Biological Screening of *Elaeagnus umbellata* Thunb.

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Abstract: The bark and fruit extracts of *Elaeagnus umbellata* have been investigated for their antibacterial, anti-fungal, insecticidal and phytotoxic activities. The petroleum ether extracts of the plant showed significant activity against *E. faecalis.* The activity of dichloromethane extract was also determined significant against *S. aureus*. The chloroform extract indicated low activity against *E. coli*, *K. pneumoniae*, *B. subtilis* and *S. flexenari*. The ethyl acetate fraction demonstrated significant activity against K. *pneumoniae* while methanolic extract exhibited significant activity against *E. coli*. All extracts showed low phytotoxic activity. The dichloromethane extract exhibited moderate insecticidal activity while other extract indicated low activity.

Keywords: *Elaeagnus umbellata*, biological activities, anti-microbial, phytotoxic, insecticidal.

INTRODUCTION

Elaeagnus umbellata Thunb. is also known as Japanese silverberry, Autumn olive and locally Known as 'Kancoli', a species of *Elaeagnus* native to eastern Asia from Himalayas east to Japan. It was cultivated in the United States in 1830 (Rehder, 1940; Eckardt and Sather, 1987). It is also present in India and Afghanistan (Kaushal and Parmar, 1982). Berries contain a variety of substances with antioxidant activity, ascorbic acid (Vitamin C), β-carotene (vitamin A), tocopherols (vitamin E) and flavonoids. The berries also contain thiamine (vitamin B_1), riboflavin (B_2), niacin (B_3), pyridoxin (B_6), biotin, folic acid and vitamin K. The berries contain oil (about 13% in seeds and 6% in whole berries). Linoleic (34%), α-linolenic (25%) and oleic (19%) are three major fatty acids in seed oil where aspalmitic (33%), oleic (26%) and palmitolcic (25%) are the three main acids in the fruit pulp oil. The seeds and flowers of *Elaeagnus umbellata* have been found to act as stimulant in coughs and its oil in pulmonary infections. It possesses anti-bacterial properties. Its flowers possess astringent properties and are also used in cardiac diseases (Watt, 1890; Kirtikar and Basu, 1938). Proteins are found abundantly in the fruits. The plant is ornamental due to its silvery foliage and fragrant flowers. Hence, it is used for making protective hedge around fields, houses and gardens. Its fruit contains carotenoid lycopene, which is widely believed to protect against mycocardial infection (Kohlmeier *et al*., 1997) and various forms of cancer (Clinton, 1998) including prostrate (Giovannnucci *et al*., 1995). It also reverses the growth of cancer. It is also used in brushing teeth and help in eradication of unpleasant and promote freshness.

Elaeagnus umbellata is an important medicinal plant. The antibacterial activity of the plant is already reported (Mubasher *et al*., 2007) but in present work antibacterial activity has been found out with extracts different from those used in earlier studies. Its anti-fungal activity has not been reported so far. So the present study will provide a broad base for the possibility of its detailed biological studies and its effectiveness against infectious diseases.

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MATERIALS AND METHODS

Collection and extraction method

The plant was collected from Jhandgranbala, Azad Kashmir, Pakistan. The plant was identified by a plant taxonomist at the Department of Botany, University of Azad Jammu and Kashmir. The voucher specimens of the plant are placed in the herbarium of the department. The dried plants material (bark) were grinded to the powdered form. About 200g of the powdered material was separately soaked in the methanol for 7 days. The extracts were then filtered and evaporated on rotary evaporator under reduced pressure to dryness. The dried plant materials were extracted in various organic solvents (petroleum ether, dichloromethane, chloroform, methanol and ethyl acetate) to afford the solvents soluble material. Antibacterial activity of crude extract and its fractions were determined by using disc diffusion method, also known as the zone of inhibition method (Bauer, *et al*., 1966).

Antibacterial activity

Nutrient broth medium was inoculated with single colony of bacterial culture and incubated at 37°C for 24 hours. After 24 hours, nutrient agar medium was taken and autoclaved at 121°C for 15 minutes. This medium was then melted and cooled up to 45°C. About 10µl of fresh bacterial culture was added, shaken and then poured on to the nutrient agar plate. The plate was rotated t o make even distribution of the culture and allowed to solidify. The test samples were added in agar plates by using 5 mm

sterile filter paper discs. The plates were incubated at 37°C for 24 hrs. Next day the results were noted in terms of zone of inhibition (expressed in mm). Reference antibacterial drug (ampicilline) and DMSO were used as positive and negative controls.

Antifungal activity

Agar tube dilution Protocol was used for analysis Sabouraud dextrose agar with acidic pH 5.5-5.6 was prepared by mixing 32.5g of glucose or maltose (2%) in 500 ml distilled water. It was then steamed to dissolve and dispense 4 ml amount in to screw capped tubes. Tubes containing media were autoclaved at 121°C for 15min. The extracts dissolved in sterile DMSO (400mg/ml) served as a stock solution. The tubes were allowed to cool to 50°C and solidified sabouraud agar media was poisoned with 66.6µl of test sample pipette out from the stock solution, which provided a final concentration of 200µg/ml of SDA for test samples. Tubes were then allowed to solidify in slanted position at room temperature. Each tube was inoculated with piece of inoculums removed from a seven days old culture of fungus, for non-mycelia growth, an agar surface streak is employed. Other media supplemented with DMSO and reference anti-fungal drugs used as negative and positive control, respectively. The standard drugs used in the

Table 1: Antibacterial activities of different fractions of *Elaeagnus umbellata*

Bacterial Strain	Extract	ZI of sample	ZI of Std. drug
Escherichia coli	P.E.	12	40
	DCM	4	40
	$CHCl_3$	8	40
	E.A.	-	40
	MeOH	20.6	40
Klebsilla pneumonia	P.E.	6	42
	DCM	4.3	42
	$CHCl_3$	6	42
	E.A.	29	42
	MeOH	-	42
Pseudomonas aeruginosa	P.E.	-	22
	DCM	-	22
	$CHCl_3$	-	22
	E.A.	-	22
	MeOH	-	22
Enterococcus faecalis	P.E.	18.6	41
	DCM	8.6	41
	$CHCl_3$	-	41
	E.A.	-	41
	MeOH	-	41
Staphylococcus aureus	P.E.	-	38
	DCM	21.3	38
	$CHCl_3$	-	38
	E.A.	-	38
	MeOH	-	38
Bacillus subtilis	P.E.	-	36
	DCM	-	36
	$CHCl_3$	6	36
	MeOH	-	36
Salmonella typhi	P.E.	-	40
	DCM	9	40
	$CHCl_3$	-	40
	MeOH	-	40
Shigella flexenari	P.E.	-	36
	DCM	-	36
	$CHCl_3$	9	36
	MeOH	-	36

ZI: Zone of inhibition, Std. drug (ampicilline); Petroleum ether (P.E), Dichloromethane (DCM), Chloroform ($CHCl_3$), Ethyl acetate (E.A), Methanol (MeOH), '-'indicated no activity.

assays were miconazole and amphotericin B. The tubes incubated at 27-29°C for 7-10 days. The growth in the compound containing media was determined by measuring the linear growth in mm and growth inhibition with reference to the negative control. The percentage inhibition was determined (Atta-ur-Rahman, *et al*., 1991).

Phytotoxic bioassay

Phytotoxic bioassays were analysed by modified protocol of *Lemna minor* (Mc Laughlin, *et al*., 1991). The medium was prepared by mixing various constituents in 1000 ml distilled water and the pH was adjusted between 6.0 to 7.0 by adding KOH solution. The medium was then autoclaved at 121°C for 15 minutes. The extracts (30mg) dissolved in any alcoholic solvent served as stock solution. Three flasks were inoculated with 10,100 and 1000µl of stock solution. The solvent was allowed to evaporate overnight under sterile conditions. Now 20ml of medium and the plant of *Lemna minor*, each containing a rosette of two to three fronds, were added to each flask (total 20 fronds). All flasks were plugged with cotton and kept in growth cabinet for seven days. The number of fronds per flasks were counted and recorded on day 7. Finally results were calculated as growth regulation in percentage**.** The results were calculated with reference to the positive and negative control. Paraquat was used as a standard drug, while paraquat and volatile solvent were used as positive and negative control.

Insecticidal activity

Contact toxicity method was used for analysis of plant extracts and fractions. The test sample was prepared by dissolving 200mg of crude fractions in 3ml volatile solvent and loaded in petri plate. After 24 hours, on complete evaporation of solvent, 10 test insects were placed in each plate (test and control) and plates were incubated at 27°C for 24 hours with 50% relative humidity in growth chamber. The results were analyzed as percentage inhibition or percentage mortality, calculated with reference to the positive and negative controls. The percentage mortality was determined. Permethrin was used as a standard drug, while permethrin, acetone and test insects were used as positive and negative controls (Ali, *et al*., 2009).

RESULTS

The antibacterial activity of the crude methanolic extract of *E. Umbellate* and its fractions were investigated against eight bacterial strains namely, *Escherichia coli, Klebsilla pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Bacillus subtilis, Salmonella typhi* and *Shigella flexenari* (table 1) which showed that all extracts inhibit the growth of all tested bacteria.

Tables 2 and 3 summarize results of anti-fungal activities of bark and fruit extracts of this plant *against* six fungal

Table 2: Anti-fungal activity of the extracts of bark of *Elaeagnus umbellata*

Name of Fungus	Extract	Linear Growth (mm)		% Inhibition	Std. Drug	Std. Drug conc. µg/ml
		Sample	Control			
Candida albicans	P.E	100	100	0	Miconazole	110.8
	DCM	100	100	0	Miconazole	110.8
	$CHCl_3$	100	100	0	Miconazole	110.8
	MeOH	100	100	0	Miconazole	110.8
Aspergillus flavus	P.E	100	100	0	Amphotericin B	20.20
	DCM	90	100	10	Amphotericin B	20.20
	$CHCl_3$	80	100	20	Amphotericin B	20.20
	MeOH	100	100	0	Amphotericin B	20.20
Microsporumcanis	P.E	85	100	15	Miconazole	98.4
	DCM	65	100	35	Miconazole	98.4
	$CHCl_3$	90	100	10	Miconazole	98.4
	MeOH	90	100	10	Miconazole	98.4
Fusariumsolani	P.E	100	100	0	Miconazole	73.25
	DCM	85	100	15	Miconazole	73.25
	$CHCl_3$	100	100	0	Miconazole	73.25
	MeOH	85	100	15	Miconazole	73.25
Candida glabrata	P.E	100	100	0	Miconazole	110.8
	DCM	100	100	0	Miconazole	110.8
	$CHCl_3$	100	100	0	Miconazole	110.8
	MeOH	100	100	0	Miconazole	110.8

Concentration of Sample: 400µg/ml of DMSO, Incubation Time: 27 (28±1C°), Incubation Period: 7 days

Table 3: Anti-fungal activity of the extracts of fruits of *Elaeagnus umbellata*

Name of Fungus	Extract	Linear Growth (mm)		% Inhibition	Std. Drug	Std. Drug conc. µg/ml
		Sample	Control			
Trichophyton longifusus	n-hex	50	100	50	Miconazole	70
	$CHCl_3$	80	100	20	Miconazole	70
	E.A	50	100	50	Miconazole	70
	MeOH	40	100	0	Miconazole	70
Candida albicans	n-hex	100	100	0	Miconazole	110.8
	$CHCl_3$	90	100	10	Miconazole	110.8
	E.A	100	100	0	Miconazole	110.8
	MeOH	50	100	2	Miconazole	110.8
Aspergillus flavus	n-hex	100	100	0	Amphotericin B	20.20
	$CHCl_3$	65	100	35	Amphotericin B	20.20
	E.A	30	100	40	Amphotericin B	20.20
	MeOH	75	100	3	Amphotericin B	20.20
Microsporumcanis	n-hex	70	100	30	Miconazole	98.4
	$CHCl_3$	60	100	40	Miconazole	98.4
	E.A	70	100	30	Miconazole	98.4
	MeOH	85	100	15	Miconazole	98.4
Fusarium solani	n-hex	30	100	70	Miconazole	73.25
	$CHCl_3$	50	100	50	Miconazole	73.25
	E.A	40	100	50	Miconazole	73.25
	MeOH	60	100	10	Miconazole	73.25
Candida glabrata	n-hex	100	100	0	Miconazole	110.8
	$CHCl_3$	100	100	0	Miconazole	110.8
	E.A	100	100	0	Miconazole	110.8
	MeOH	100	100	0	Miconazole	110.8

Concentration of Sample: 400 µg/ml of DMSO, Incubation Time: 27 (28±1C°), Incubation Period: 7 days

Table 4: Phytotoxic activities of different fractions of *Elaeagnus umbellata*

Plant	Fraction	Conc. (µg/ml)	No. of Fronds		% Growth Regulation	Std. Drug (µg/ml)
			Sample	Control		
Lemna minor	Petroleum ether	1000	12	18	33.33	0.015
		100	18		11.11	
		10	20		0	
	Dichloromethane	1000	14	19	26.3	0.015
		100	18		5.26	
		10	19		0	
	Chloroform	1000	16	18	22	0.015
		100	19		15	
		10	20		6	
	Methanol	1000	15	18	25	0.015
		100	17		14	
		10	19		6	

strains like *Trichophytonlongifusus, Candida albicans*, *Aspergillus flavus*, *Microsporumcanis*, *Fusariumsolani* and *Candida glabrata.* Bark extracts were found to be effective against some strains while fruit extracts showed significant anti-fungal activity.

For phytotoxic bioassay, the content values of each fraction are depicted in the table 4. The results of insecticidal activity given in the table 5 indicate that among all extracts, dichloromethane fraction showed significant activity.

DISCUSSION

Antibacterial activity

Medicinal plants are valuable source of biologically active compounds. These plants prove the irworth in curing infectious diseases. *The* petroleum ether fraction

Table 5: Insecticidal activities of different fractions of *Elaeagnus umbellata*

Insect	% Mortality		% Sample mortality			
	Std	NC	Petroleum ether	Dichloromethane	Chloroform	Methanol
Triboliumcastaneum	100	0	0 %	20 %	0 %	0 %
Rhyzoperthadominica	100	0	0 %	40 %	20 %	0 %
Callosbruchusanalis	100	0	0 %	20 %	0 %	20 %

Concentration of sample (1019.1µg/cm^2), Concentration of std. drug (239.5µg/cm^2)

indicated good activity against *E. faecalis*. It exhibited low activity against *K. pneumoniae while* moderate activity against *E. coli*. The fraction showed no activity against *S. aureus, S. typhi*, *S. flexenari, B. subtilis* and *P. aeruginosa*. The dichloromethane fraction exhibited good activity against *S. aureus.* The fraction indicated low activity against *E. coli*, *K. pneumoniae, E. faecalis* and *S. typhi*. It showed no activity against *P. aeruginosa*, *B. subtilis* and *S. flexenari*. Low activity was indicated by the chloroform fraction against *S. flexenari* while no activity was observed against *E. coli*, *K. pneumoniae, P. aeruginosa*, *E. faecalis*, *S. aureus, B. subtilis* and *S. typhi*. The ethyl acetate fraction showed *significant* activity against *K. pneumoniae. Itdid not exhibit any activity* against *E. coli*, *P. aeruginosa*, *E. faecalis* and *S. aureus.* The methanolic *fraction* indicated moderate activity against *E. coli*. The fraction showed no activity against *K. pneumoniae, P. aeruginosa*, *E. faecalis*, *S. aureus, S. typhi*, *S. flexenari* and *B. subtilis*.

Antifungal activity

The petroleum ether extract of the bark of the plant showed low activity against *M. canis.* The fraction showed no activity against *C. albicans, A. flavus, F. solani* and *C. glabrata.* The dichloromethane fraction indicated significant activity against *M. canis* while it exhibited low activity against *A. flavus* and *F. solani.* The fraction did not show any activity against *C. albicans* and *C. glabrata*. The chloroform fraction indicated moderate activity against *A. flavus.* The fraction showed low activity against *M. canis.* It exhibited no activity against *C. albicans, F. solani* and *C. glabrata*. The methanolic fraction of *Elaeagnus umbellata* Thunb. indicated low activity against *M. canis* and *F. solani*. The fraction did not show any activity against *C. albicans, A. flavus* and *C. glabrata*.

Fruit

The n-hexane extracts of the fruits of the plant indicated moderate activity against *T. longifusus* and *M. canis* while it showed significant activity against *F. solani*. The fraction showed no activity against *C. albicans, A. flavus* and *C. glabrata*. The chloroform exhibited moderate activity against *A. flavus, M. canis* and *F. solani* while fraction showed low activity against *T. longifusus* and *C. albicans.* The fraction exhibited no activity against *C. glabrata.* The ethyl acetate fraction showed moderate activity against *T. longifusus, A. flavus* and *M. canis* while it indicated no activity against *C. albicans* and *C. glabrata*. The methanolic fraction showed low activity against *C. albicans, A. flavus, M. canis* and *F. solani* while it indicated no activity against *C. albicans* and *C. glabrata.*

Phytotoxic bioassay

The petroleum ether fraction indicated low activity. The dichloromethane and chloroform fraction also exhibited low phytotoxic activity. The crude methanolic extract showed low phytotoxic activity.

Insectcidal bioassay

The petroleum ether fraction indicated no activity against *T. castaneum, R. dominica* and *C. analis.* The dichloromethane fraction showed significant activity against *R. dominica.* The fraction indicated moderate activity against *T. castaneum* and *C. analis*. The chloroform fraction showed moderate activity against *R. dominica* while it did not show any activity against *T. castaneum* and *C. analis*. The methanolic fraction exhibited moderate activity against *C. analis*. The fraction did not exhibit any activity against *T. castaneum* and *R. dominica.*

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