium foetidum*	
		Aqueous	**Chloroform**	**Aqueous**	**Chloroform**
1.	Amino acid	+	+	+	+
2.	Alkaloids	-	-	-	-
3.	Acidic compounds	-	-	-	-
4.	Anthroquinone	+	-	+	-
5.	Anthrocyanines	+	-	+	-
6.	Coumarins	+	-	+	-
7.	Cardiac glycosides	+	+	+	+
8.	Carbohydrates	+	+	+	+
9.	Essentials oil	+	-	+	-
10.	Flavonoids	-	-	-	-
11.	Glycosides	-		-	+
12.	Phenol	+	-	+	-
13.	Phlobatannins	+	-	+	-
14.	Proteins	+	-	-	-
15.	Quinones	+	-	+	-
16.	Reducing sugar	+	-	+	-
17.	Starch	+	+	-	+
18.	Steroids	-	+	-	+
19.	Sterols	+	-	+	-
20.	Saponins	+	-	+	-
21.	Tannins	+	+	+	+
22.	Terpenoids	+	-	+	-

+ presence - absence

Among the secondary metabolites, alkaloids, phenols, saponins amd terpenoids were present only in the aqueous extracts of both *Coriandrum sativum* and *Eryngium foetidum.* Flavonoids and glycosides were present in all the three extract except in the chloroform extracts of *Eryngium foetidum*. Coumarins are found only in the aqueous extract of *Eryngium foetidum.* Steroids were observed in the chloroform root extracts of *Coriandrum sativum* and *Eryngium foetidum.* Anthroquinones, anthrocyanins, cardiac glycosides, phlobatannin, quinines, sterols and tannins were found to be absent in the root of *Coriandrum sativum.*

The result obtained in the present investigation is in accordance with the report by Nithya (2015). The aqueous and Chloroform extracts of the leaves of *Coriandrum sativum Linn*., showed the presence of alkaloids, amino acids, flavonoids, glycosides, phytosterols, saponins, steroids, tannins and triterpenoids.

Preliminary analysis demonstrated that methanolic extracts of both *E. foetidum* and *C. sativum* contains the presence of alkaloids, terpenoids, phlobatannins, coumarins, tannins, flavonoids, saponins and anthroquinones (Anusha *et al.,* 2015). The phytochemical analysis of ethanolic extracts of *Coriandrum sativum* showed the presence of flavonoids, alkaloids, tannins, saponins, steroids and glycosides (Mohan *et al.,* 2015).

Similar result was observed by Pathak *et al*., (2011). The phytochemical screening of *Coriandrum sativum* showed the presence of carbohydrates, reducing sugars, glycosides, triterpenes, proteins and essential oil. Similarlt the result of Kousar *et al*., (2011) revealed the presence of flavanoids of 5%, glycosides of 16%, alkaloids of 0.20%, sterols of 11.13% and tannins of 3.4% and the absence of anthroquinones and saponins in *C. sativum* leaf extract. The present result is on par with the finding of Sindhu *et al.* (2012). The analysis of *Coriandrum sativum* reported the presence of cardiac glycosides, terpenoids,flavonoids, saponins and anthroquinones.

Similar view was expressed by Nirmala *et al*., (2013) and Smitapadma (2013). Their study revealed the presence of alkaloids, carbohydrate, glycosides, saponins, flavonoid and tannin in the leaves of *Coriandrum sativum.* Rajeswari *et al*., (2013) reported that *Coriandrum sativum* seeds revealed the presence of carbohydrate

derivatives, phenolic compounds, tannins, flavonoids, steroids, terpenoids, saponins, glycosides and alkaloids.

Nithya and Sumalatha, (2014) and Zohreh *et al*., (2014) during their study on phytochemical analysis of *Coriandrum sativum* leaf extract found to contain tannins, alkaloids, steroids, essential oils, flavonoids, fatty acids and sterols.

Table IV

Photochemical screening test of *Coriandrum sativum* and *Eryngium foetidum* Root

S.No.	Phytochemical constituent	*Coriandrum sativum*		*Eryngium foetidum*	
		Aqueous	**Chloroform**	**Aqueous**	**Chloroform**
1.	Amino acid	+	-	+	-
2.	Alkaloids	-	+	-	+
3.	Acidic compounds	+	-	+	-
4.	Anthroquinone	-	-	-	-
5.	Anthrocyanines	-	-	-	-
6.	Coumarins	-	-	+	-
7.	Cardiac glycosides	-	-	-	-
8.	Carbohydrates	+	+	+	+
9.	Essentials oil	+	+	+	+
10.	Flavonoids	+	+	+	-
11.	Glycosides	-	+	-	+
12.	Phenol	+	-	+	-
13.	Phlobatannins	-	-	-	-
14.	Proteins	-	-	-	-
15.	Quinones	-	-	-	-
16.	Reducing sugar	-	-	+	-
17.	Starch	-	-	-	-
18.	Steroids	-	+	-	+
19.	Sterols	-	-	-	-
20.	Saponins	+	-	+	-
21.	Tannins	-	-	-	-
22.	Terpenoids	+	-	+	-

+ presence – absence

Similar result was obtained by Chandira and Jaykar (2013). Their study on the leaf extract of *Eryngium foetidum* revealed the presence of carbohydrates, glycosides, proteins and amino acids, saponins, phytosterols, flavonoids, gum and mucilages and phenolic compounds and tannins. Okon *et al.*, (2013) analysed phytochemical constituents of *Eryngium foetidum* and revealed the presence of saponins, flavonoids, alkaloids, tannins anthroquinones, gardiac glycosides and terpenes. Rajagopal *et al*., (2015) reported the presence of secondary compounds like flavonoids, tannins, saponin and several terpanoids in *Eryngiun foetidum* L. leaves.

3. Quantitative phytochemical analysis

Carbohydrate content

It may be deduced from the Table V and Figure 1 that among the aqueous and chloroform leaf extracts, a significantly highest amount of carbohydrates was found in aqueous of *Eryngium foetidum* (9.30±0.33 mg/g) and *Coriandrum sativum* (9.20±0.06 mg/g) root extract. The least value of 1.25±0.06 mg/g was recorded in the chloroform leaf extracts of *Coriandrum sativum.*

Table V

Estimation of carbohydrates content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Carbohydrate (mg/g)			
		Leaf		Root	
		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Coriandrum sativum*	9.20 ± 0.06	1.25 ± 0.06	9.15 ± 0.05	1.70 ± 0.06
2.	*Eryngium foetidum*	9.17 ± 0.07	8.52 ±0.41	9.30 ± 0.33	1.61 ±4.88
	SEd	0.174		1.999	
	CD (p<0.05)	0.402		4.609	

Similar result was observed by Chowdhury *et al.* (2007) and Mohammad *et al.* (2012). They obtained total carbohydrates content of 6.5% in the leaves of *Eryngium foetidum.* Sasi Kumar *et al.* (2014) also reported higher amount of carbohydrates in the leaves of *Coriandrum sativum.*

Protein content

A significantly higher amount of total protein content of 6.50±0.20 mg/g and 6.50±0.11 mg/g were recorded in the chloroform extracts of leaf and root of *Coriandrum sativum* followed by aqueous extracts of root of *C. sativum* (3.90±0.10 mg/g) and chloroform root extracts of *Eryngium foetidum* (3.74±0.41 mg/g).

Table VI

Estimation of Protein content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Protein (mg/g)			
		Leaf		Root	
		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Coriandrum sativum*	2.71±0.30	6.50±0.20	3.90±0.10	6.50±0.11
2.	*Eryngium foetidum*	2.98±0.41	2.22±0.23	3.74±0.41	1.41±0.42
	SEd	0.240		0.246	
	CD (p<0.05)	0.553		0.568	

Similar result was obtained by Garcia *et al.* (1997) and Mohammad *et al.* (2012) with a total protein content of 3.3% in the fresh leaves of *Eryngium foetidum.*

Figure I

Estimation of Carbohydrate content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

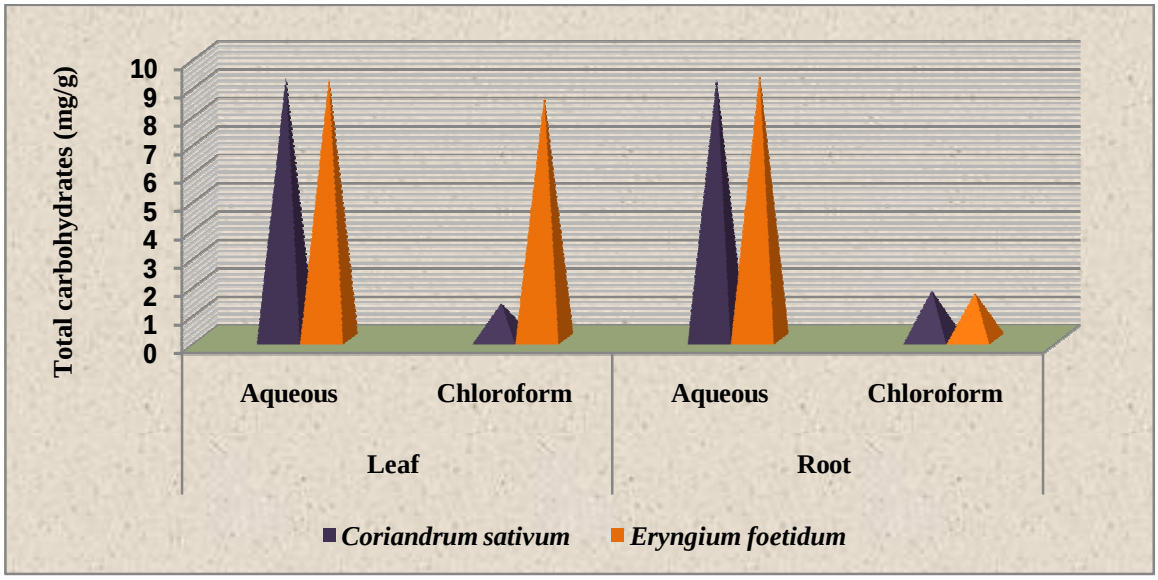
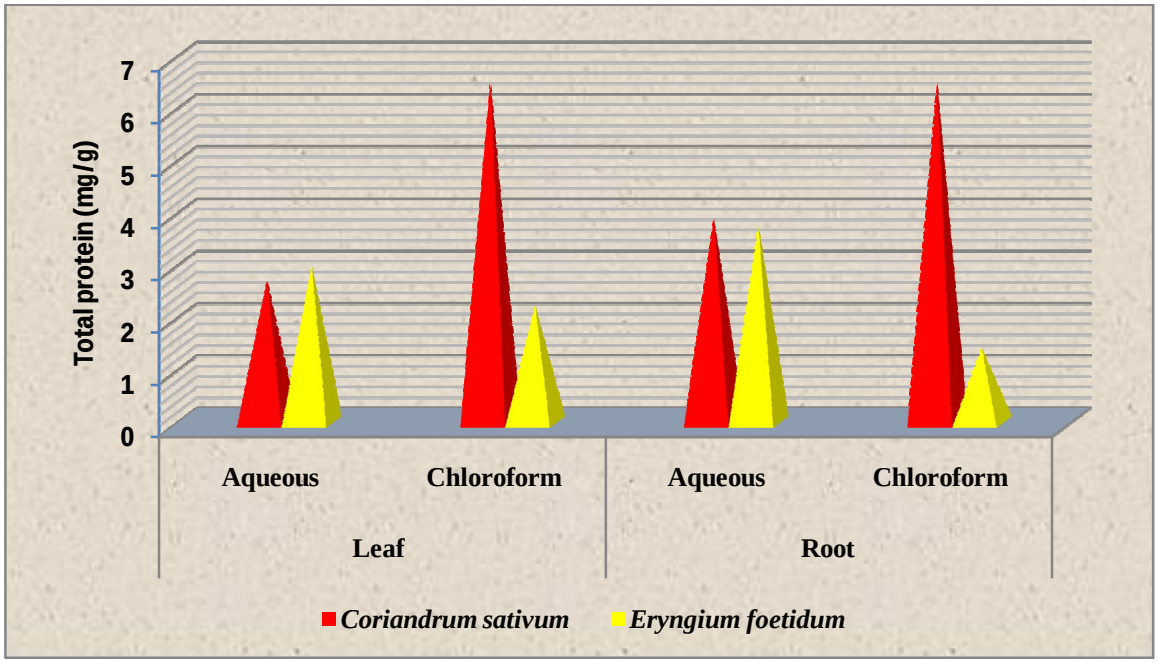


Figure II

Estimation of Protein content of Coriandrum sativum and Eryngium foetidum leaf and root



Total flavonoid content

Total flavonoid content was observed to be 0.69±0.04 RE/g in aqueous extracts of

Coriandrum sativum. The flavonoid content of chloroform leaf extracts of *Coriandrum sativum* and *Eryngium foetidum* were similar with each other (0.67±0.07 RE/g) and 0.67±0.09 RE/g). Similarly the flavonoid content values of aqueous root extract of *Eryngium foetidum* and chloroform extract of *Coriandrum sativum* were also on par with each other (0.64±0.08 RE/g and 0.64 RE/g. The least value of 0.49±0.06 RE/g was found in the aqueous root of *Coriandrum sativum.*

Table VII

Estimation of Total Flavonoid content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Total Flavonoid (RE/g)			
		Leaf		Root	
		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Coriandrum sativum*	0.67±0.04	0.69±0.07	0.49±0.06	0.64±0.06
2.	*Eryngium foetidum*	0.65±0.12	0.67±0.09	0.64±0.08	0.63±0.08
	SEd	0.070		0.577	
	CD (p<0.05)	0.161		0.133	

Similar result was obtained by Kousar *et al.* (2011). The phyto screening analysis conducted by them reported the presence of total flavonoid content of 5% in the leaf extracts of *Coriandrum sativum.* Rajeswari *et al.* (2013) also obtained a highest flavonoid content of 3.30±0.09mg/g (RE) in n-butanol extract sample of *Coriandrum sativum* seeds.

The present result is on par with the finding of Mohan *et al*. (2015). They reported the flavanoid content of *C. sativum* as 12.6 QE/g. Saciu *et al.* (2006) reported that the total flavonoid content in dried aerial parts of *Eryngium planum*, *Eryngium campestre* and *Eryngium maritinum* ranged from 0.32 to 56% (RE/g).

The present result is on par with the investigation by Bhavana *et al.* (2013). They reported the occurrence of highest total flavonoid content of 34.36±0.19 mg QE/g in *Eryngium foetidum* collected from Darjeeling. Similar result was observed by Anusha *et al.* (2015). They obtained the total flavonoid content of 0.315±0.00 and 0.254±0.00 QE/g on methanolic extracts of *Eryngium foetidum* and *Coriandrum sativum.*

Total phenol content

A significantly higher total phenolic content of 2.65±0.07 GAE/g was recorded in the aqueous leaf extracts of *Coriandrum sativum.* The total phenolic content in the chloroform leaf extract of *Coriandrum sativum* and *Eryngium*

foetidum were on par with each other (1.92±0.10 and 1.92±0.05 GAE/g). The least amount was found in the aqueous root extract of *Coriandrum sativum,* (1.07±0.10 GAE/g).

Table VIII

Estimation of Total Phenol content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Total Phenol (GAE/g)			
		Leaf		Root	
		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Coriandrum sativum*	2.65±0.07	1.92±0.10	1.07±0.10	1.50±0.05
2.	*Eryngium foetidum*	2.08±0.07	1.92±0.05	1.32±0.07	2.10±0.06
SEd		0.060		0.055	
CD (p<0.05)		0.138		0.128	

Similar result was reported by Bhavana *et al.* (2013). They observed high total phenol content in Darjeeling population (41.61±0.29) followed by Andaman (24.95±0.33) and the least value in Karnataka population (18.09±0.23) of dried plant extracts of *E. foetidum.*

Figure III

Estimation of Total Flavonoid content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

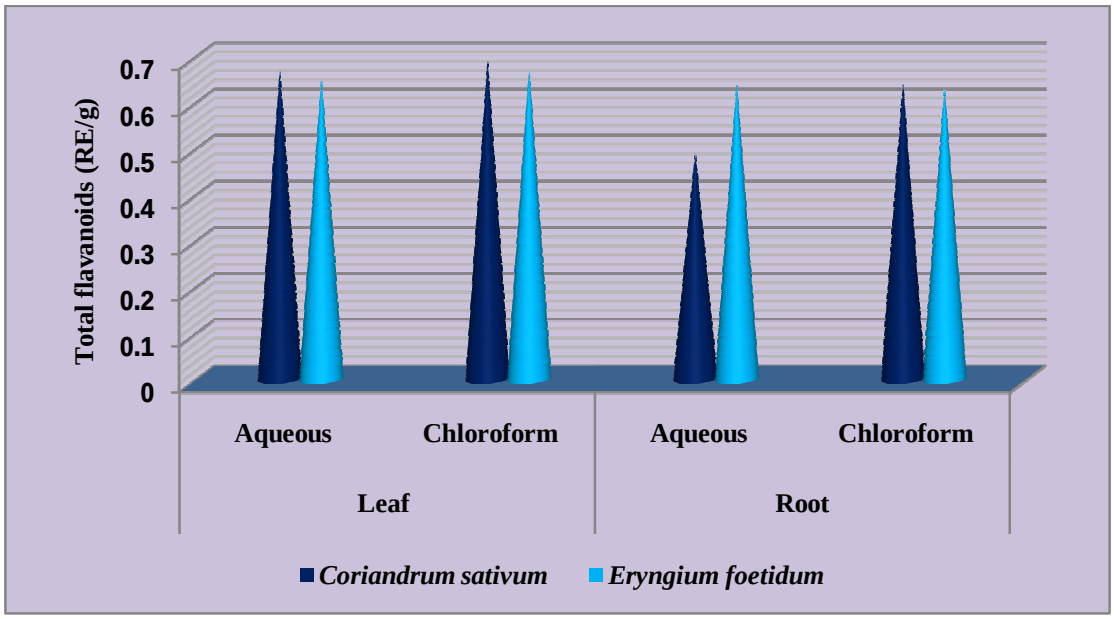
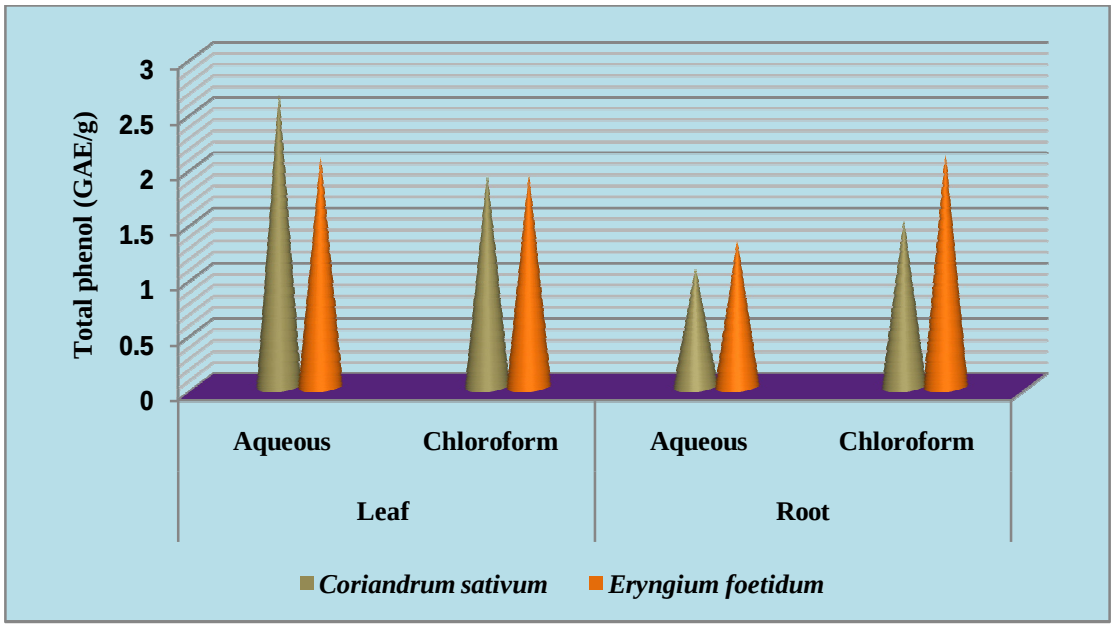


Figure IV

Estimation of Total Phenol content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root



The present result is in accordance with the investigation by Aly (2010) who obtained the total phenolic content of 8.91±0.01 GAE/g in *Eryngium foetidum*. Rajeswari *et al.* (2013) reported high phenolic content of 5.76±1.07mg/100g (GAE) in n-butanol extract of *Coriandrum sativum*.

Similar result was observed by Mohan *et al.* (2015). They obtained the phenolic content of 12.2 mg/g (GAE) in the ethanol extract of *C. sativum* seed. Bhavana *et al.* (2013) reported highest total phenolic content of 45.03±0.23 GAE/g in *Eryngium foetidum* collected from Andaman. The present result is in agreement with the study by Anusha *et al.* (2015). They reported that the methanolic extracts of *E. foetidum* and *C. sativum* showed the total phenolic content of 5.25±0.2 and 2.8±0.1 mg/g (GAE).

Total tannin content

The quantitative analysis of total tannin content in aqueous and chloroform extracts of *Coriandrum sativum* and *Eryngium foetidum* were analysed in the leaf and root (Table IX and Figure 5).

Table IX

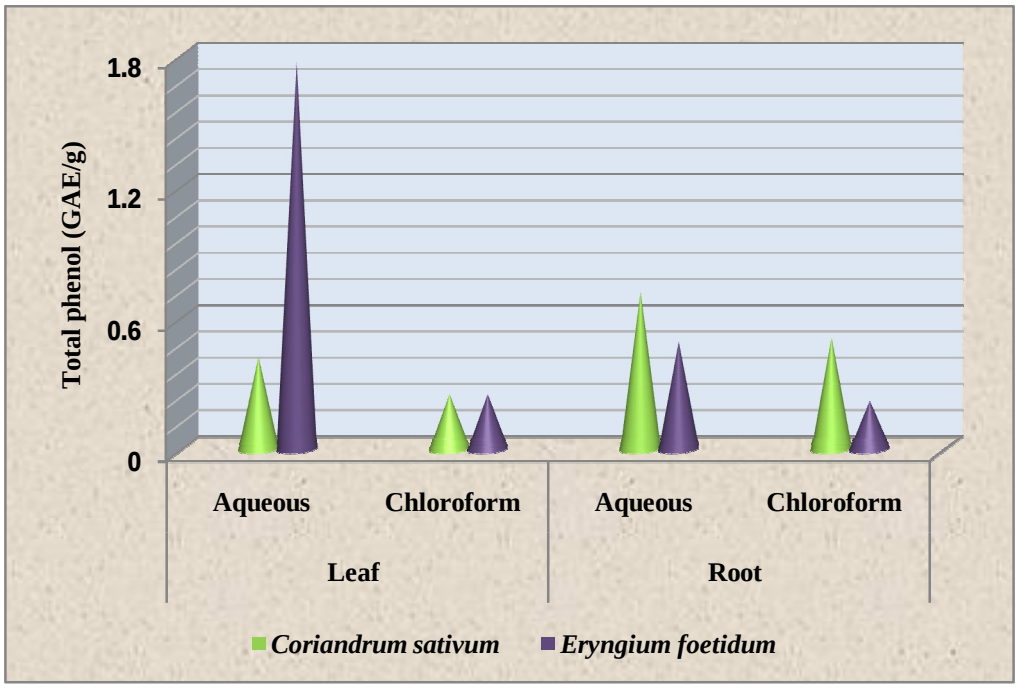
Estimation of Total Tannin content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root

S. No.	Plants	Total Tannin (GAE/g)			
		Leaf		Root	
		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Coriandrum sativum*	0.42±0.11	0.25±0.10	0.72±0.05	0.51±0.08
2.	*Eryngium foetidum*	1.77±0.08	0.25±0.07	0.49±0.07	0.22±0.07
SEd		0.076		0.053	
CD (p<0.05)		0.167		0.123	

The results of the study revealed the occurrence of higher amount of 1.77±0.08 GAE/g total tannin content in the aqueous leaf extracts of *Eryngium foetidum* followed by 0.72±0.05 GAE/g in the aqueous root extracts of *Coriandrum sativum.* The least value of 0.22±0.07 GAE/g was recorded in *Eryngium foetidum* root.

Figure V

Estimation of Total Tannin content of *Coriandrum sativum* and *Eryngium foetidum* leaf and root



The result is in agreement with the finding of Bhavana *et al.* (2013). They reported a significantly high tannin content in Andaman population (34.35±0.19) followed by Darjeeling and Karnataka population of dried *E. foetidum* plant extracts.

Antimicrobial activity of *Coriandrum sativum* and *Eryngium foetidum*

In order to assess the efficacy of phytochemical present in *Coriandrum sativum* and *Eryngium foetidum*, antibiosis of aqueous extracts of root and leaf against two fungal strains, *Aspergillus flavus* and *Trichoderma harzianum* and two bacterial strains, *Staphylococcus aureus* and *Escherichia coli* were undertaken by agar well and disc diffusion methods.

Antibacterial activity

Among the two extracts, chloroform and aqueous leaf extract of *Coriandrum sativum* and *Eryngium foetidum*, higher zone of inhibition of 6.00±2.65mm diameter was recorded in the aqueous leaf extract of *Eryngium foetidum* with the positive drug control, chloramphenicol showing 7.33±5.5mm diameter against *Staphylococcus aureus.* The least zone of inhibition was found in the chloroform extract of *Eryngium foetidum* (2.00±1.00 mm diameter) against *E. coli*. The inhibition zone of positive drug control used for the inhibition of *E. coli* showed 5.00±1.00 mm diameter by agar well diffusion method.

The aqueous root extract of *Eryngium foetidum* showed highest antibacterial activity with the inhibition zone of 5.33±2.08 mm diameter and the positive drug control showed 7.33±5.51 mm diameter against bacterial strains. The inhibitory activity of *Coriandrum sativum* aqueous root extract was found to be 5.00±2.65 mm diameter. The least antibacterial activity with the inhibition zone of 2.33±1.53 mm diameter was found in the chloroform root extract of *Coriandrum sativum* against *S. aureus.* The antibacterial activity of chloroform root extract of *Eryngium foetidum* was found to be high with the inhibition zone of 4.00±2.00 mm diameter against *E. coli* by agar well diffusion method. The least inhibitory antimicrobial activity with a minimum zone of inhibition of 2.33±1.53mm diameter was observed in the chloroform extract of *Coriandrum sativum* against *E. coli.*

In case of disc diffusion method, the higher antimicrobial activity with the inhibition zone of 4.00±1.00mm diameter in the chloroform extracts of both leaf and root against *Staphylococcus aureus.* The inhibition zone of positive drug control was 4.60±1.00 mm incase of leaf and 5.67±1.52 mm in root. The aqueous and chloroform extract of *Coriandrum sativum* root showed inhibition zone 4.67±1.53 mm and 4.33±1.53 mm diameter against E. *coli.* The least antibacterial activity was found in *Eryngium foetidum* with the inhibition zone of 3.00±1.00 mm diameter in aqueous leaf extract and 2.00±1.00mm diameter in chloroform root extract.

The present result is in agreement with report of Dash *et al.* (2011). They have found that the crude methanol extract of *Coriandrum sativum* leaves produced inhibitory zone of 7 mm diameter against *Shigella dysentriae* and *Salmonella typhi* and 7 mm and 9 mm diameter against *E. coli* and *Pseudomonas* spp respectively.

Similar result was observed by Darshan *et al.* (2016). They have reported that the leaf of *Trigonella foenum-graceum* and *Serratia marcescens* exhibited minimum zone of inhibition of 12.33±0.57 mm diameter by aqueous extracts followed by 11.50±0.50 mm diameter by methanol extract against *Bacillus cereus.*

The present result is on par with the finding of Vijayan *et al.* (2003). They have obtained a zone of inhibition of 10.0 mm and 10.5 mm diameter by water and acetone extracts of *Coriandrum sativum* leaves against *Salmonella typhi* and *Staphylococcus aureus.*

Similar result was obtained by Joglekar *et al.* (2012), who found that the methanol extracts of *Coriandrum sativum* showed inhibition zone of 3.5 mm diameter against *Staphylococcus aureus* at 500 µl/ml concentrations.

Antifungal activity

The antifungal activity of aqueous and chloroform extracts of root and leaf of *Coriandrum sativum* and *Eryngium foetidum* were carried out against two fungal strains, *Aspergillus flavus* and *Trichoderma harzianum* by agar well and disc diffusion methods.

The result of the study revealed that higher antifungal activity with the inhibition zone of 6.00±1.00 mm diameter was registered in the chloroform leaf extract of *Coriandrum sativum* against the fungus, *Aspergillus flavus.*

The inhibitory zone obtained by the aqueous root extract of *Coriandrum sativum* was 5.33±1.53 mm diameter. The least antifungal activity with the minimum zone of inhibition was recorded in the chloroform root extract of *Coriandrum sativum* and leaf extract of *Eryngium foetidum* against the fungal strain, *Aspergillus flavus.* The inhibition zone of positive drug control for *Aspergillus flavus* was 8.60±2.00 mm diameter in leaf and 6.00±2.00 mm diameter in root by agar well diffusion method

Table X

Inhibition zone (mm) of Bacteria in leaf and root of *Coriandrum sativum* and *Eryngium foetidum* (Agar well diffusion method)

S. No.	Test organism	Control	Leaf				Control	Root			
			Coriandrum sativum		*Eryngium foetidum*			*Coriandrum sativum*		*Eryngium foetidum*	
			Aqueous	Chloroform	Aqueous	Chloroform		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Staphylococcus aureus*	7.33±5.51	4.67±2.08	3.67±1.53	6.00±2.65	3.33±1.53	7.33±5.51	5.00±2.65	2.33±1.53	5.33±2.08	3.00±1.73
2.	*E. coli*	5.00±1.00	3.67±1.53	3.00±1.00	3.00±1.00	2.00±1.00	4.33±2.08	3.33±1.53	3.67±1.53	3.67±1.53	4.00±2.00
SEd		2.143			2.211		2.277			2.317	
CD(p>0.05)		4.669			4.818		4.956			5.049	

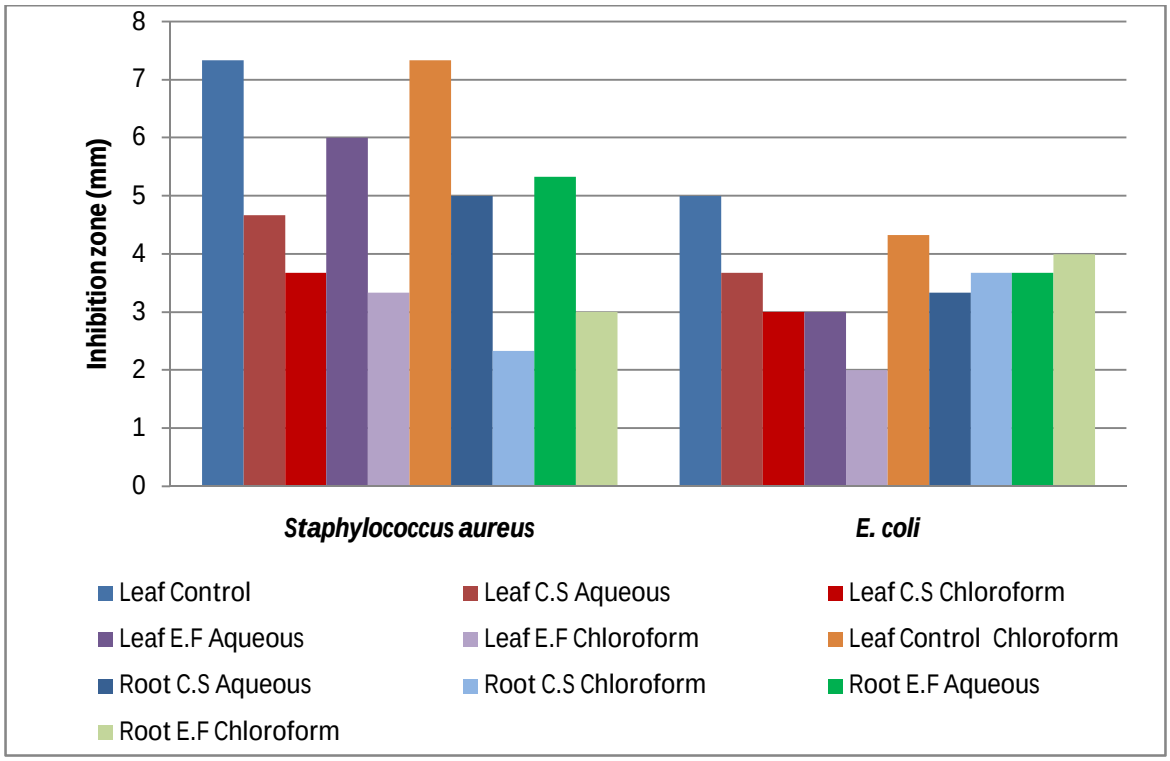
TableXI

Inhibition zone (mm) of Bacteria in leaf and root *Coriandrum sativum* and *Eryngium foetidum* (Disc diffusion method)

S. No.	Test organism	Control	Leaf				Control	Root			
			Coriandrum sativum		*Eryngium foetidum*			*Coriandrum sativum*		*Eryngium foetidum*	
			Aqueous	Chloroform	Aqueous	Chloroform		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Staphylococcus aureus*	4.60±1.00	3.67±1.53	3.33±2.08	3.79±1.53	4.00±1.00	5.67±1.52	3.00±1.00	2.67±1.53	3.00±1.00	4.00±1.00
2.	*E. coli*	5.00±2.00	4.00±2.00	3.33±2.08	3.00±1.00	4.00±1.00	5.00±2.00	4.67±1.53	4.33±1.53	3.00±1.00	2.00±1.00
SEd		1.414			1.072		1.202			1.072	
CD(p>0.05)		3.081			2.335		2.619			2.335	

Figure VI

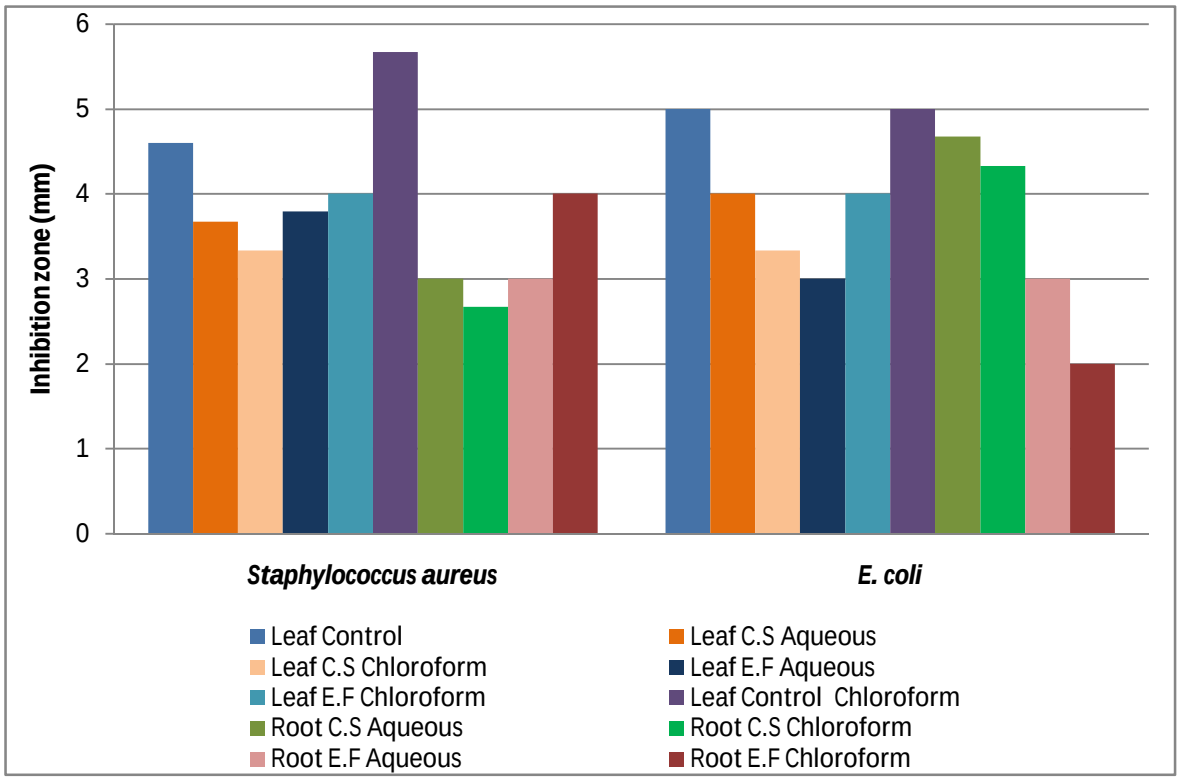
Inhibition zone (mm) of Bacteria in leaf and root of *Coriandrum sativum* and *Eryngium foetidum* (Agar well diffusion method)



C.S- *Coriandrum sativum*, E.F- *Eryngium foetidum*

Figure VII

Inhibition zone (mm) of Bacteria in leaf and root of *Coriandrum sativum* and *Eryngium foetidum* (Disc diffusion method)



C.S- *Coriandrum sativum,* E.F- *Eryngium foetidum*

Plate VII

Zone of inhibition of *Corinadrum sativum* L. against *Staphylococcus aureus* and *E. coli*

(Agar well diffusion method)

Staphylococcus aureus ***E.coli***

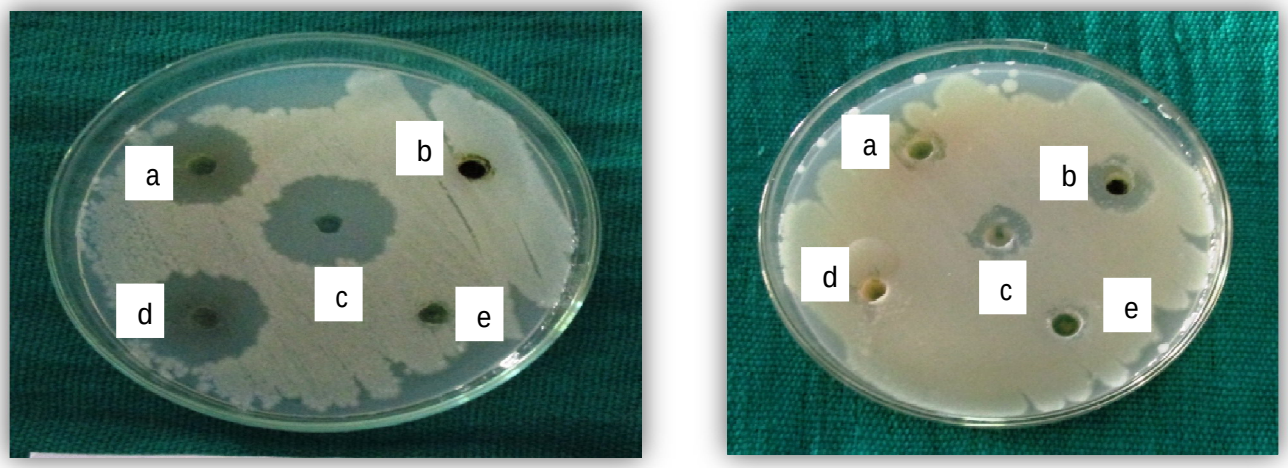
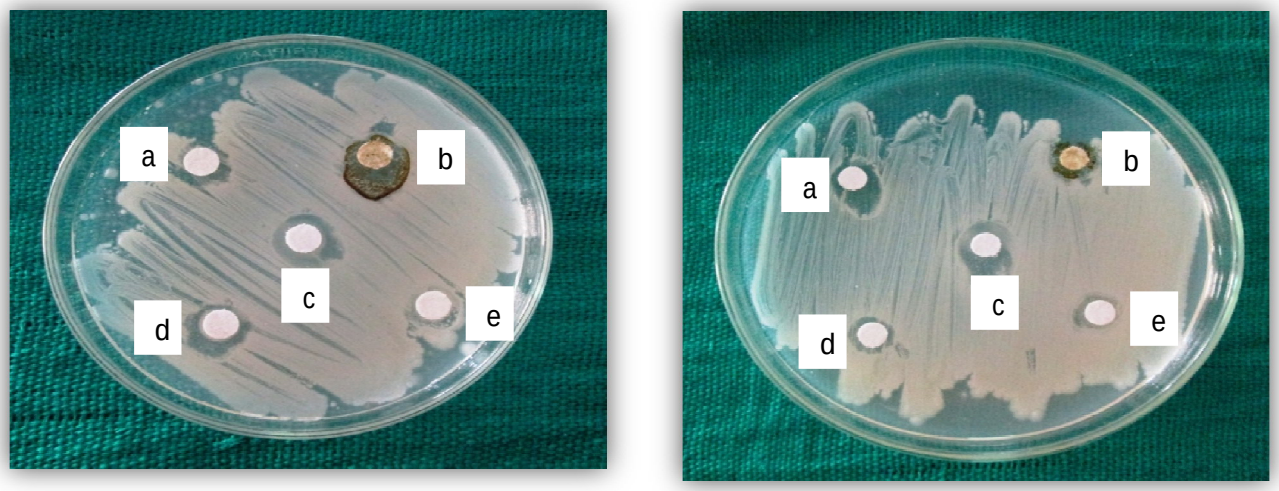


Plate VIII

Zone of inhibition of *Corinadrum sativum* L. against *Staphylococcus aureus* and *E. coli*

(Disc diffusion method)

Staphylococcus aureus ***E. coli***



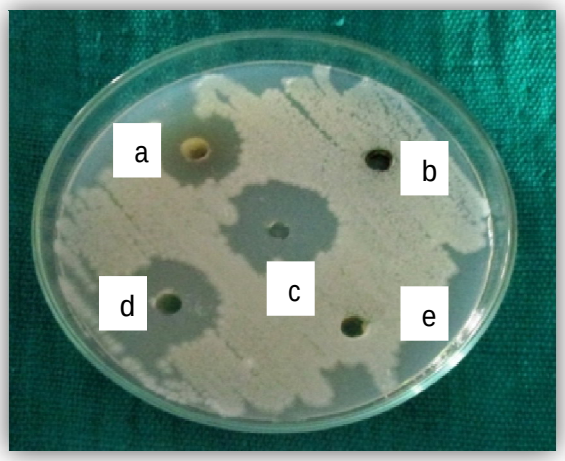
a-Aqueous leaf b- Chloroform leaf c- Control d-Aqueous root e- Chloroform root

Plate IX

Zone of inhibition of *Eryngium foetidum* L. against *Staphylococcus aureus* and *E. coli*

(Agar well diffusion method)

Staphylococcus aureus ***E. coli***



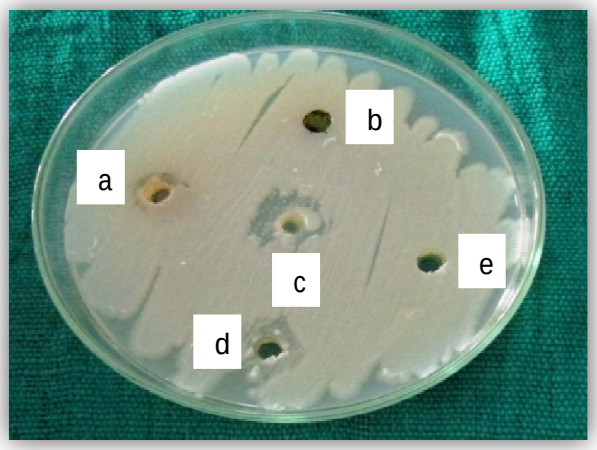
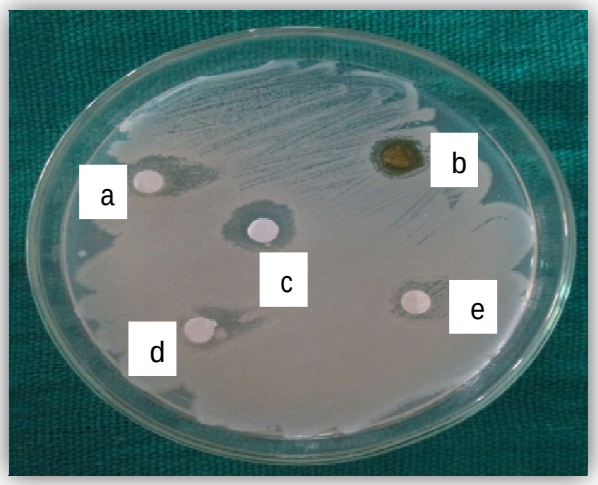


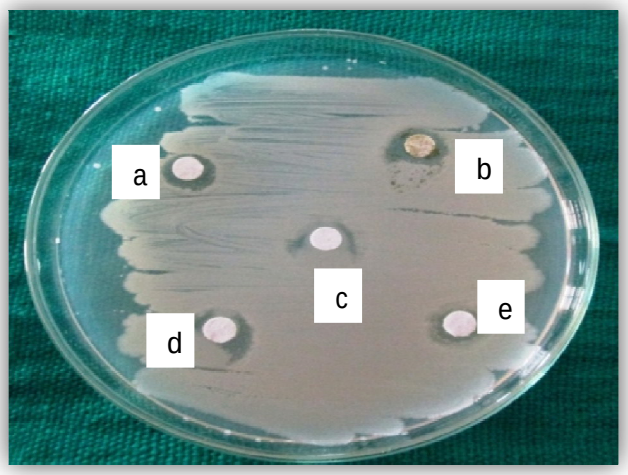
Plate X

Zone of inhibition of *Eryngium foetidum* L. against *Staphylococcus aureus* and *E. coli*

(Disc diffusion method)

Staphylococcus aureus ***E. coli***





a-Aqueous leaf b- Chloroform leaf c- Control d-Aqueous root e- Chloroform root

Table XII

Inhibition zone (mm) of Fungi in leaf and root *Coriandrum sativum* and *Eryngium foetidum* (Agar well diffusion method)

S. No.	Test organism	Control	Leaf				Control	Root			
			Coriandrum sativum		*Eryngium foetidum*			*Coriandrum sativum*		*Eryngium foetidum*	
			Aqueous	Chloroform	Aqueous	Chloroform		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Aspergillus flavus*	8.60±2.00	5.33±1.53	3.60±1.00	4.00±2.00	3.33±1.53	6.00±2.00	4.00±1.00	5.00±1.00	5.00±1.00	5.00±1.00
2.	*Trichoderma harzianum*	3.67±1.53	4.00±1.00	4.67±1.53	3.00±1.00	5.00±1.00	5.60±1.00	5.00±1.00	3.33±1.53	2.33±1.53	4.00±2.00
SEd			1.139		1.337			1.202		1.217	
CD(p>0.05)			2.481		2.935			2.652		2.652	

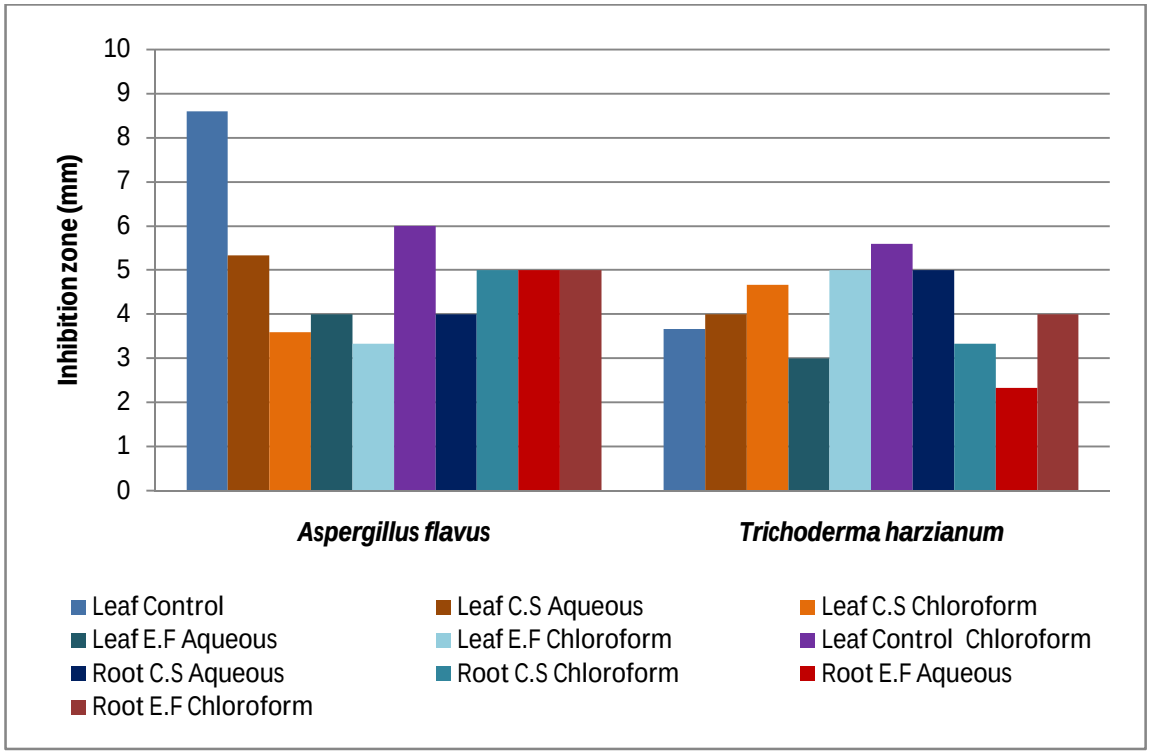
Table XIII

Inhibition zone (mm) of Fungi in leaf and root *Coriandrum sativum* and *Eryngium foetidum* (Disc diffusion method)

S. No.	Test organism	Control	Leaf				Control	Root			
			Coriandrum sativum		*Eryngium foetidum*			*Coriandrum sativum*		*Eryngium foetidum*	
			Aqueous	Chloroform	Aqueous	Chloroform		Aqueous	Chloroform	Aqueous	Chloroform
1.	*Aspergillus flavus*	5.67±1.52	3.67±1.53	3.33±2.08	3.67±1.52	4.00±1.00	8.00±1.00	2.67±1.53	3.00±1.00	3.00±1.00	4.00±2.00
2.	*Trichoderma harzianum*	3.00±1.00	4.00±1.00	3.33±2.00	3.00±1.00	4.00±2.00	9.33±1.53	4.67±1.53	4.33±1.53	3.33±1.00	2.00±1.00
SEd			1.414		1.072			1.202		1.072	
CD(p>0.05)			3.081		2.335			2.619		2.335	

Figure VIII

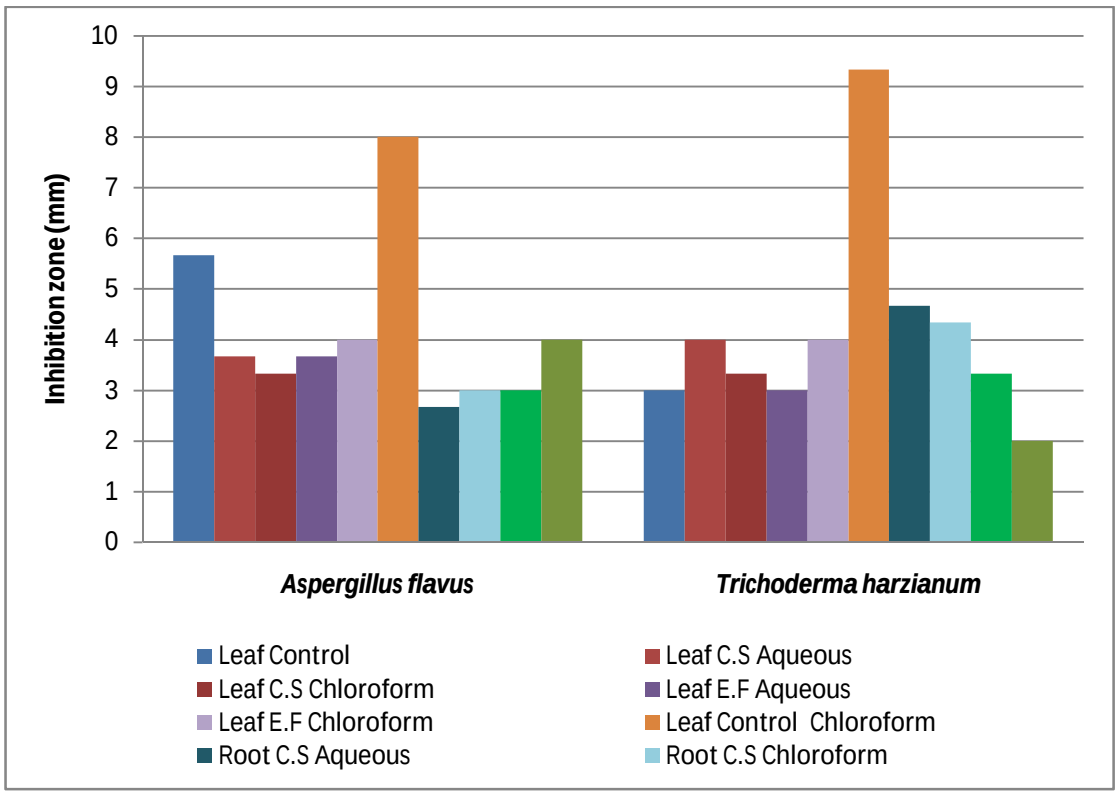
Inhibition zone (mm) of Fungi in leaf and root of *Coriandrum sativum* and *Eryngium foetidum* (Agar well diffusion method)



C.S- *Coriandrum sativum,* E.F- *Eryngium foetidum*

Figure IX

Inhibition zone (mm) of Fungi in leaf and root of *Coriandrum sativum* and *Eryngium foetidum* (Disc diffusion method)



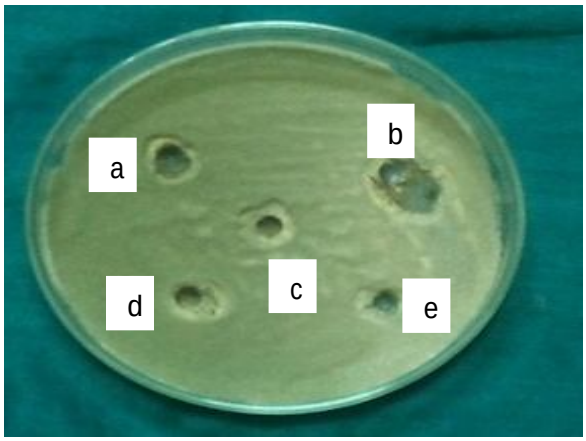
C.S- *Coriandrum sativum,* E.F- *Eryngium foetidum*

Plate XI

Zone of inhibition of *Coriandrum sativum* L. against *Aspergillus flavus* and *Trichoderma harzianum*

(Agar well diffusion method)

Aspergillus flavus ***Trichoderma harzianum***



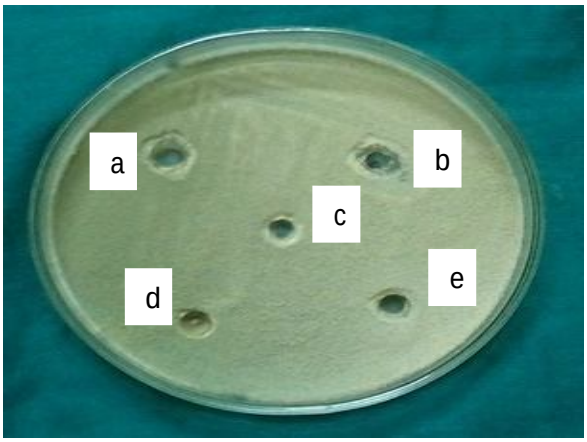
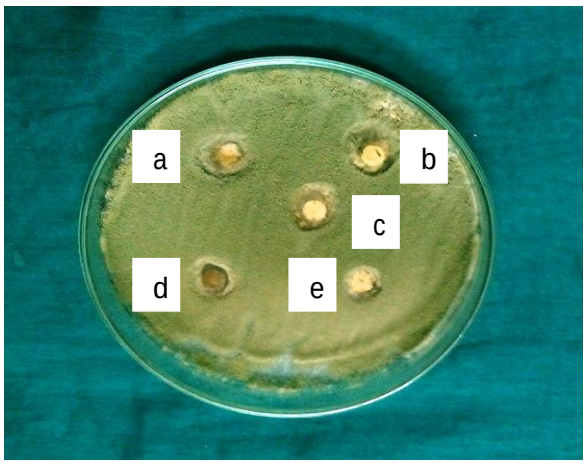


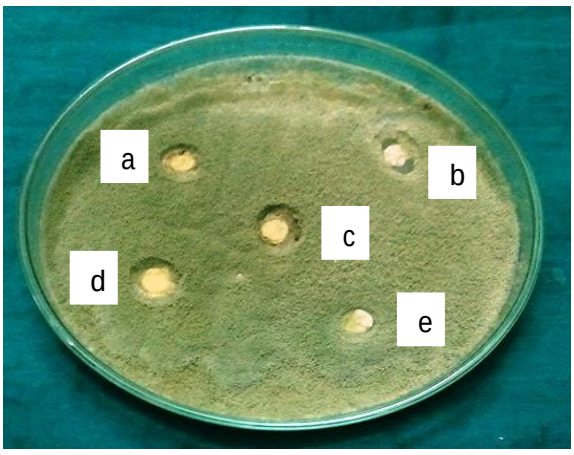
Plate XII

Zone of inhibition of *Coriandrum sativum* L. against *Aspergillus flavus* and *Trichoderma harzianum*

(Disc diffusion method)

Aspergillus flavus ***Trichoderma harzianum***





a-Aqueous leaf b- Chloroform leaf c- Control d-Aqueous root e- Chloroform root

Plate XIII

Zone of inhibition of *Eryngium foetidum* L. against *Aspergillus flavus* and *Trichoderma harzianum*

(Agar well diffusion method)

Aspergillus flavus ***Trichoderma harzianum***

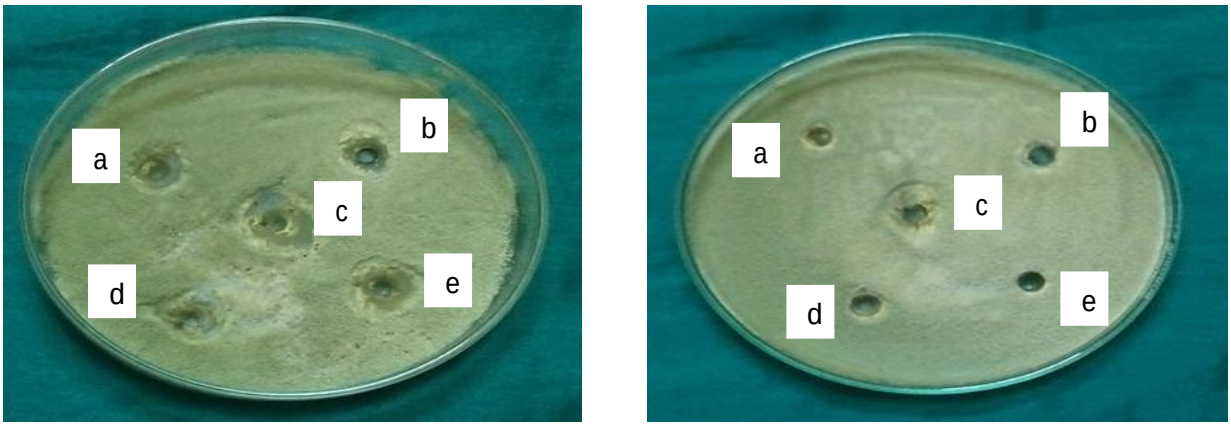
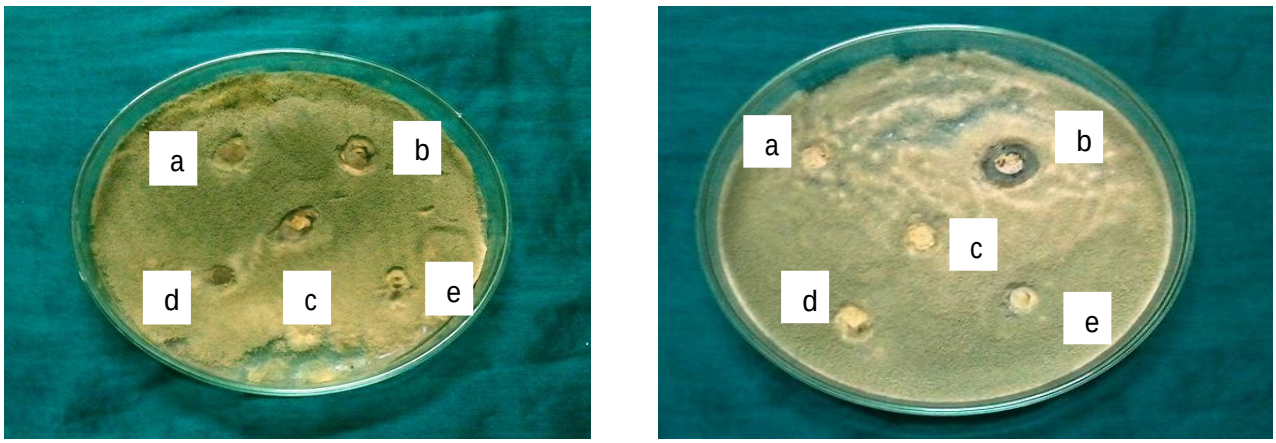


Plate XIV

Zone of inhibition of *Eryngium foetidum* L. against *Aspergillus flavus* and *Trichoderma harzianum*

(Disc diffusion method)

Aspergillus flavus ***Trichoderma harzianum***



a-Aqueous leaf b- Chloroform leaf c- Control d-Aqueous root e- Chloroform root

In case of disc diffusion method, highest zone of 7.67±1.53 mm diameter was found in the root chloroform extract of *Coriandrum sativum,* followed by 5.77±1.55 mm diameter inhibition zone in the chloroform root extract of *Eryngium foetidum.* The least antimicrobial activity with the inhibition zone of 3.33±1.53 mm diameter was recorded in the aqueous root extract of *Eryngium foetidum* against *Aspergillus flavus.* The positive drug control showed the inhibitory zone of 8.00±1.00 mm diameter. The inhibitory activity of leaf extract against *Aspergillus flavus* was found to be highest (5.00±1.00 mm diameter) in the chloroform extracts of *Eryngium foetidum* leaf. The least inhibitory zone of 3.00±1.00 mm diameter was recorded in the aqueous extract of *Coriandrum sativum.*

Among the aqueous and chloroform extracts of leaf and root of *Coriandrum sativum* and *Eryngium foetidum* against the fungus *Trichoderma harzianum,* highest zone of inhibition 0f 7.00±2.00 mm diameter was obtained by the chloroform extract of *Coriandrum sativum* root followed by 5.67±1.53 mm diameter in the chloroform extract of *Eryngium foetidum*. The least antimicrobial activity with the minimum zone of inhibition of 2.33±1.53 mm diameter was observed in the aqueous leaf extract of *Coriandrum sativum*. The positive drug control showed an inhibitory zone of 7.33±1.53 mm diameter in the leaf extract and 9.33±1.53 mm diameter against *Trichoderma harzianum*.

The antifungal activity was carried out by Darshan *et al.* (2016) showed maximum zone of inhibition of 14.5±.0.5 mm diameter by methanol extract followed by the inhibition zone of 12.0±1.0 mm diameter by ethyl acetate extract of *Trigonella foenum-graceum* and *Serratia marcescens* against the fungus *Trichoderma viridae.*

Marcetic *et al.* (2013) reported that the root and leaves of *Eryngium foetidum* exerted a significant effect with a minimum inhibitory concentration ranging from 3.5 to 15.6µg/ml.

SUMMARY AND CONCLUSION

Chapter V

SUMMARY AND CONCLUSION

Plants are the storehouse of natural products which differ widely in their structures, biological properties and mechanism of action. Natural products have been a major source of new drugs (Vourelaa *et al.*, 2004). The phytochemical analysis result indicated the presence of alkaloids, flavanoids, phenolic compounds and glycosides in the plant extracts, these compounds might be responsible for antimicrobial activity against micro-organism (Gothandam *et al.*, 2010).

Antimicrobial properties of medicinal plant are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80%of the world population. There are about 45,000 plant species in India with capacity to produce large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity (Mahato *et al.*, 2005).

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials (Iwu *et al.*, 1999).

Physiochemical analysis

1. The moisture content was found to be higher in *Coriandrum sativum* than the leaves and root of *Eryngium foetidum.*
2. The maximum solubility tendency was found in chloroform leaf extracts of *Coriandrum sativum* and the least solubility percetage was observed in aqueous and chloroform extracts of *Eryngium foetidum.*

Phytochemical Analysis

The preliminary investigation of the leaf and root extract of *Coriandrum sativum* and *Eryngium foetidum* reported the presence of carbohydrate, anthroquinone, anthrocyanin, coumarins, phenol, phlobatannins, quinines, sterols, saponins and terpenoids in aqueous of leaf extract.

Quantitative Analysis

1. Among the aqueous and chloroform leaf extracts, a significantly highest amount of carbohydrates was found in aqueous of *Eryngium foetidum* and *Coriandrum sativum* root extracts.
2. A significantly higher amount of total protein content were recorded in the chloroform extracts of leaf and root of *Coriandrum sativum*..
3. Total flavanoid content was observed in aqueous extracts of *Coriandrum sativum* followed by chloroform leaf extract of E. foetidum
4. A significantly higher Total phenolic content was recorded in the aqueous leaf extracts of *Coriandrum sativum* when compare to *E. foetidum* extracts.
5. A higher amount of Total tannin content was observed in the aqueous leaf extracts of *Eryngium foetidum* followed by the aqueous root extracts of *Coriandrum sativum.*

Antimicrobial Activity

The antimicrobial activity was carried out using two strains of bacteria, (*Staphyllococcus aureus* and *E. coli*), and two fungus, (*Aspergillus flavus* and *Trichoderma harzianum*).

Antibacterial Activity

1. Among the two extracts, chloroform and aqueous leaf extract of *Coriandrum sativum* and *Eryngium foetidum*, higher zone of inhibition owas recorded in the aqueous leaf extract of *Eryngium foetidum* with the positive drug control, chloramphenicol against *Staphylococcus aureus.*
2. The highest zone of inhibition was recorded in the aqueous leaf extract of *Eryngium foeitidum* against Staphylococcus aureus and he least zone of inhibition was also seen in chloroform extract of *Eryngium foetidum* against *E. coli* by agar well diffusion method.

3. The higher antimicrobial activity with the inhibition zone was recorded in the chloroform extracts of both leaf and root against *Staphylococcus aureus* and the least antibacterial activity was found in *Eryngium foetidum* in aqueous leaf extract followed by chloroform root extract by disc diffusion method.

Antifungal Activity

1. A higher antifungal activity was registered in the chloroform leaf extract of *Coriandrum sativum* and least antifungal activity with the minimum zone of inhibition was recorded in the chloroform root extract of *Coriandrum sativum* and leaf extract of *Eryngium foetidum* against the fungal strain, *Aspergillus flavus* respectively by agar well diffusion method.
2. The highest inhibition zone was found in the root chloroform extract of *Coriandrum sativum,* followed by chloroform root extract of *Eryngium foetidum* and the least was recorded in the aqueous root extract of *Eryngium foetidum* against *Aspergillus flavus* by disc diffusion method.

Conclusion:

Eryngium foetidum L. and *Coriandrum sativum* L. are two commonly used herbs or spice with two fold purposes. Traditionally, they are used in culinary and nowadays, they are used as medicine to cure various diseases as it possesses antioxidant, antimicroibial, anticancer, anti-inflammator and anti-diabetic properties.

Thus, it can be inferred from the present finding that the presence of various phytochemical constituent like alkaloid, flavanoid, phenol, etc., present in the plant may be responsible for the various activities.

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