Research Article

Anti-microbial Activity of Hydro-alcoholic Extracts of Some Traditionally Important Medicinal Plants

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ABSTRACT

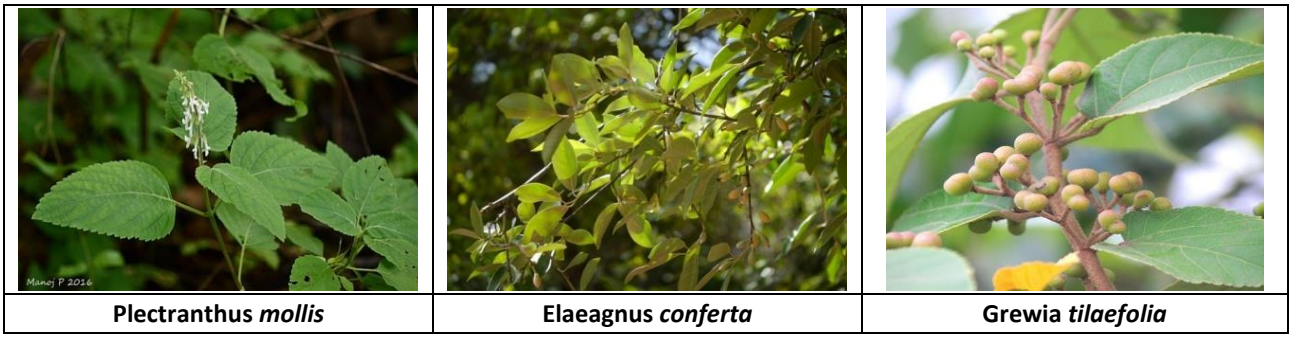
Plants are the rich natural source of bioactive compounds. The more diversified composition of the plants makes their role as biomedicine. These bioactive molecules are often lethal to both plants and animals. Based on ethnomedical use, the leaves *Plectranthus mollis*, *Elaeagnus conferta* and *Grewia tilaefolia* leaf extracts were extracted successively with organic solvents. These plants are reported to exhibit relaxant activity on smooth and skeletal muscles, and has cytotoxic and anti-tumour promoting activity, and can be used in the treatment of cancer. These crude extracts were screened for their toxic potential against three Gram- positive bacteria, five Gram- negative bacteria and two fungus by using disc diffusion method. The hydro alcoholic extracts of the plant possessed significant antimicrobial activities on both Gram- positive and Gram- negative bacteria. The hydro alcoholic of the plant exhibited prominent activities against all the tested bacteria. Hydro alcoholic extracts also showed considerable activity against fungus and bacteria. The leaves extracts of the plant were found more active against the microorganisms.

Keywords: *Elaeagnus umbellata*, extracts, fungi, yeast, antibiotic discs.

INTRODUCTION

Plectranthus is a large and widespread genus of Lamiaceae family with a diversity of ethnobotanical uses. In traditional medicine, the juice of stem and leaves of *Plectranthus hadiensis* which is mixed with honey is taken as a remedy for diarrhea. The aim of the present study is to determine the chemical composition of the essential oil from the seed of *P. hadiensis* and to evaluate antimicrobial efficacy of the oil. The essential oil of the seeds from *P. hadiensis* is obtained by hydro-distillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS). It results in the identification of 25 compounds representing 99.3%, of the total oil. The main compound is Piperitone oxide (33.33%). Antibacterial activity of the essential oil of *P. hadiensis* is tested against two Gram-positive and two Gram-negative bacteria, using zone of inhibition method. The essential oils inhibit the organisms and shows the zone of inhibition in the range of 20-35mm. The essential oil can serve as an antibacterial agent[1].

Essential oils are natural, complex volatile compound mixtures characterized by a strong odour. Essential oils are composed mainly of terpenoids, including monoterpenes and sesquiterpenes, their oxygenated derivatives and a variety of molecules such as aliphatic hydrocarbons, acids, alcohols, aldehydes, acyclic esters or lactones, and exceptionally nitrogen- and Sulphur containing compounds and coumarins. Known for their antimicrobial medicinal properties and their fragrance, they are invariably used in preservation of foods and as antimicrobial, analgesic, sedative, anti-inflammatory, spasmolytic and local anesthetic remedies[2-3]. The genus Plectranthus consists of 300 species, distributed from Africa to Asia and Australia[4].



Plectranthus *mollis*	Elaeagnus *conferta*	Grewia *tilaefolia*

Figure 1: Plant Sample images

In India, about 30 Plectranthus species are known[5], of which *P. amboinicus, P. vettiveroides, P. barbatus, P. mollis, P. coetsa*, and *P. incanus* are the most common species used in the traditional Indian Ayurvedic medicine since ancient times to cure many disorders and diseases[6,7]. Phytochemical studies of the genus reveals that Indian Plectranthus species are rich in essential oil. The essential oil is obtained only from very few species *P. amboinicus Lour., P. barbatus Andrews, P. fruticosus L He, P. incanus Link, P. japonicus Burm. F., P. melissoides Benth. P. rugosus* Wall and their composition have been reviewed and reported[8].

Plectranthus hadiensis is reportedly cultivated in Tamil Nadu on river banks and sandy loams. The root and stem of this plant has a quite distinct and specific aroma. The herb accepts as the source of Hribera (Iruveli) in Kerala is *Coleus zeylanicus* (Benth.) Cramer (syn. *Plectranthus zeylanicus* Benth). This species is reportedly an endemic taxon of Sri Lanka, where it is known by the Sinhalese name Iruveriya, the juice of stem and leaves of which is mixed with honey is taken as a remedy for diarrhea. This plant belongs to the Lamiaceae family and is used in Ayurveda. So far only one report on its phytochemical analysis[9] was available and that to no reports are available with respect to its essential oil. Recently in our laboratory we studied the essential oil composition from the aerial parts of *P. hadiensis*. This prompts us to carry out the present work to study the essential oil from the seeds of *P. hadiensis*.

Dental caries is associated with acidogenic and aciduric bacteria that adhere to the tooth surface as an oral biofilm (dental plaque)[10]. Because this pathology can destroy dental hard tissues[11-12], it has become a major public health concern worldwide. The most efficient way to prevent caries and periodontal diseases is to reduce and eliminate bacterial accumulation on the top of and between teeth by brushing the teeth on a daily basis and conducting periodic dental cleaning or prophylaxis. Unfortunately, most people fail to maintain a sufficient level of oral hygiene[13], which has called for the use of oral products containing antimicrobial ingredients as a complementary measure to diminish biofilm formation on the tooth surface[14].

Chlorhexidine has been the most effective antiplaque agent tested to date, but some reversible local side effects have led dentists to recommend its use for short periods only[15]. Several other antimicrobial agents including fluorides, phenol derivatives, ampicillin, erythromycin, penicillin, tetracycline, and vancomycin can inhibit bacterial growth[16]. Nevertheless, excessive use of these chemicals can disturb the oral and intestinal flora and cause microorganism susceptibility, vomiting, diarrhea, and tooth staining[17].

Plectranthus barbatus Andr. (Syn.*Coleus forskohlii* Briq.), that belongs to the family Lamiaceae. It is commonly known as Coleus, Pashanbhedi (Sanskrit), Patharchur (Hindi), Makandi beru or Mangani beru (Kannada) and is grown throughout the country. Its tuberous roots are found to be a rich source of forskohlin (coleonol) used as a potential drug for hypertension, congestive heart failure, eczema, colic, respiratory disorders, painful urination, insomnia, and convulsions[18-20]. Clinical studies of the plant and the forskolin constituent support these traditional uses, but also indicate it may have therapeutic benefit in asthma, angina, psoriasis, and prevention of cancer metastases[21].

The aim of the present study was to identify chemical composition of the essential oil of *P. mollis* and to evaluate antimicrobial efficacy of the oil. The essential oil of the flowering aerial parts of *P. mollis* was obtained by hydro-distillation and analyzed by gas chromatography equipped with a flame ionization detector (GC-FID) and gas chromatography coupled with mass spectrometry (GC/MS). Twentyseven compounds were identified, which comprised 98.6% of the total constituents. The main compound was identified as fenchone (32.3%), followed by α-humulene (17.3%), piperitenone oxide (8.5%), *cis*-piperitone oxide (6.0%) and *E*-β-farnesene (5.9%). The oil was found rich in oxygenated monoterpenes type constituents (52.0%), followed by sesquiterpene hydrocarbons (40.2%), oxygenated sesquiterpenes (4.9%), and monoterpene hydrocarbons (1.5%). Antimicrobial activity of the essential oil of *P. mollis* was tested against six Gram-positive and eight Gram-negative bacteria, and three fungi, by using the tube dilution method. The oil was active against the tested Gram-positive and Gram-negative bacteria, and fungi at a concentration range of 0.065±0.008- 0.937±0.139mg/mL, 0.468±0.069-3.333±0.527 mg/mL and 0.117±0.017-0.338±0.062mg/mL, respectively. The present study revealed that the oil constituents somehow were qualitatively similar and quantitatively different than earlier reports from different parts of the world. The essential oil of *P. mollis* has found to be antimicrobial activity which can be usefulness in the treatment of various infectious diseases caused by bacteria and fungi [22].

Biological screening is an important step in the evaluation of medicinal plants activity[23]. Thus, any phytochemical investigation of a given plant will reveal a spectrum of its bioactive chemical constituents. Natural products represent virtually inexhaustible reservoir of molecules, most of which are hardly explored and could constitute lead molecules for new antimalarial drugs, such as artemisinin, isolated from *Artemisia annua*[24]. Historically, pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics[25-26]. Even now, contrary to common belief, drugs from higher plants continue to occupy an important niche in modern medicine.

On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons[27]. In present time, multiple drug resistance in microbial pathogens become a serious health problem to humankind world-wide[28]. It is aroused due to indis-criminate and

repetitive use of antimicrobial drugs[29]. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. Due to the same reason, during the past decade, traditional systems of medicines have become increasingly important in view of their safety[30] and research is carried out in order to determine antimicrobial potential of medicinal plants. Bioassay has been used successfully to monitor the isolation of cytotoxic, antimalarial, insecticidal and antifeedent[31-34].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs[35]. A wide range of medicinal plant parts is used for extracts as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local use, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries[36].

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated[37]. Medicinal plants have been relied upon by 80% of the world population for their basic health care needs. Pakistan is no exception, as it has a variety of plants of medicinal importance[38]. The herbs are extensively used for treating diseases, however their commercial exploitation is limited due to the lack of scientific knowledge for their use.

Among these plants, *Elaeagnus umbellata* Thunb, also called cardinal olive, autumn olive or autumn Elaeagnus[39], a wild shrub belonging to the family Elaeagnaceae, is native to China, Japan and Korea, and is also found in Afghanistan and India[40]. The plant was introduced to the US in the 1830s from East Asia as an ornamental plant [39].

The fruit / berries are silvery with brown scales when immature and ripen to a speckled red in September – October[41]. Its berry is an excellent source of vitamins A, C, E, flavonoids, essential fatty acids[42], lycopene, carotene, lutein, phytofluene and phytoene. The lycopene content of the *E. umbellata* fruit is 17 times greater than that of tomato[43-44]. Many studies have proved that lycopene protects against myocardial infarction[43] and various forms of cancers including prostate cancer[45-46]. Various phytochemicals including palmitic acid (16.9%), eugenol (11.1%), methyl palmitate (10.5%), 4-methyl anisole (33-42.7%) and 4-methyl phenol (10.9-13.3%) have been isolated from the flowers of the plant [47].

The extracts of the plant and its chemical constituents exhibit antimicrobial properties, which may be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency[48]. Many plants have been used due to their antimicrobial traits, which are due to the compounds synthesized in the secondary metabolism processes, that is, phenolics and tannins. *E. umbellata* is one of such plants which are being used against infectious diseases. Although anti-bacterial activity of the aerial parts of the plant had been studied by[49] against four bacteria, a detailed antimicrobial potential of aerial and ground parts of *E. umbellata* has not been studied, the *in vitro* antimicrobial activity of the leaves and roots of the plant growing wild in Azad Jammu and Kashmir was evaluated by using disc diffusion method against eight bacteria, one fungus and one yeast. The present work appears to be the first detailed antimicrobial bioassay report on aerial as well as ground part of the plant.

MATERIALS AND METHODS

Collection of plant materials

Materials of Three plants (leaves) were harvested in March to May from Trichy and Perambalur region. They were carefully washed, oven-dried for 1 h at 120°C and put in the shade in an aerated place till complete drying, then were ground into a fine powder.

Preparation of plant extracts

The prepared powder was soaked in each of Hydroalcoholic extract solvents (plant material to solvent ratio was 1:10, w/v) and extracted for 24 h at room temperature with shaking at 150 rpm. Filtrates of the extracts were dried at 40°C. Then, samples were centrifuged (3000 rpm, 5 minutes) to eliminate the impurities and suspended solids. The supernatants were used as aqueous crude extract in this study.

Hydro-Alcoholic Extraction

The plants powders were prepared as explained in previous section. For hydro-alcoholic extraction, the powders were placed in flasks individually. Water and alcohol at a ratio of 50:50 in specified deal were added to the flasks and kept 24 hours in dark. Samples were poured in rotary evaporator to remove as much as possible extra water and alcohol. Then, the concentrated samples were centrifuged (3000 rpm, 5 minutes) and supernatants were used as hydro-alcoholic crude extract in this study.

Antimicrobial procedure

Screening of antibacterial activity

The bacterial strains were used throughout investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India. The young bacterial broth cultures were prepared before the screening procedure

Preparation of inoculums

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures of experiment were prepared by transferring a loopful of cells from the stock cultures to test tube of Muller-Hinton broth (MHB) for bacteria that were incubated without agitation for 24 hrs at 37°C and 25°C respectively. The cultures were diluted with fresh Muller-

Hinton broth to achieve optical densities corresponding to 2.0 X 10^6 colony forming units (CFU/ml) for bacteria.

Antimicrobial susceptibility test

The disc diffusion method (Bauer *et al.,* 1966) was used to screen the antimicrobial activity. *Invitro* antimicrobial activity was screened by using Muller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1% inoculums suspension was swabbed uniformly and the inoculums were allowed to dry for 5 minutes. The concentration of extracts is 40 mg/disc was loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the extract was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter.

Screening of antifungal activity

Fungi tested

The fungal strains were used throughout investigation. All the fungal cultures were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology and Chandigarh, India. The young fungal broth cultures were prepared before the screening procedure.

a) Culture Media

The media used for antifungal test was Sabouraud's dextrose agar/broth of Hi media Pvt. Bombay, India.

b) Preparation of Inoculums

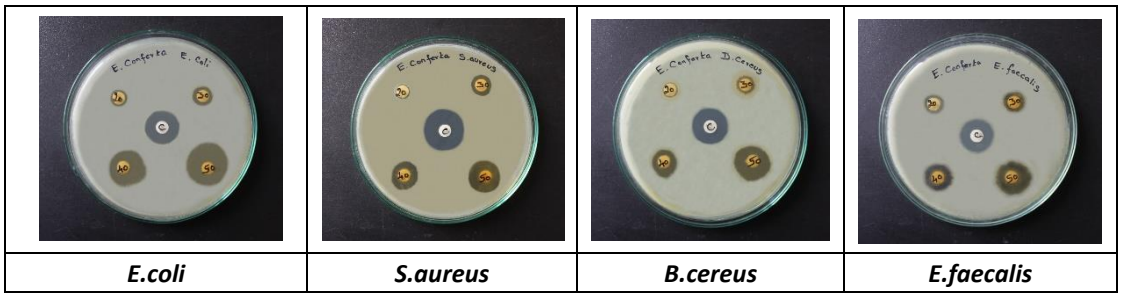
The fungal strains were inoculated separately in Sabouraud's dextrose broth for 6 hrs and the suspensions were checked to provide approximately 10^5 CFU/ml.

c) Determination of antifungal activity

The agar well diffusion method (Perez, 1993) was modified. Sabouraud's dextrose agar (SDA) was used for fungal cultures. The culture medium was inoculated with the fungal strains separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks (methanol, ethyl acetate and hexane). Standard antibiotic (Fucanazole, concentration 1 mg/ml) was used as positive control and fungal plates were incubated at 37°C for 72 hrs. The diameters of zone of inhibition observed were measured.

RESULTS AND DISCUSSION

Antibacterial Activity - *Elaeagnus conferta (E.conferta)*



E.coli	*S.aureus*	*B.cereus*	*E.faecalis*

Figure 2: Anti-bacterial activity for Hydroalcoholic extract of *E.conferta* plants by disc diffusion method

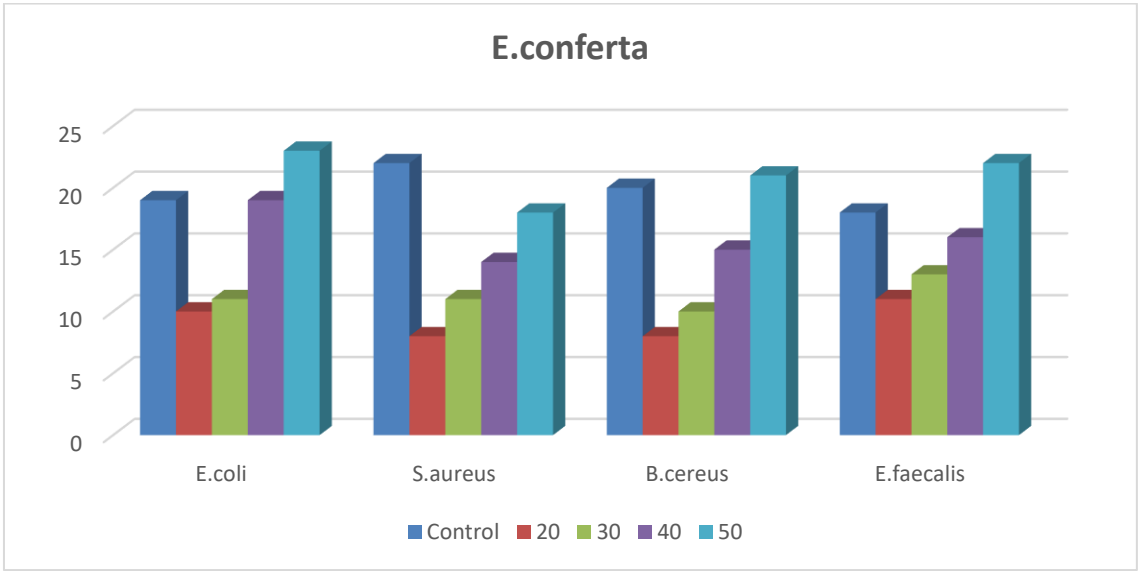
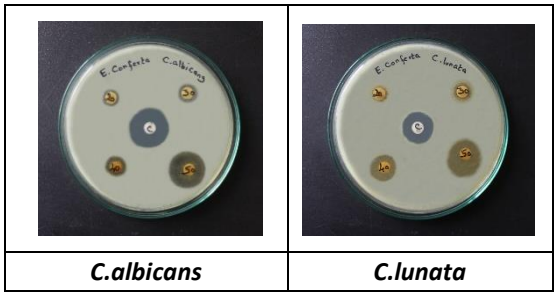


Figure 3: Graphical representation of Anti-bacterial activity for Hydroalcoholic extract of *E.conferta* plants

Table 1: Anti-bacterial activity for Hydroalcoholic extract of *E.conferta* plants

Organisms	Control	20	30	40	50
E.coli	19	10	11	19	23
S.aureus	22	08	11	14	18
B.cereus	20	08	10	15	21
E.faecalis	18	11	13	16	22

Antifungal Activity - *Elaeagnus conferta (E.conferta)*



C.albicans ***C.lunata***

Figure 4: Anti-fungal activity for Hydroalcoholic extract of *E.conferta* plants by disc diffusion method

In the present study, Hydroalcoholic extract of *E.conferta* leaves exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table 1,2 and Figure 2-5, that the Hydroalcoholic extract of leaves possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 10 mm, 08 mm, 08 mm and 11 mm, for 30 mg/ml as 11 mm,11 mm , 10 mm and 13 mm, for 40 mg/ml showing 19 mm, 14 mm, 15 mm and 16 mm, for 50 mg/ml as 23 mm, 16 mm, 21 mm and 22 mm, for Hydroalcoholic extract of Leaves against E. coli, *S.aureus*, B.cereus and E. faecalis, respectively when compared with standard drug of chloromphenicol showing 19 mm, 22 mm, 20 mm and 18 mm zone of inhibition respectively. It is evident from the data presented in Table 1,2 and Figure 2-4, that the Hydroalcoholic extract of leaves possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 09 mm and 09 mm, for 30 mg/ml as 11 mm and 12 mm, for 40 mg/ml as 13 mm and 15 mm, for 50 mg/ml as 21 mm and 20 mm for Hydroalcoholic extract of leaves against C.albicans and C.lunata respectively when compared with standard drug Fucanazole showing 23mm and 19 mm of inhibition respectively, for Elaeagnus confertaleaf extracts. The result indicates that all the test extracts show good inhibitory activity against all these bacterial and fungal strains.

Table: 2 Anti-fungal activity for Hydroalcoholic extract of *E.conferta* plants

S.No	Organisms	Control	20	30	40	50
1	*C.albicans*	23	09	11	13	21
2	*C.lunata*	19	09	12	15	20

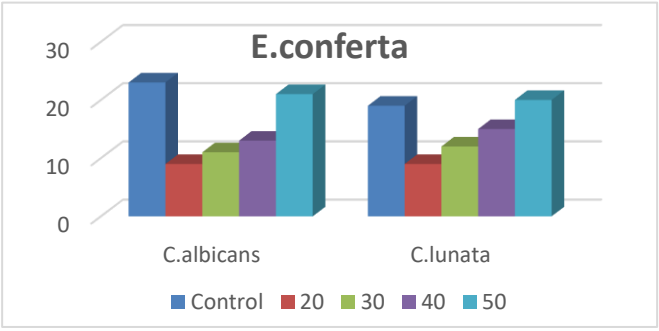
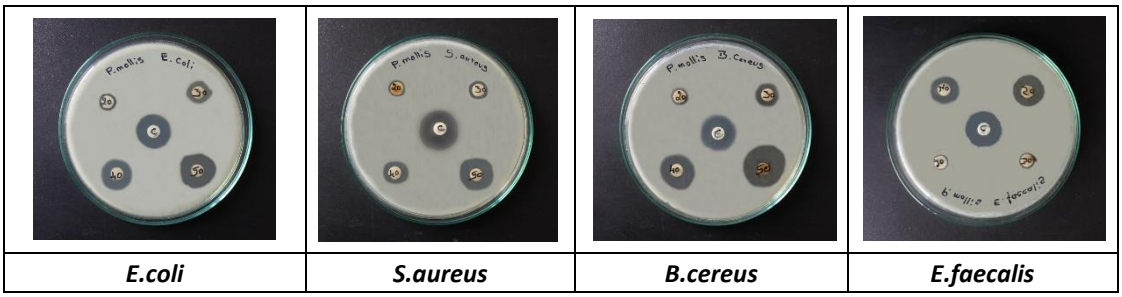


Figure 5: Graphical representation of Anti-fungal activity for Hydroalcoholic extract of *E.conferta* plants

Antibacterial Activity - *Plectranthus mollis (P.mollis)*



E.coli ***S.aureus*** ***B.cereus*** ***E.faecalis***

Figure 6: Anti-bacterial activity for Hydroalcoholic extract of *P.mollis* plants by disc diffusion method

Table 3: Anti-bacterial activity for Hydroalcoholic extract of *P.mollis*plants

S.No	Organisms	Control	20	30	40	50
1	*E.coli*	20	10	15	19	21
2	*S.aureus*	23	09	12	15	20
3	*B.cereus*	21	11	14	20	24
4	*E.faecalis*	21	09	10	17	19

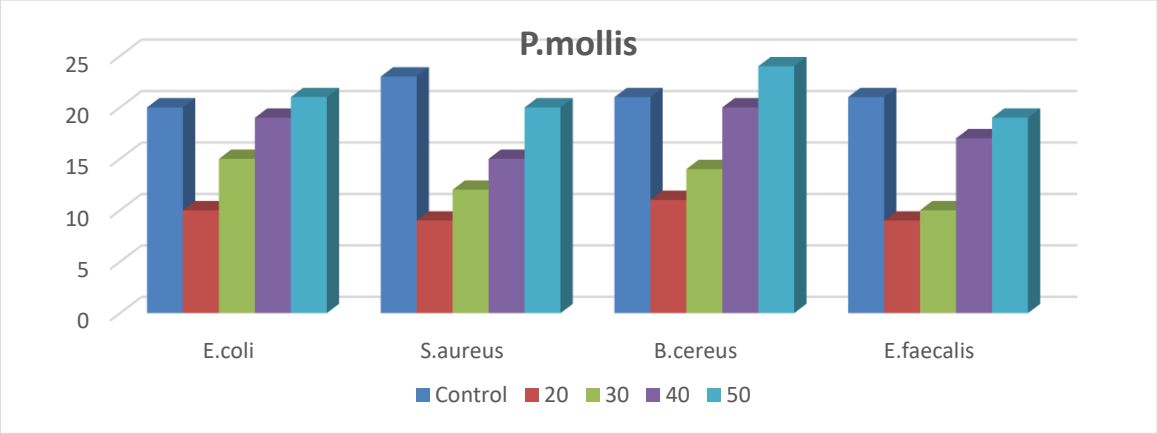
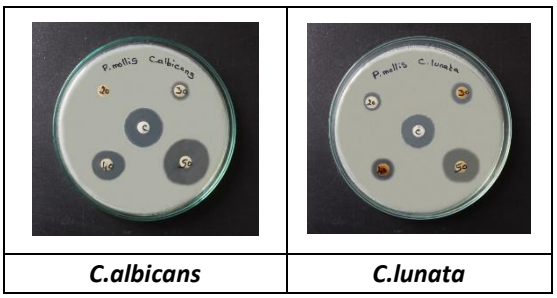


Figure 7: Graphical representation of Anti-bacterial activity for Hydroalcoholic extract of *P.mollis*plants

Antifungal Activity - Plectranthus *mollis (P.mollis)*



C.albicans	***C.lunata***

Figure 8: Anti-fungal activity for Hydroalcoholic extract of *P.mollis* plants by disc diffusion method

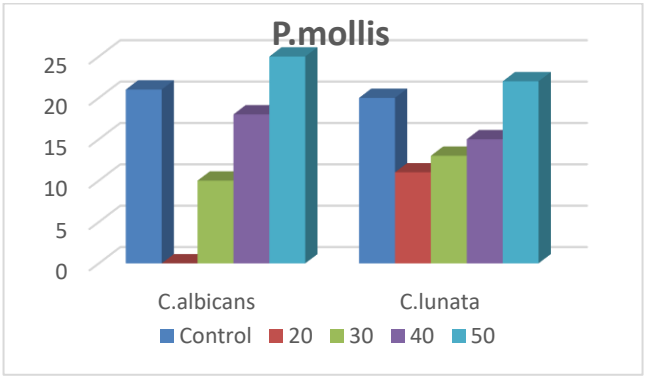


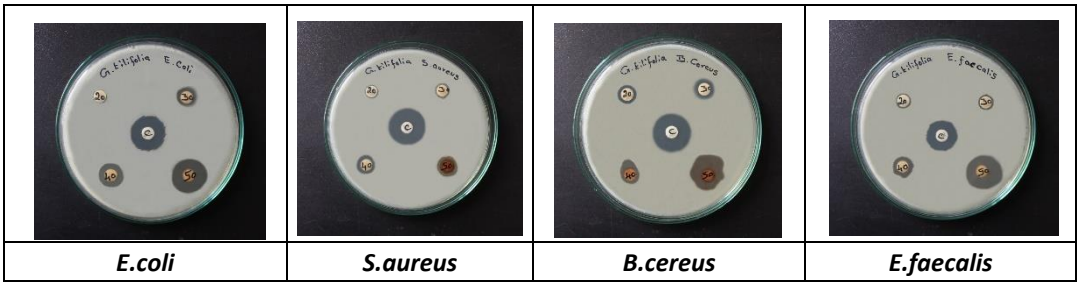
Figure 9: Graphical representation of Anti-fungal activity for Hydroalcoholic extract of *P.mollis* plants

In the present study, Hydroalcoholic extract of *P.mollis* leaves exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table 3,4 and Figure 6-9, that the Hydroalcoholic extract of leaves possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 10 mm, 09 mm, 11 mm and 09 mm, for 30 mg/ml as 15 mm,12 mm , 14 mm and 10 mm, for 40 mg/ml showing 19 mm, 15 mm , 20 mm and 17 mm, for 50 mg/ml as 21 mm, 20 mm , 24 mm and 19 mm, for Hydroalcoholic extract of leaves against *E. coli, S.aureus, B.cereus* and *E. faecalis*, respectively when compared with standard drug of chloromphenicol showing 20 mm, 23 mm, 21 mm and 21 mm zone of inhibition respectively. It is evident from the data presented in Table 1,2 and Figure 2-4, that the Hydroalcoholic extract of leaves possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 00 mm and 11 mm, for 30 mg/ml as 10 mm and 13 mm, for 40 mg/ml as 18 mm and 15 mm, for 50 mg/ml as 25 mm and 22 mm for Hydroalcoholic extract of leaves against *C.albicans* and *C.lunata* respectively, When compared with standard drug Fucanazole showing 21mm and 20 mm of inhibition respectively, for *Plectranthus mollis,* leaf extracts. The result indicates that all the test extracts show good inhibitory activity against all these bacterial and fungal strains.

Table 4: Anti-fungal activity for Hydroalcoholic extract of *P.mollis* plants

Organisms	Control	20	30	40	50
C.albicans	21	0	10	18	25
C.lunata	20	11	13	15	22

Antibacterial Activity - *Grewia tilaefolia (G.tilifolia)*



E.coli	***S.aureus***	***B.cereus***	***E.faecalis***

Figure 10: Anti-bacterial activity for Hydroalcoholic extract of *G.tilifolia* plants by disc diffusion method

Table 5: Anti-bacterial activity for Hydroalcoholic extract of *G.tilifolia* plants

S.No	Organisms	Control	20	30	40	50
1	*E.coli*	19	08	12	15	20
2	*S.aureus*	22	09	10	11	13
3	*B.cereus*	23	10	11	13	21
4	*E.faecalis*	19	09	10	12	21

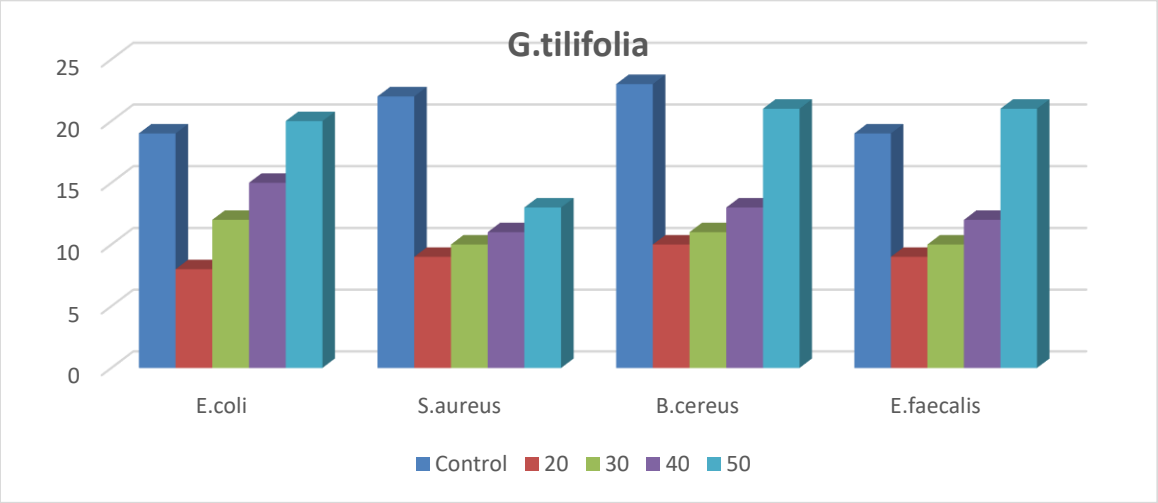
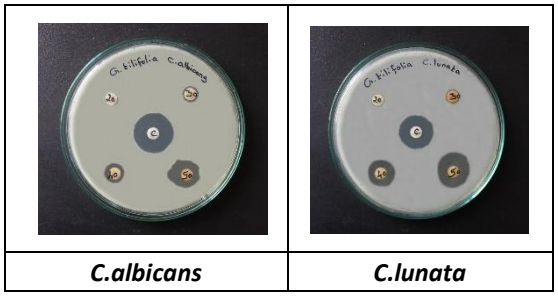


Figure 11: Graphical representation of Anti-bacterial activity for Hydroalcoholic extract of *G.tilifolia* plants

Antifungal Activity

Grewia *tilaefolia (G.tilifolia)*



C.albicans	***C.lunata***

Figure 12: Anti-fungal activity for Hydroalcoholic extract of *G.tilifolia* plants by disc diffusion method

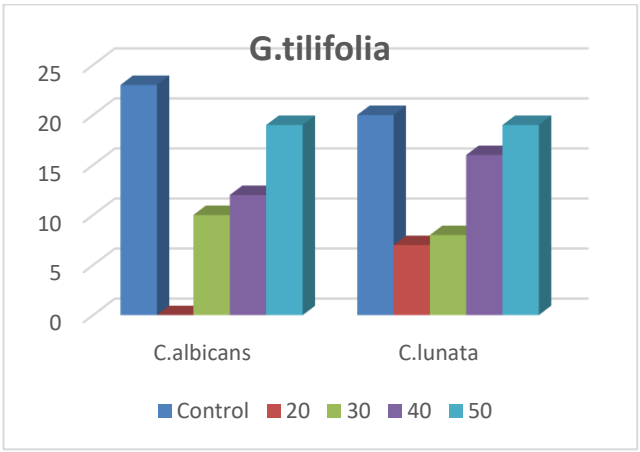


Figure 13: Graphical representation of Anti-fungal activity for Hydroalcoholic extract of *G.tilifolia* plants

Table 6: Anti-fungal activity for Hydroalcoholic extract of *G.tilifolia* plants

S.No	Organisms	Control	20	30	40	50
1	*C.albicans*	23	0	10	12	19
2	*C.lunata*	20	07	08	16	19

In the present study, **Hydroalcoholic extract of *G.tilifolia* leaves** exhibited significant antimicrobial activity when compared with standard drug. It is evident from the data presented in Table 5,6 and Figure 10-13, that the **Hydroalcoholic** extract of **leaves** possesses antibacterial activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 08 mm, 09 mm , 10 mm and 09 mm, for 30 mg/ml as 12 mm,10 mm , 11 mm and 10 mm, for 40 mg/ml showing 15 mm, 11 mm , 13 mm and 12 mm, for 50 mg/ml as 20 mm, 13 mm , 21 mm and 21 mm, for **Hydroalcoholic extract** of leaves against E. coli, *S.aureus*, B.cereus and E. faecalis, respectively when compared with standard drug of chloromphenicol showing 19 mm, 22 mm , 23 mm and 19 mm zone of inhibition respectively. It is evident from the data presented in Table 1,2 and Figure 2-4, that the **Hydroalcoholic extract** of leaves possesses antifungal activity. The disc diffusion method result showed the zone of inhibition for 20 mg/ml as 00 mm and 07 mm, for 30 mg/ml as 10 mm and 08 mm, for 40 mg/ml as 12 mm and 16 mm, for 50 mg/ml as 19 mm and 19 mm for **Hydroalcoholic** extract of leaves against C.albicans and C.lunata respectively, When compared with standard drug Fucanazole showing 21mm and 20 mm of inhibition respectively, for ***Grewia tilaefolia*** leaf extracts. The result

indicates that all the test extracts show good inhibitory activity against all these bacterial and fungal strains.

The observed percentage of inhibition indicated that the antimicrobial activities are dose dependent one and independent of the length of the time. In lower concentrations gram-negative group has been inhibited to a lesser extent as compared to the gram-positive group. This may be attributed to that cell-wall thickness of the bacteria. The cell wall of the Gram-positive organisms in general is thin compared to the cell wall of the gram-negative bacteria. It is a general phenomenon observed among most of the antibiotics. The drugs employed manifest their activity in one of the several ways.

The drugs would have interacted with the cell wall materials causing its lysis. The integrity of the cytoplasmic membrane might have got damaged causing death of the cell. Alteration of protein and nucleic acid molecule is another possibility. Apart from these things enzyme action, which is the potential target, can be modified with drugs causing a serious repair in the cell.

- In general, all the plants have better inhibition activity when compared with standard chloramphenicol.
- Among all, For *E.coli*, the plant leaves extracts of *E.conferta* leaves, are showing good inhibition against bacteria. *S.aureus*, the plant leaves extracts of *P.mollis* leaves are showing good inhibition against bacteria. For *B.cereus*, the plant leaves extracts of *P.mollis* leaves are showing good inhibition against bacteria. For *E.faecalis*, the plant leaves extracts of *E.conferta* leaves are showing good inhibition against bacteria.
- They possess very good anti-fungal activity when compared with standard fluconazole.
- Among all, For *C.albicans*, the plant leaves extracts of *P.mollis* leaves, are showing good inhibition against fungal. For *C.lunata*, the plant leaves extracts of *P.mollis* leaves, are showing good inhibition against fungal.
- Specifically all the compounds have a good anti-fungal activity against the fungal infection caused by *C.albicans*.
- On the whole, all the samples act as anti-fungal, especially *E.conferta* and *P.mollis* have good inhibition against Bacteria and fungal infections.

This study shows the presence of different phytochemicals with the biological activity that can be of the valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components such as saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, gum, mucilage, proteins and aminoacids. From the above results, the activity of hydroalcoholic extracts of *plant extracts* shows significant antibacterial and antifungal activity. Results shows that, *E.conferta* and *P.mollis* plant extracts are shown to possess antimicrobial activities against a number of microorganisms.

CONCLUSION

Antimicrobial resistance is a global problem. Emergence of multidrug resistance has limited the therapeutic options. Hence, monitoring resistance is of paramount importance. Hence, this study was aimed to focus the antimicrobial properties. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic anti-microbials. The extracts of the leaves of *E.conferta* and *P.mollis* plant extracts showed excellent antimicrobial activity against the tested bacteria and fungus. Therefore, it is concluded that the extract of the leaves of the plant can be regarded as good natural antibiotics with considerable degree of antimicrobial activity and that they can be used in the treatment of various infectious diseases caused by resistant microorganisms.

The results also revealed that the use of leaves of *E.conferta* and *P.mollis* are may be more beneficial than the aerial parts against infectious diseases. Further investigations are showing large inhibitory activity against various microorganisms as well as other pharmacological or toxicological properties aimed. Moreover, various parts of the plant may be used to treat various ailments as reported in the literature. Cultivation of this plant on commercial basis may also be employed so as to further increase the availability and to reduce the cost. Such usage may be more effective due to synergetic effect of various components rather than stand-alone use of a pure compound. However, further studies are needed to better evaluate the potential effectiveness of the crude extracts as the antimicrobial agents.

REFERENCES

1. Raju Sripathi, Subban Ravi, Chemical Composition and Antibacterial Activity of the Essential Oil from the Seeds of *Plectranthus hadiensis*, International Journal of Pharmacognosy and Phytochemical Research, 9(5), 2017, 637-639
2. Burt S. Essential oils: their antibacterial properties and potential applications in foods a review. Journal of Food Microbiology, 94, 2004, 223-253.
3. K. A. Hammer KA, Carson CF, Riley TV. Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 86,1999, 985-990.
4. L. E. Codd, in _Flora of Southern Africa_, Balogh Scientific Books, 28, 1985, 37.
5. P. Weyerstahl, V. K. Kaul, N. Meier, M. Weirauch, H. Marschall, Volatile Constituents of Plectranthus rugosus Leaf Oil, Planta Med. 48, 1983, 99.
6. H. P. Ammon, F. H. Kemper, Ayurveda: 3000 years of Indian traditional medicine, Med. Welt, 33, 1982,148.
7. N. J. De Souza, V. Shah, Forskolin-an adenylate cyclase activating drug from an Indian herb. Econ. Med. Plant Res. 2, 1988, 18-25.
8. Shobha Waldia, Bipin C. Joshi, Uma Pathak, and Mukesh C. Joshi, The Genus Plectranthus in India and Its Chemistry, Chemistry and Biodiversity, 8, 2011, 244-252.

9. R. Mehrotra, R. A. Vishwakarma, R. S. Thakur, Abietane diterpenoids from Coleus Zeylanicus, Phytochemistry, 28, 1989, 3135.
10. G. P. Aguiar, C. E. Carvalho, H. J. Dias et al., "Antimicrobial activity of selected essential oils against cariogenic bacteria," *Natural Product Research*, 27(18), 2013, 1668–1672.
11. B. R. da Silva, V. A. A. de Freitas, V. A. Carneiro et al., "Antimicrobial activity of the synthetic peptide Lys-a1 against oral streptococci," *Peptides*, 42(4), 2013,78–83.
12. B. Kouidhi, T. Zmantar, and A. Bakhrouf, "Anticariogenic and cytotoxic activity of clove essential oil (*Eugenia caryophyllata*) against a large number of oral pathogens," *Annals of Microbiology*, 60(4), 2010, 599–604.
13. W. G.Wade, "Characterisation of the human oral microbiome," *Journal of Oral Biosciences*, 55(3), 2013, 148.
14. J. J. Jardim, L. S. Alves, and M. Maltz, "The history and global market of oral home-care products," *Brazilian Oral Research*, vol. 23, supplement 1, 2009, 17–22.
15. A. Furiga, A. Lonvaud-Funel, G. Dorignac, and C. Badet, "*In vitro* anti-bacterial and anti-adherence effects of natural polyphenolic compounds on oral bacteria," *Journal ofAppliedMicrobiology*, 105(5), 2008, 1470–1476.
16. K.W. Albertsson, A. Persson, and J.W. V. van Dijken, "Effect of essential oils containing and alcohol-free chlorhexidine mouthrinses on cariogenic micro-organisms in human saliva," *Acta Odontologica Scandinavica*, 71 (3), 2013, 883–891.
17. S. E.Moon,H.Y.Kim, and J. D. Cha, "Synergistic effect between clove oil and its major compounds and antibiotics against oral bacteria," *Archives of Oral Biology*, 56(9), 2011, 907–916.
18. Adnot S, Desmier M, Ferry N, Hanoune J and Sevenet T. Forskolin- a powerful inhibitor of human platelet aggregation. Biochem. Pharmacol. 31, 1982, 4071-4074.
19. Agarwal KC and Parks RE. Forskolin: a potential antimetastatic agent. Int. J. Cancer. 32, 1983, 801-804.
20. Ammon HP and Kemper FH. Ayurveda: 3000 years of Indian traditional medicine. Med. Welt. 1982,33,148-153.
21. Ammon HP and Muller AB. Forskolin: from an Ayurvedic remedy to a modern agent. Planta. Med. 6, 1985, 473-477.
22. Rajesh K. Joshi, Chemical composition and antimicrobial activity of the essential oil of *lectranthus mollis* (Lamiaceae) from Western Ghats region, Karnataka, India, Rev. Biol. 62(2), 2015, 423-431.
23. Nisar M, Kaleem WA, Qayum M, Hussain A, Zia-Ul-Haq M, Ali I, Choudhary MI. Biological screening of *Zizyphus oxyphylla* Edgew stem. Pak. J. Bot, 43(1), 2011, 311- 317.
24. Kayser O, Kiderlen AF, Croft SL. Natural products as anti-parasitic drugs. Parasitol. Res. 2003, 90, 55-S62.
25. Gerhartz W, Yamamota YS, Campbell FT, Pfefferkorn R, Rounsaville JF. Ullmann's Encyclopedia of Industrial, 1985.
26. Kroschwitz, JI, Howe-Grant M. Kirk-Othmer encyclopedia of chemical Technology. 2, 1992, 893.
27. Newman DJ, Cragg GM, Snader KM. The influence of natural products upon drug discovery. Nat. Prod. Res. 17, 2000, 15-234.
28. Peng Y, Rakowskim SA, Filutowiez M . Small deletion variants of the replication protein Pi and their potential for over- replication-based antimicrobial activity. FEBS Microbiol Lett. 261(2), 2006, 245-252
29. Shariff ZU. Modern Herbal Therapy for Common Ailments. Nature Pharmacy, 1, 2001, 9684.
30. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju G .Biological screening of medicinal plants collected from Eastern Ghats of India using *Artemia salina* (brine shrimp test). Int. J. Appl. Sci. Eng. 4, 2006, 115-125.
31. Siqueira MJ, Bomm D M, Pereira, NF G, Gareez W S, Boaventura MA D. Estudo fitoquimico de Unonopsis lindmanii- Annonaceae, biomonitorado peloensaio de toxicidade sobre Artemia salina LEACH. *Quimica Nova*. 21,1998, 557-559.
32. Perez H, Diaz F, Medina JD. Chemical investigation and in vitro antimalarial activity of *Tabebuia ochracea* ssp. neochrysantha. Int. J. Pharmacog. 35, 1997, 227-231.
33. Oberlies N H, Rogers LL, Martin JM, McLaughlin JL. Cytotoxic and insecticidal constituents of the unripe fruit of *Persea Americana* J. Nat. Products. 61, 1998, 781-785
34. Labbe C, Castillo M, Connoly JD. Mono and sesquiterpenoids from *Satureja gilliesii.* Phytochemistry. 34, 1993, 441-444.
35. Srivastava J, Lambert J, Vietmeyer N. Medicinal plants, an expanding role in development. World Bank Technical Paper. No. 1996, 320.
36. Uniyal SK, Singh KN, Jamwal P, Lal B. Traditional use of medicinal plants among the tribal communities of Chhota Bhangal, Western Himalayan. J. Ethnobiol. Ethnomed. 2,2006, 1-14.
37. Balandrin MF, Klocke JA, Wurtele ES, Bollinger WH . Natural plant chemicals: Sources of Industrial and Medicinal Materials. Science. 228, 1985, 1154-1160.
38. Tareen RB, Mohammad K, Zaidi MI. Plant communities, species diversity, medicinal plants and soil water relationship of the watercourses of Shireen valley Juniper ecosystem Ziarat, Balochistan. Res. J. University of Balochistan. 1,2002, 41-49.
39. Dirr MA. Manual of Woody Landscape Plants, Stipes Publ. Co., Champaign, IL, US, 1983.
40. Potter TL . Floral volatiles of *Elaeagnus umbellata* Thunb. J. Essent. Oil Res. 7(4), 1995, 347-354.
41. Sternberg G. *Elaeagnus umbellata* in Illinois conservation practice. Prelim Report 111. Dept. of Conservation,Verginia: 1982,251-178.
42. Chopra RN, Nayar SL, Chopra LC . Glossary of Indian medicinal plants. New Delhi; Council of Scientific and Industrial Research. 1986, 238-240.
43. Kohlmeier L, Kark JD, Gomez GE, Martin BC, Steck SE. Lycopene and myocardial Infarction risk in the EURAMIC study. Am. J. Epidemiol. 146, 1997, 618-626.
44. Fordham IM, Clevidence BA, Wiley ER, Zimmerman RH. Fruit of autumn olive, a rich source of lycopene. Hort. Sci. 36, 2001, 1136-1137.
45. Clinton SK. Lycopene: chemistry, biology, and implications for human health and disease. Nutr. Rev. 56, 1998, 35-51.
46. Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC. Intake of carotenoids and retinol in relation to risk of prostate cancer. J. Natl. Cancer Inst. 87, 1995, 1767-1776.
47. Matthews V. Chemical composition of Elaeagnus umbellata. The new plantsman. London Royal Horticultural Society. 1994, 1352-4186.
48. Nabeela A, Zaheer-ud-din K. Effect of host species on antimicrobial activity of the ethanolic extracts of *Cuscuta reflexa Roxb*. Mycopath. 1, 2003, 99-104.
49. Sabir SM, Dilnawaz S, Imtiaz A, Hussain M, Kaleem MT. Antibacterial activity of *Elaeagnus umbellata* (*Thunb.*) a medicinal plant from Pakistan. Saudi Med J. 28(2), 2007, 259- 263.

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