Antibacterial activity of *Elaeagnus umbellata* (*Thunb.*) a medicinal plant from Pakistan

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ABSTRACT

Objective: To evaluate the biological activity of *Elaeagnus umbellata* extracts on standard microorganism strains as well as multi-drug resistant bacteria isolated from hospitals.

Methods: We carried out this study at the Plant Pathology Laboratory of the University College of Agriculture, Rawalakot Pakistan during the period between September - November 2004. Flowers, leaves, and berries of the plant were extracted in different solvents and tested for their antibacterial activity by disc diffusion method on selected organisms like methicillin resistant *Staphylococcus aureus (S. aureus)*, multi-drug resistant *Pseudomonas aeruginosa (P. aeruginosa)*, and enterohemorrhagic *Escherichia coli (E. coli)*.

Results: The ether extract of flower was found to be highly effective against *E. coli, P. aeruginosa, S. aureus,* and *Bacillus subtilis (B. subtilis)*. The alcohol extract of these leaves also demonstrated strong activity against gram positive and negative bacteria. The aqueous extract from the berry strongly inhibited the growth of *E. coli* and *S. aureus* whereas, it exhibited a very small zone of inhibition against *B. subtilis*. Multi-drug resistant *P. aeruginosa* was found completely resistant to aqueous extract. The acetone extract of the berry showed good activity against *P. aeruginosa.*

Conclusions: The present study reports the antibacterial activity of *Elaeagnus umbellata*. Most of the extracts displayed broad-spectrum activity, since gram positive bacteria including *S. aureus, B. subtilis* and gram-negative bacteria including *E. coli* and *P. aeruginosa* were inhibited. These preliminary findings may provide the basis for traditional use of this plant in the treatment of infectious diseases.

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Pharmacological industries have produced a large number of new antibiotics in the last 3 decades, but resistance to these drugs by microorganisms has increased. Bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents.[1] This fact is cause for concern, due to the number of patients in hospitals who have suppressed immunity, and are infected by multi-resistant organisms. Consequently, new infections may occur in hospitals resulting in high mortality. Therefore, actions must be taken to control the use of antibiotics, to understand the genetic mechanisms of bacterial resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal should be to offer appropriate and efficient antimicrobial drugs to the patients. Plants have been an integral part of human society since the start of civilization. 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.[2] The herbs are extensively used for treating diseases; however, their commercial exploitation is limited due to the lack of a scientific knowledge for their use.[3] Among these plants *Elaeagnus umbellata* (*thunb.*) (*E. umbellata*) a wild shrub belonging to the family *Elaeagnaceae*, is widely distributed at a height of 4500-6000 feet above sea level in Azad Kashmir.[4] It is abundantly found in Himalayan regions of Pakistan.[4] 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.[5] The fruit/berries are silvery with brown scales when immature and ripens to a speckled red in September to October.[6] The berry is an excellent source of vitamins A, C, E, flavonoids, essential fatty acids,[7] lycopene, β-carotene, lutein, phytofluene, and phytoene. The lycopene content of the *E. umbellata* fruit is 17 times greater than that of tomato.[8,9] Many studies have proved that lycopene protects against myocardial infarction,[8] and various forms of cancers

including prostrate cancer.[10-12] 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.[7] Various photochemicals 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.[13] Various plant extracts and phytochemicals 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.[14-18] Many plants have been used due to their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant (Phenolics, tannins). Although the antibacterial activity of *E. umbellata* has not been studied, the in vitro antibacterial activity of different parts of the plant growing in Kashmir was evaluated using disc diffusion method.

Methods. Fresh plants or parts (**Figure 1 & 2**) were collected randomly from different locations of Rawalakot, Azad Kashmir, Pakistan in August 2004 on the same day. The plants were identified at the University College of Agriculture, Rawalakot by a Senior Botanist, where a voucher specimen (No. UCR 101) was deposited. Fresh plant materials (flowers, berries and leaves) were washed under running tap water, air dried, and then homogenized to fine powder and finally stored in airtight bottles.

Aqueous extraction. For aqueous extraction, 10 g of air-dried powder was placed in distilled water and boiled for 6 hours. At intervals of 2 hours, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 minutes. The supernatant was collected and after 6 hours was concentrated to make the final volume one-fourth of the original volume. Finally, the material was extracted in 25 ml of distilled water giving a concentration of 4 mg/20 µl.

Solvent extractions. Ten grams of air dried powder was placed in 100 ml of organic solvents in a conical flask, plugged with cotton and then kept on a rotary shaker at 190-220 rpm for 24 hours. After 24 hours, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 x g for 15 min. The supernatant was collected, and the solvent was evaporated to make the final volume one-fourth of the original volume, giving a concentration of 4 mg/20 µl

Test microorganisms. Three clinical strains were used in the study: methicillin-resistant *Staphylococcus aureus*, multi-drug resistant *Pseudomonas aeruginosa* (namely, resistant to ampicillin, cefuroxime, cefotaxime, gentamicin, amikacin, erythromycin, clindamycin, ofloxacin, nalidixic acid, norfloxacin, ciprofloxacin, and amoxicillin-clavulanic acid), and enterohemorrhagic *E. coli* O157 (EHEC). A reference strain of *Bacillus subtilis* ATCC 6633 was also tested.

Antibacterial activity. A loop full of the strain was inoculated in 30 ml of nutrient broth in a conical flask and incubated on a rotary shaker for 24 hours to activate the strain. Mueller Hinton Agar No. 2 was prepared for the study. The assay was performed using 2 methods. Agar disk diffusion[19] for aqueous extract and Agar ditch diffusion[20] for solvent extract. The media and the test bacterial cultures were poured into Petri dishes (Hi-Media). The test strain (0.2 ml) was inoculated into the media (inoculum size 108 cells/ml) when the temperature reached 40-42^{O}C. Care was taken to ensure proper homogenization. The experiment was performed under strict aseptic conditions. For the Agar disk diffusion method, the test compound (20 µl) was introduced onto the disk (0.7 cm) (Hi-Media), and then allowed to dry. Thus, the disk was completely saturated with the test compound. Then, the disk was introduced onto the upper layer of the medium with the bacteria. The plates were incubated overnight at 37^{O}C. For the Agar ditch diffusion method, after the medium was solidified, a ditch was made in the plates with the help of a cup-borer (0.85 cm). The test compound was introduced into the well, and the plates were incubated overnight at 37^{O}C. Microbial growth was determined by measuring the diameter of the zone of inhibition. A standard 30 µg tetracycline disk was used as positive control. Pure solvents and distilled water were used as the negative control. The control activity was deducted from the test, and the result obtained was plotted.

Statistical analysis. Statistical analysis was conducted using ANOVA ($p \leq 0.05$) for antibacterial data (**Table 1**) to compare between plant regions, solvents used, and also tetracycline.

Results. The results of antibacterial activity were recorded as zone of inhibition in mm for all the materials used as follows. Antibacterial activity of crude flower extract. Ether, chloroform, methanol, and ethanol extracts of the flower were found to be highly effective against all of the gram positive and negative bacteria (**Table 2**). However, ether extract showed considerably more activity against all tested bacteria. The maximum zone of inhibition was produced by ether extract against *E. coli* whereas, the minimum zone was produced by chloroform extract against *Bacillus subtilis* among the different extracts used. Among the alcohol extracts, methanol was found more effective in producing a zone of inhibition against ethanol.

Antibacterial activity of crude leaves extract. Methanol, chloroform and acetone extracts showed mild activity whereas, ethanol showed strong activity against

Table 1 - Analysis of variance for antibacterial activity of *Elaeagnus umbellata.*

Source of variation	Standard score	Degrees of freedom	MS	F	*P*-value	F crit
Between groups	929.605	12	77.4671	4.654	0.0001	2.01
Within groups	649.0625	39	16.6426			
Total	**1578.6675**	**51**				

MS - mean square, F - calculated, Fcrit - critical value of F.

Table 2 - Antibacterial activity of plant extracts of *Elaeagnus umbellata* recorded as zone of inhibition in mm.

Extracts	*Escherichia Coli*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Bacillus subtilis*
Flower				
Ether	15	12	14	11
Chloroform	10	11	9	7
Methanol	8	8.5	10	12
Ethanol	7	7	5	8
Leaf				
Acetone	5.5	4	6	8
Chloroform	8.5	10	7	12
Methanol	10	12	9	11
Ethanol	8	9	7	14
Berry				
Aqueous	15	Nil	14	4
Acetone	15	13	10	8
Chloroform	7	7	6	7
Methanol	8	9	6	10

All the values are significantly different from each other ($p \leq 0.05$).

Figure 1 - Photograph showing ripe berries and leaves of *Elaeagnus umbellata.*

Figure 2 - Photograph showing mature flowers of *Elaeagnus umbellata.*

pathogenic bacteria (**Table 2**). Among the extracts, ethanol produced the maximum zone of inhibition against *Bacillus subtilis* whereas, acetone produced the minimum zone of inhibition against methicillin-resistant *Staphylococcus aureus*.

Antibacterial activity of crude berry extract. Aqueous extract strongly inhibited the growth of *E. coli* and *Staphylococcus aureus* whereas, it exhibited a very small zone of inhibition against *Bacillus subtilis*. *Pseudomonas aeruginosa* was found resistant to aqueous extract (**Table 2**). Chloroform and methanol extract showed mild activity against bacteria whereas, acetone extract was found equally effective in inhibiting the growth of *Pseudomonas aeruginosa* as well as *E. coli*, *Staphylococcus aureus*, and *Bacillus subtilis*.

Antibacterial activity of standard antibiotics. Inhibition zones of different test organisms for extracts, standard reference discs and solvents were significantly different at $p \leq 0.05$. Tetracycline very strongly inhibited the growth of *E. coli* and *Staphylococcus aureus* whereas, it exhibited a very small zone of inhibition against *Pseudomonas aeruginosa*, and *Bacillus subtilis*.

Discussion. *Elaeagnus umbellata* is one of the medicinal plants that are commonly used by the local practitioners for various human ailments, but no attempt has been made to study its antimicrobial activity. In the present study, crude extracts of the plant material obtained in polar and non-polar solvents were tested against 4 types of the test organisms. The antibacterial activity was compared with the standard antibiotic (tetracycline). Out of 3 plant materials screened, the flower of *E. umbellata* indicated 100% activity. However, the ether extract showed maximum inhibition against all tested organisms when compared with other solvents. This shows that the oil of *E. umbellata* has a potent antibacterial activity against gram positive and negative bacteria. Although, the antibacterial activity of essential oils from many plant species has been extensively surveyed, their antimicrobial mechanism has not been reported in great detail. Since the active antimicrobial compounds of essential oils are phenolics and terpenes in nature,[21,22] it seems reasonable that their mode of action might be similar to that of other phenolic compounds.

The leaf extract of *E. umbellata* also inhibited 100% of microorganisms. Among the different solvents, ethanol showed maximum inhibition. Flavonoids and phenolic compounds have already been reported in leaves of the plant.[7] These compounds have antibacterial and antifungal activities. The compounds are polar and are usually well extracted in solvents such as alcohol and chloroform. Most of the studies on the mechanism of phenolic compounds focused on their effects on cellular membranes. Actually, phenolics not only attacked the cell wall and cell membrane, thereby destroying its permeability and releasing the intracellular constituents (ribose, sodium, glutamate, and so forth) but also interfered with membrane function, for example, electron transport, nutrient uptake, protein, and nucleic acid synthesis, and enzyme activity. The bioactive compounds might have several invasive targets that could lead to inhibition of the bacteria. Furthermore, leakage of intracellular material was a general phenomenon induced by many antibacterial substances, the level of leakage from *E. coli* and *Staphylococcus aureus* observed was in correlation with the concentration of phenolics present.[23,24] The berry extract of the plant inhibited 99% of organisms, only *Pseudomonas aeruginosa* was resistant to aqueous extract. The resistance of *Pseudomonas aeruginosa* to plant extracts was not unexpected as, in general, this class of bacteria is more resistant than gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism.[25] Infections caused by *Pseudomonas aeruginosa*, especially those with multi-drug resistance, are among the most difficult to treat with conventional antibiotics.[26] In this study, acetone extract showed strong activity against *Pseudomonas aeruginosa*. It seems very likely, therefore, that the antibacterial compound extracted from *E. umbellata* may inhibit bacteria by a different mechanism than that of currently used antibiotics and may have therapeutic value as an antibacterial agent against multi-drug resistant bacterial strains. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers use the method of treating a bacterial infection by administering a decoction of the plant or by using a part by boiling it in water. According to our results, an organic solvent is more beneficial. The berries of *E. umbellata* are astringent, indicating that fruit may be high in phenolic compounds and were found to be high in flavanols and hydroxybenzoic acids (33 rutin and 31 gallic acid mg/kg equivalents), while the seeds were high in hydroxy cinnamic acids and extremely high in hydroxybenzoic acids (35 chlorogenic acid and 184 gallic acid mg/kg equivalents).[27] The fatty acid composition of phospholipids and glycolipids in *Elaeagnus angustifolia Linn* fruit were also reported, which may also be responsible for the antibacterial activity of berry extracts.[28]

From the above results it can be concluded that plant extracts have great potential as antimicrobial compounds against microorganisms, and that they can be used in the treatment of infectious diseases caused by resistant microorganisms.

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