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## Antimicrobial activities of the leaves and roots of *Elaeagnus umbellata* Thunb

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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 and root of *Elaeagnus umbellata* (Elaeagnaceae) were extracted successively with various organic solvents and water. These crude extracts were screened for their toxic potential against three Gram- positive bacteria, five Gram- negative bacteria, one yeast, and one fungus by using disc diffusion method. The acetone, petroleum ether, ethyl acetate, chloroform, ethanol and methanol extracts of the plant possessed significant antimicrobial activities on both Gram- positive and Gram- negative bacteria. The acetone, petroleum ether, ethyl acetate and methanol extracts of leaves and roots of the plant exhibited prominent activities while chloroform, ethanol extracts showed moderate activity and water extract showed no activity against all the tested bacteria. Ethanolic and methanolic extracts also showed considerable activity against fungus and yeast. The root extracts of the plant were found more active against the microorganisms.

**Key words:** *Elaeagnus umbellata*, extracts, fungi, yeast, antibiotic discs.

### INTRODUCTION

Biological screening is an important step in the evaluation of medicinal plants activity (Nisar et al., 2011; Qayum et al., 2012). 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* (Kayser et al., 2003). 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 (Gerhartz et al., 1985; Kroschwitz, and Howe-Grant, 1992). 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 (Newman et al., 2000). In present time, multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng et al., 2006). It is aroused due to indiscriminate and repetitive use of antimicrobial drugs (Shariff, 2001). 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 (Krishnaraju et al., 2006) and research

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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 (Siqueira et al., 1998; Perez et al., 1997; Oberlies et al., 1998; Labbe et al., 1993).

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 (Srivastava et al., 1996). 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 (Uniyal et al., 2006).

Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin et al., 1985). 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 (Tareen et al., 2002). The herbs are extensively used for treating diseases, however their commercial exploitation is limited due to the lack of scientific knowledge for their use (Ahmad et al., 2003).

Among these plants, *Elaeagnus umbellata* Thunb, also called cardinal olive, autumn olive or autumn Elaeagnus (Dirr, 1983), a wild shrub belonging to the family Elaeagnaceae, is native to China, Japan and Korea, and is also found in Afghanistan and India (Potter, 1995). The plant was introduced to the US in the 1830s from East Asia as an ornamental plant (Dirr, 1983).

*E. umbellata* is widely distributed at a height of 5000-7500 feet above sea level in Muzaffarabad Azad Kashmir. It is abundantly found in Himalayan regions of Pakistan (Hensley, 1984; Ahmad et al., 2005). The *E. umbellata* is a large spreading, spiny-branched shrub often obtaining 3.5-5.5 m in height, and 3.5-5.5 mm in width. The foliage is light green on top and a silvery green on the bottom. Leaves are alternate and petiolated in small lateral clusters on twigs (Eckardt, 1987).

The fruit / berries are silvery with brown scales when immature and ripen to a speckled red in September - October (Sternberg, 1982). Its berry is an excellent source of vitamins A, C, E, flavonoids, essential fatty acids (Chopra et al., 1986), lycopene, carotene, lutein, phytofluene and phytoene. The lycopene content of the *E. umbellata* fruit is 17 times greater than that of tomato (Kohlmeier et al., 1997; Fordham et al., 2001). Many studies have proved that lycopene protects against myocardial infarction (Kohlmeier et al., 1997) and various forms of cancers including prostate cancer (Clinton, 1998; Giovannucci et al., 1995). The seeds of the plant are used as a stimulant in the treatment of coughs and seed oil is used in the treatment of pulmonary affections

(Chopra et al., 1986). 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 (Matthews, 1994).

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 (Nabeela and Zaheer, 2003).

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 antibacterial activity of the aerial parts of the plant had been studied by Sabir et al. (2007) 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

Fresh plant parts were collected randomly from different localities of Muzaffarabad, Azad Jammu and Kashmir, Pakistan. The plant was identified by a Senior Botanist at the Department of Botany, where a voucher specimen (No. Bot. UAJK 1021) is deposited. The collected plant parts were separately air dried under shade and then homogenized to fine powder and finally stored in air tight bottles at 4°C.

### Aqueous extracts

Forty-five gram (45 g) of each ground plant part material was extracted with distilled water in soxhlet extraction apparatus (Thomas, 1977). These extracts were collected separately and each extract was dried on rotary evaporator under reduced pressure. The last traces of the water were evaporated at water bath, which was used as a source of heat (Rawlins and Tindall, 1977).

### Organic solvent extraction

A portion (25 g) of each dried powdered plant material was soaked separately in 250 ml petroleum ether, acetone, ethyl acetate, chloroform, ethanol and methanol for ten days at room temperature (25±2°C). The solvents extracted material was filtered in flasks (Rawlins and Tindall, 1977). The extracts were then filtered through Whatman filter paper No.1. All organic extracts were dried on a rotary evaporator under reduced pressure, weighed and stored at 4°C till further analysis. .

### Preparation of dilution

The dried aqueous, methanol, ethanol, petroleum ether, acetone,

ethyl acetate and chloroform extracts were then dissolved in their respective solvents in a proportion of 10 mg/ml. The concentration of reference antibiotics, that is, ciprofloxacin was 100 µg/ml and nystatin 1500 u/ml. *aureus*, *Bacillus subtilis*, *Enterococcus faecium*, five Gram-negative bacteria, *Escherichia coli*, *Bordetella bronchiseptica*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Pseudomonas syringae* (local isolate), one yeast *Saccharomyces cerevisiae* (local isolate) and one fungus *Aspergillus flavus* (local isolate), were used to check the antimicrobial potential of different extracts of the selected plant parts.

The pure bacterial, fungal and yeast strains were obtained from the Department of Pathology Muzaffarabad Medical College Teaching Hospital, Muzaffarabad Azad Jammu and Kashmir. Bacterial strains were cultured overnight at 37°C in nutrient agar (NA, Oxoid, Hampshire, UK) while fungal strains were cultured overnight at 28°C using sabouraud's dextrose agar (SDA, Oxoid, Hampshire, UK).

### Antimicrobial assay

The antimicrobial activity was determined by disc diffusion method (Vander and Vlietnck, 1991). Briefly, 100 µl of suspension of tested microorganisms, containing 10<sup>8</sup> colony-forming units (cfu)/ml of bacteria cells and 10<sup>5</sup> spores/ml of fungi was spread on sterilized nutrient agar (NA) and SDA medium, respectively. The disc (6 mm in diameter) was individually impregnated with extract samples, placed on the agar plates which had previously been inoculated with the tested microorganisms. A disc without compound was used as a negative control. In the second series of experiment, antibiotic discs prepared from the dilution of commercially available standard reference antibiotics, that is, ciprofloxacin and nystatin were placed on top of the medium in the center of Petri dishes following the disc diffusion method (Vander and Vlietnck, 1991).

The purpose of this experimental set was to compare the antimicrobial activity of the standard reference antibiotics with that of the solvent extracts of leaves and roots of *E. umbellata*. Plates, after 2 h at 4°C, were incubated at 37°C for 24 h for bacteria and at 28°C for 72 h for fungal strains in incubator (Synou, Germany). Antimicrobial activity was evaluated by measuring the diameter of the growth inhibition zones by zone reader (MAS GmbH, Germany) in millimeters for the organisms and comparing to the controls (Rehman et al., 2001).

### Statistical analysis

All values were expressed as means ± standard error. The data for each microorganism were analyzed by using one way analysis of variance (ANOVA) technique and means were compared by using least significant difference (LSD) at 5% (0.05) probability level (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

Plant extracts are being studied against bacteria for years and in a more intensified way in the last three decades. During this period, a lot of antimicrobial screening evaluations has been published based on the traditional use of Chinese, African and Asian plant drugs (Forestiere et al., 1988). In the present study, the antimicrobial activities of acetone, petroleum ether, ethyl acetate, chloroform, ethanol, methanol and water extracts of *E. umbellata* leaves and roots were determined. The results of the antimicrobial screening of different solvents

extracts of leaves and root of *E. umbellata* against 8 bacteria, 1 yeast and 1 fungus are presented in Tables 1, Figure 1 and Table 2, Figure 2.

According to previous investigations of Sabir et al. (2007), the samples prepared from the aerial parts of *E. umbellata* specie growing in Rawalakot Azad Jammu and Kashmir showed antimicrobial activity against four bacteria tested. They studied antibacterial activity of acetone, chloroform, methanol and ethanol extracts of leaves along with various extracts of berries and flowers against four bacteria namely *E. coli*, *P. aeruginosa*, *S. aureus* and *B. subtilis*. The present study shows 50 to 100% better results than the previous antibacterial activity. The acetone extracts of leaves showed antimicrobial activity against *S. aureus*, *B. subtilis*, *E. coli*, *B. bronchiseptica*, while root extract exhibited activity against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *B. bronchiseptica*, *P. aeruginosa*, *P. syringae*, and *S. typhae*. The results are presented in Tables 1, Figure 1 and Table 2 and Figure 2.

The petroleum ether extract of the leaves of *E. umbellata* exhibited antimicrobial activities against *S. aureus*, *E. coli* and *B. bronchiseptic* while root extract showed remarkable activity against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *B. bronchiseptica*, *P. aeruginosa*, *P. syringae* and *S. typhae*. The results are presented in Table 1, Figure 1 and Table 2, Figure 2.

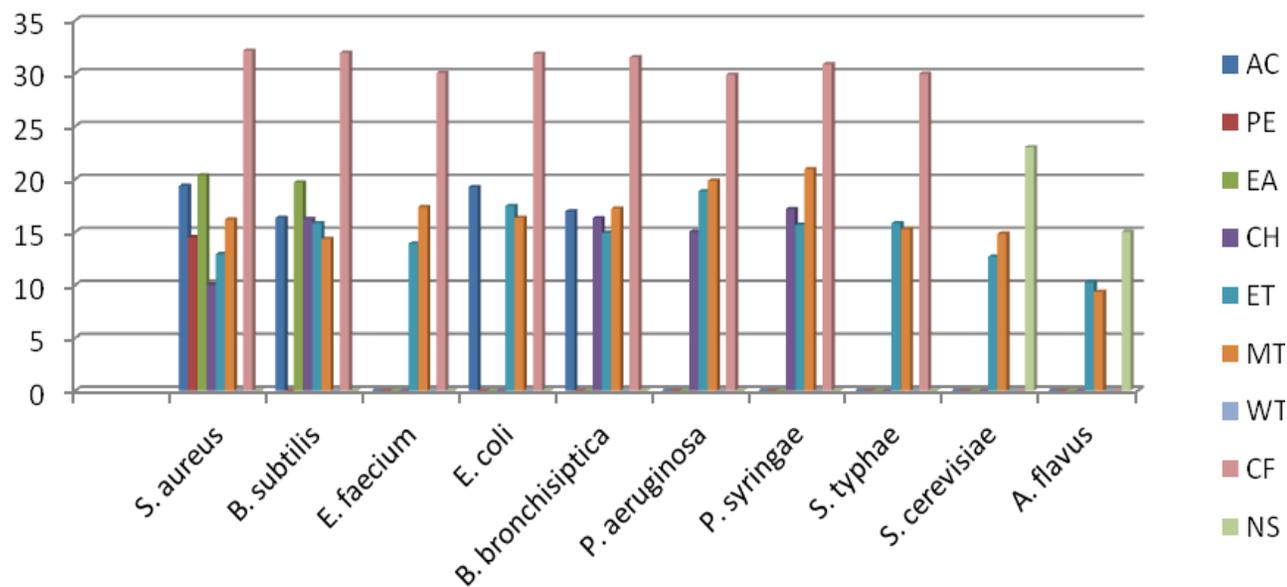
The ethyl acetate extract of the leaves of the plant showed high antibacterial activity against *S. aureus*, *B. subtilis*, *E. coli*, *B. bronchiseptica*, *P. aeruginosa*, and *P. syringae* while the extract of root also showed promising activity against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *B. bronchiseptica*, *P. aeruginosa*, *P. syringae*, and *S. typhae* (Table 1, Figure 1 and Table 2, Figure 2). The chloroform extract of the leaves showed activity against *E. coli* (20.56±0.23 mm), *P. aeruginosa* (15.00 ±0.00 mm), *P. syringae* (17.16 ±0.16 mm), *S. aureus*, (10.00 ±0.00 mm) *B. subtilis* (16.22 ±0.08 mm), *B. bronchiseptica* (16.30 ±0.16 mm), while *S. typhae*, *E. faecium*, *S. cerevisiae* and *A. flavus* were found inactive (Table 1, Figure 1). The chloroform extract of the root of *E. umbellata* exhibited moderate activity against all the tested microorganisms used except *S. cerevisiae* and *A. flavus*. The mean diameter of zones of inhibition of the extract against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *B. bronchiseptica*, *P. aeruginosa*, *P. syringa* and *S. typhae* were 10.00 ±0.00, 9.66 ±0.33, 9.66 ±0.33, 20.06 ±0.06, 10.06 ±0.06, 15.1 ±0.1, 9.66 ±0.33 and 9.66 ±0.33 mm, respectively (Table 2, Figure 2). The chloroform extracts showed comparatively more *in vitro* antimicrobial activity against bacteria as compared to the previous work on leaf extract by Sabir et al. (2007) which may be due to topographic variation on the chemical constituents of the plant.

The methanolic extracts of the leaves and root of the plant showed considerable activity not only against all tested bacteria but also exhibited considerable activity against the fungus and yeast. The zones of inhibition

**Table 1.** Antimicrobial activity profile of the leaves of *E. umbellata* Thunb.

S/N	Strain	Zones of inhibition (mm) ± standard error (S E M)								
		AC	PE	EA	CH	ET	MT	WT	CF	NS
1	<i>S. aureus</i>	19.35±0.15	14.5±0.05	20.33±0.17	10.00±0.00	12.9±0.35	16.16±0.08	0.00±0.00	32.13±0.13	0.00±0.00
2	<i>B. subtilis</i>	16.33±0.17	0.00±0.00	19.66±0.33	16.22±0.17	15.83±0.16	14.33±0.17	0.00±0.00	31.93±0.06	0.00±0.00
3	<i>E. faecium</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	13.9±0.1	17.33±0.19	0.00±0.00	30.03±0.03	0.00±0.00
4	<i>E. coli</i>	19.22±0.11	16.11±0.10	22.96±0.23	20.56±0.23	17.46±0.33	16.33±0.16	0.00±0.00	31.83±0.17	0.00±0.00
5	<i>B. bronchisiptica</i>	16.93±0.06	20.33±0.15	20.83±0.17	16.30±0.16	14.9±0.2	17.19±0.16	0.00±0.00	31.5±0.1	0.00±0.00
6	<i>P. aeruginosa</i>	0.00±0.00	0.00±0.00	17.66±0.33	15.00±0.00	18.83±0.16	19.83±0.17	0.00±0.00	29.83±0.17	0.00±0.00
7	<i>P. syringae</i>	0.00±0.00	0.00±0.00	22.66±0.33	17.16±0.16	15.66±0.33	20.93±0.06	0.00±0.00	30.83±0.17	0.00±0.00
8	<i>S. typhae</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	15.83±0.17	15.26±0.13	0.00±0.00	29.93±0.06	0.00±0.00
9	<i>S. cerevisiae</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.66±0.06	14.82±0.18	0.00±0.00	0.00±0.00	21.00±0.33
10	<i>A. flavus</i>	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	10.23±0.1	09.33±0.17	0.00±0.00	0.00±0.00	15.00±0.26

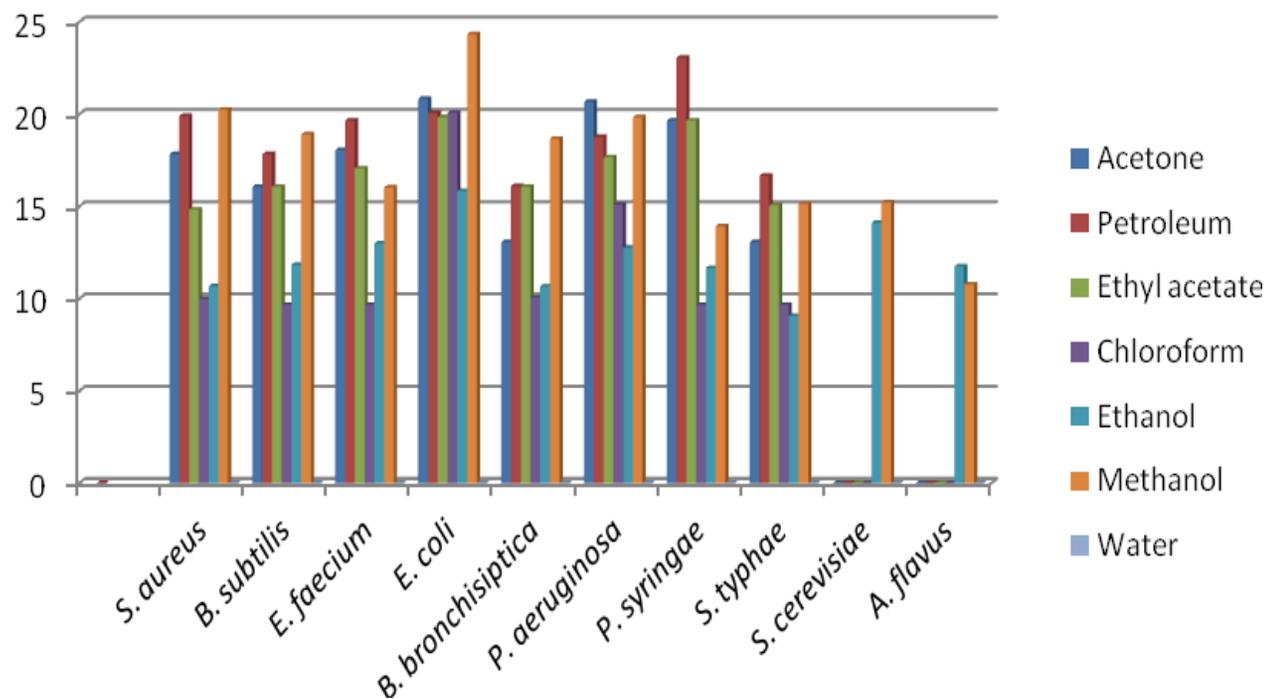
AC, Acetone; PE, petroleum ether; EA, ethyl acetate; CH, chloroform; ET, ethanol ; MT, methanol; WT, water; CP, ciprofloxacin; NS, nystatin. Concentration of crude extracts, 100 mg/ml; ciprofloxacin, 100 µg/ml; nystatin, 1500 u/ml.



**Figure 1.** Antimicrobial activity of the leaves of *Elaeagnus umbellata*. AC: Acetone, PE: petroleum Ether, EA: ethyl acetate, CH: chloroform, ET: ethanol, MT: methanol, WT: water, CF: ciprofloxacin, NS: nystatin.

**Table 2.** Antimicrobial activity of the Roots of *E. umbellata* Thunb. with concentration of crude extracts of 100 mg/ml.

S/N	Microorganism	Zones of inhibition (mm) $\pm$ standard error (S E M)						
		Acetone	Petroleum ether	Ethyl acetate	Chloroform	Ethanol	Methanol	Water
1	<i>S. aureus</i>	17.83 $\pm$ 0.17	19.9 $\pm$ 0.1	14.83 $\pm$ 0.17	10.00 $\pm$ 0.00	10.67 $\pm$ 0.33	20.24 $\pm$ 0.06	0.00 $\pm$ 0.00
2	<i>B. subtilis</i>	16.06 $\pm$ 0.06	17.83 $\pm$ 0.17	16.06 $\pm$ 0.06	9.66 $\pm$ 0.33	11.83 $\pm$ 0.17	18.9 $\pm$ 0.1	0.00 $\pm$ 0.00
3	<i>E. faecium</i>	18.06 $\pm$ 0.06	19.66 $\pm$ 0.33	17.06 $\pm$ 0.06	9.66 $\pm$ 0.33	13.00 $\pm$ 0.00	16.03 $\pm$ 0.3	0.00 $\pm$ 0.00
4	<i>E. coli</i>	20.83 $\pm$ 0.17	20.06 $\pm$ 0.06	19.83 $\pm$ 0.17	20.06 $\pm$ 0.06	15.83 $\pm$ 0.16	24.33 $\pm$ 0.17	0.00 $\pm$ 0.00
5	<i>B. bronchisiptica</i>	13.06 $\pm$ 0.03	16.1 $\pm$ 0.01	16.06 $\pm$ 0.03	10.06 $\pm$ 0.06	10.66 $\pm$ 0.33	18.66 $\pm$ 0.15	0.00 $\pm$ 0.00
6	<i>P. aeruginosa</i>	20.66 $\pm$ 0.33	18.76 $\pm$ 0.23	17.66 $\pm$ 0.33	15.1 $\pm$ 0.10	12.76 $\pm$ 0.23	19.83 $\pm$ 0.17	0.00 $\pm$ 0.00
7	<i>P. syringae</i>	19.66 $\pm$ 0.33	23.06 $\pm$ 0.06	19.66 $\pm$ 0.33	9.66 $\pm$ 0.33	11.66 $\pm$ 0.33	13.93 $\pm$ 0.16	0.00 $\pm$ 0.00
8	<i>S. typhae</i>	13.06 $\pm$ 0.06	16.66 $\pm$ 0.33	15.06 $\pm$ 0.06	9.66 $\pm$ 0.33	9.06 $\pm$ 0.06	15.13 $\pm$ 0.17	0.00 $\pm$ 0.00
9	<i>S. cerevisiae</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	14.11 $\pm$ 0.1	15.22 $\pm$ 0.16	0.00 $\pm$ 0.00
10	<i>A. flavus</i>	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	11.75 $\pm$ 0.15	10.77 $\pm$ 0.13	0.00 $\pm$ 0.00

**Figure 2.** Antimicrobial activity of the roots of *Elaeagnus umbellata*.

produced by the leaves extract against *E. faecium*, *P. aeruginosa*, *P. syringae*, *S. aureus*, *B. subtilis*, *E. coli*, *S. typhae* and *B. bronchisiptica* were  $17.33 \pm 0.19$ ,  $19.83 \pm 0.17$ ,  $20.93 \pm 0.06$ ,  $16.16 \pm 0.08$ ,  $16.33 \pm 0.16$ ,  $15.26 \pm 0.13$  and  $17.19 \pm 0.16$  mm, respectively. The zone of inhibition produced by the methanolic extract of leaves against *S. cerevisiae* and *A. flavus* were found to be  $14.82 \pm 0.18$  and  $09.33 \pm 0.17$  mm, respectively. The zones of inhibition produced by the methanolic extract of root against *E. faecium*, *P. aeruginosa*, *P. syringae*, *S. aureus*, *B. subtilis*, *E. coli*, *S. typhae* and *B. bronchisiptica* were found to be  $16.03 \pm 0.3$ ,  $19.83 \pm 0.17$ ,  $13.93 \pm 0.16$ ,  $20.24 \pm 0.06$ ,  $18.9 \pm 0.1$ ,  $24.33 \pm 0.16$ ,  $15.13 \pm 0.17$  and  $18.66 \pm 0.15$  mm, respectively. The zones of inhibition against *S. cerevisiae* and *A. flavus* were  $15.22 \pm 0.16$  and  $10.77 \pm 0.13$  mm, respectively (Table 1, Figure 1 and Table 2, Figure 2).

Sabir et al. (2007) described that the ethanolic extract of the leaves of *E. umbellata* did not show any significant antimicrobial activity against *P. aeruginosa*, *S. aureus* and *E. coli* except *B. subtilis*. While in the present study, it was observed that the ethanolic extracts of the leaves and roots were found to be more active against Gram-positive and Gram-negative bacteria as well as against fungus and yeast. The ethanolic extract of the leaves showed antimicrobial activity against *E. coli* ( $17.46 \pm 0.16$  mm), *P. aeruginosa* ( $18.83 \pm 0.06$  mm), *P. syringae* ( $15.66 \pm 0.33$  mm), *S. aureus*, ( $12.90 \pm 0.35$  mm), *B. subtilis* ( $15.83 \pm 0.16$  mm), *B. bronchisiptica* ( $14.09 \pm 0.2$  mm), *S. typhae* ( $15.83 \pm 0.17$  mm), *E. faecium*, ( $13.90 \pm 0.1$  mm), *S. cerevisiae*, ( $12.66 \pm 0.06$  mm) and *A. flavus*, ( $10.23 \pm 0.1$  mm). The ethanolic extract of the root of *E. umbellata* also exhibited moderate activity against all the tested microorganisms including *S. cerevisiae* and *A. flavus*. The mean diameter of zones of inhibition of the extract against *S. aureus* ( $10.67 \pm 0.33$  mm), *B. subtilis* ( $11.83 \pm 0.17$  mm), *E. faecium* ( $13.00 \pm 0.00$  mm), *E. coli* ( $15.83 \pm 0.16$  mm), *B. bronchisiptica* ( $10.66 \pm 0.33$  mm), *P. aeruginosa* ( $12.76 \pm 0.23$  mm), *P. syringae* ( $11.66 \pm 0.33$  mm), *S. typhae* ( $9.06 \pm 0.06$  mm), *S. cerevisiae* ( $14.11 \pm 0.1$  mm) and *A. flavus* ( $11.75 \pm 0.15$  mm), respectively. The detail results are shown in Table 1, Figure 1 and Table 2, Figure 2.

The water extracts of the leaves and roots represented no activity against the microorganisms (Table 1, Figure 1 and Table 2, Figure 2). The antibiotic, ciprofloxacin showed high activity against all the microorganisms used except *S. cerevisiae* and *A. flavus*. The activity profile against *S. aureus*, *B. subtilis*, *E. faecium*, *E. coli*, *B. bronchisiptica*, *P. aeruginosa*, *P. syringae*, *S. typhae* are presented in Table 1. The nystatin exhibited activity against the yeast and fungus while all the bacteria were resistant to nystatin (Table 1, Figure 1). Avato et al. (1997) reported that extracts of *Bellis perennis* have a high antimicrobial activity against bacteria than fungus. The results of Zavala et al. (1997) were similar to ours.

They showed that extracts from some plants have high activity against bacteria than yeast and fungus. On the other hand the antimicrobial activity against Gram-negative bacteria was more effective than Gram-positive bacteria (Table 1, Figure 1 and Table 2, Figure 2).

The antibacterial and antifungal activity of the plant extracts may be due to the flavonoids and phenolics which have already been reported in the leaves of the plant (Chopra et al., 1986). These compounds are usually extracted in organic solvents. Actually, phenolics not only attack the cell wall and cell membrane, thereby destroying its permeability and releasing the intracellular constituents (ribose, sodium, glutamate, and so forth) but also interfere with membrane function, for example, electron transport chain, nutrient uptake, protein and nucleic acid synthesis, and also affect enzyme activity. The bioactive compounds might have several invasive targets that could lead to inhibition of the bacteria and fungi (Sabir et al., 2007). The results reveal that the extent of inhibition is variable for different extracts against different microbes. It seems very likely, therefore, that the antimicrobial compounds extracted from *E. umbellata* may inhibit bacteria and fungi by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antimicrobial agent against multidrug resistant microbial strains. The method used by traditional healers for treating a bacterial and fungal infections, is administering a decoction of the plant or by using a part by boiling it in water. According to our results, an organic solvent extract is more effective in reducing microbial infections compared to water. We have recently isolated three coumarins, namely, 7-Hydroxy-chromen-2-one, 7, 8-Dihydroxychromen-2-one, 3-(2,2,3,4,5-Pentahydroxy-hexyloxy)-chromen-2-one and an anthraquinone from *E. umbellata* which showed partial suppression of parasitic growth against *Plasmodium falciparum* (Fiaz et al., 2013).

## Conclusion

Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The extracts of the leaves and roots of *E. umbellata* showed excellent antimicrobial activity against the tested bacteria, fungus and yeast. Therefore, it is concluded that the extract of the leaves and roots 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 roots of *E. umbellata* may be more beneficial than the aerial parts against infectious diseases. Further investigations are directed at isolation of pure compounds which are present in frac-

tions 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.

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