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Full Length Research Paper

Phytochemical screening and biological activity of the aerial parts of *Elaeagnus umbellata*

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The methanolic crude extract of the aerial parts of *Elaeagnus umbellata* and its various solvent fractions was screened for the secondary metabolites and biological activity. Phytochemical analysis of the aerial parts showed the presence of bioactive secondary metabolites; alkaloids, steroids, terpenoids, saponins while flavonoids, anthraquinones, tannins, phlobatanins and glycosides were absent. The ethyl acetate fraction and methanolic extract of *E. umbellata* showed moderate antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis and Bacillus subtilis.* The isolated fractions; *n*-hexane, ethyl acetate and methanolic extract exhibited significantly 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging activity while chloroform showed low activity. The EC_{50} of the fractions and extract ranged between 5.5 and 250.6 µg/ml and that of querceitin was 4.12 µg/ml. This study reveals that the consumption of the plant would exert several beneficial effects by virtue of their antioxidant and antibacterial activities.

Key words: Phytochemical screening, *Elaeagnus umbellate*, antibacterial activity, antioxidant activity, Infrared spectroscopic analysis.

INTRODUCTION

Elaeagnus umbellata (*Thumb*) is a deciduous thorny wild shrub species, which belongs to *Elaeagnaceae* family, found at a height of 4500 to 6000 feet above the sea level in the various regions of Azad Kashmir and Himalayan regions of Pakistan (Ahmad et al., 2005). The berries of *E. umbellat*a are silvery with brown scales in an immature stage and ripen to speckled red in the months of September to October (Sternberg, 1982), which is a rich source of vitamins A, C, E, flavonoids, and essential fatty acids (Chopra et al., 1986). These barriers have been shown to have about 17 times the amount of the antioxidant lycopene than tomatoes (Kohlmeier et al., 1997; Fordham et al., 2001), which acts against myocardial infection as well as various forms of cancers including prostate cancer (Clinton, 1998; Giovannucci et al., 1995). The seeds and flowers of *E. umbellata* are used as a stimulant in the treatment of coughs and expressed seeds oil is used to treat pulmonary affections (Clinton, 1998). The medicinal values of *E. umbellata* lie in their phytochemical compositions such as alkaloids, steroids, terpenoids, saponins and phenolic chemical constituents. This paper presents a preliminary phytochemical investigation on *E. umbellata* associated with selected microorganism and antioxidant activities which could be further exploited for the isolation and characterization of biologically active chemical constituents in the treatment of infectious diseases.

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MATERIALS AND METHODS

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, Streptomycin, Dragendrof's reagent, Fehling's solution A and B and acetic anhydride were obtained from Sigma-Aldrich. All other chemicals

Table 1. Weight and percentage yield of the methanolic crude extract and fractions of *E. umbellata.*

Fraction/Extract	Weight of fraction/extract (g)	Percentage yield
n-Hexane	22.4	22.2
Chloroform	3.8	34.1
Ethyl acetate	2.8	25.3
Methanol	27	19.5

and reagents used were analytical grade.

Plant materials

The aerial parts of plant were collected from Swat, Khyber Pakhtunkhwa province of Pakistan in the month of June, 2009. The plant was identified by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan. The voucher specimen (Rf-257) has been deposited in the herbarium of the Department of Botany, University of Peshawar. The aerial parts (barriers, leaves and twigs) were shade dried and grounded into powder. Two kilogram of powdered plant material was extracted with methanol (x3) for ten days. The methanolic extract was concentrated in a rotary evaporator, partition into *n*-hexane, chloroform and ethyl acetate and preserved for further use.

Phytochemical screening

Phytochemical analysis were carried out on the methanolic extract, *n*-hexane, chloroform and ethyl acetate fractions of the screened plant was done for the presence or absence of secondary metabolites followed by the standard procedures (Sofora 1993; Trease and Evans, 1989; Herborne, 1973; Uddin et al., 2011).

Micro-organism

Authentic cultures of selected human pathogenic bacteria; *Staphylococcus aureus*, *Staphylococcus epidermis, Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* were obtained from microbial culture collection, Centre for Phytomedicine and Medicinal Organic Chemistry, University of Peshawar, Peshawar.

Antimicrobial activity

The antibacterial activity of the different fractions of the plant was determined by modified agar well diffusion method. The Müller-Hinton agar was used as medium. The cultures were taken in triplicates at incubation temperature of 37°C for 24 to 72 h. The broth culture (0.6 ml) of the test organism was placed in a sterile Petri-dish and 20 ml of the sterile molten Müller-Hinton agar (MHA) was added. Holes were bored in to the medium using 0.2 ml of each extract. Streptomycin was the standard antimicrobial agent at concentration of 2 mg/ml. Inoculation was done for 1 h to make possible the diffusion of the antimicrobial agent into the medium. Incubation was done at 37°C for 24 h and the diameters of the zone of inhibition of microbial growth were measured in the plate in millimeters.

Antioxidant activity

The hydrogen atom or electron donation abilities of the corresponding plant extracts against 2, 2-diphenyl-1-picrylhydrazyl (DPPH.) was determined by UV spectrophotometry at 517 nm. Radical scavenging activity was measured by a slight modified described by Blois (1958). Briefly, 1 mM solution of DPPH radical solution in methanol was prepared as standard solution and 1 ml of the standard solution was mixed with 3 ml of each sample solution in ethanol (containing 20 to 100 *u*g), and standard drug (querceitin) as control separately. The reaction mixture was allowed to react completely at room temperature for 30 min and absorbance of the resulting mixture was measured spectrophotometrically at 517 nm and converted to percentage DPPH radical scavenging activity.

RESULTS AND DISCUSSION

Table 1 shows the weight percentage yield of *n*-hexane, chloroform and ethyl acetate fractions of the aerial parts of *E. umbellata.* The chloroform and ethyl acetate fraction contains a greater proportion of the phytoconstituents. Different solvents fractions were obtained from the methanolic extract which was evaluated for their phytoconstituents. Steroids, terpenoids, alkaloids and saponins are the major constituents present in the fraction of chloroform and ethyl acetate while *n*-hexane fraction has only steroid (Table 2). The presences of these secondary metabolites were confirmed for their respective functional groups by IR spectroscopy analysis. The IR spectrums (Table 3) were exhibited strong absorption bands at 3398, 3398.3323 and 3365 cm^{-1}, indicated O-H stretching and hydroxyl groups; 1737, 1732 and 1708 indicated the presence of C=O aldehyde stretching and ketonic stretching respectively while 1653, 1605, 1558, 1516. 1558, 1556, and 1367 cm^{-1} indicated the presence of amide stretching; C=C stretching and NH stretching, respectively.

The antibacterial value of various fractions (*n*-hexane, chloroform, ethyl acetate and methanolic extract) of the plant was tested against the selected human pathogenic bacteria; *S. aureus*, *S. epidermis, B. subtilis*, *E. coli* and *K. pneumonia* (Table 4 and Figure 1) which showed varied level of inhibition. All the fractions were found active against Gram positive while totally remain inert in Gram negative bacteria. The highest activity was shown by ethyl acetate fraction against *S. epidermis.* Significant activity against *S. aureus, S. epidermis* and *B. subtilis* were also shown by ethyl acetate fraction and methanolic extract. The DPPH antioxidant assay provides information on the reactivity of the fractions with a stable

Table 2. Phytochemical screening of *n*-hexane, chloroform, ethyl acetate and methanolic crude extract of *E. umbellate.*

Chemical components	*n*-Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	+	+	+
Steroids	+	+	+	+
Terpenoids	-	+	+	+
Flavonoids	-	-	-	-
Anthraquinones	-	-	-	-
Tannins	-	-	-	-
Phlobatanins	-	-	-	-
Saponins	-	+	+	+
Glycoside	-	-	-	-
Reducing sugars	-	-	+	+

– = absent, + = present.

Table 3. IR Spectroscopic data of the methanolic crude extract and fractions of *E. umbellate.*

Components	Region (cm^{-1})			
	n-Hexane	Chloroform	Ethyl acetate	Methanol
OH	3398, 1238	3398, 1261	3323, 1255	3365, 1261
CH	2922	2927	2929	2926
CHO	2852, 1737	2850, 1732	-	-
C=O ketone	1708	-	-	-
C=O α, β unsaturated -		1687	-	1687
C=O amide	1653	-	-	-
C=C	-	1604	-	-
NH def	1558	1516	1558, 1517	-
NO_2	1456, 1375	1456, 1375	1440, 1375	1456, 1367
Ar-O	-	1271, 1238	-	1271, 1242
C-O-C	1240, 1165	1238	1197	1242, 1197

Table 4. Antimicrobial activity of the methanolic crude extract and fractions of *E. umbellate.*

Microorganism	Gram	Hexane ext	$CHCl_3$ ext	EtOAc ext	MeOH ext
Escherichia coli (E. c)	-	NA	NA	NA	NA
Staphylococcus aureus (S. a)	+	NA	12	18	18
Klebsiella pneumonia (K. p)	-	NA	NA	NA	NA
Staph epidermis (S. e)	+	16	14	20	16
Bacillus subtilis (B. s)	+	8	10	18	14

NA, Not active, ext-extract; Well size: 4 mm.

free radical. DPPH gives a strong absorption band at 517 nm in visible region and showed activity at different level (Table 5 and Figure 2). The highest anti-radical activity was found in the methanolic extract (EC 50: 50.5 µg/ml) which is almost equal to the activity shown by standard querceitin (EC 50: 50.5 µg/ml). Ethyl acetate and *n*-hexane fraction also showed moderate activity whereas chloroform fraction was the least active fraction among the entire fractions.

The medicinal value of the plant can be correlated to the presence of various bioactive secondary metabolites. The *n*-hexane, chloroform, ethyl acetate fractions and methanolic extract of the *E. umbellata* showed the presence of terpenoids which exhibits antiviral and

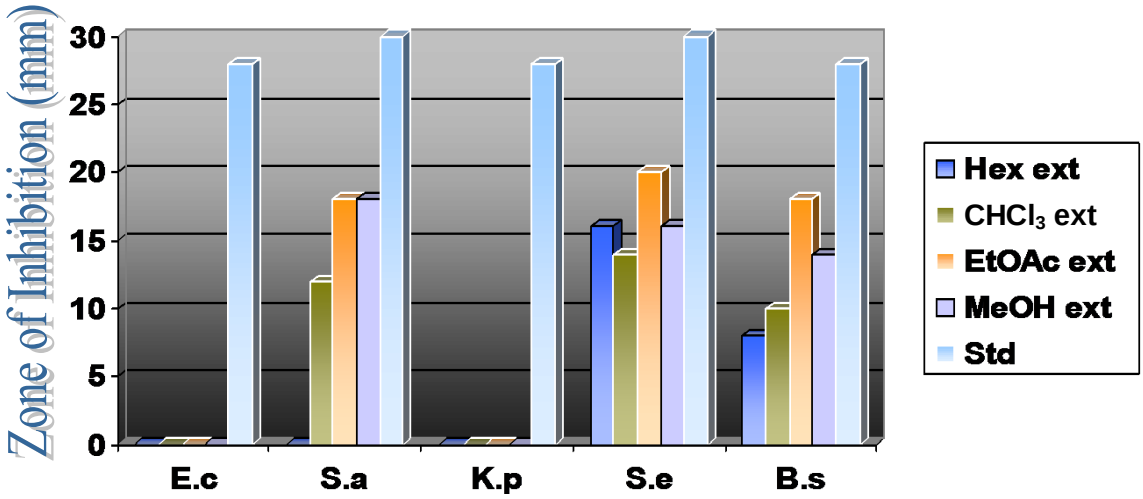

Figure 1. Antibacterial activity of the crude extracts of *E. umbellata* with comparison of streptomycin standard.

Table 5. Antiradical activity of various extracts of *E. umbellata* against DPPH radical.

Fraction	RSA at 100 µg/ml (%)	EC_{50} (µg/ml)
n-Hexane	86.9 ± 3.8	65.4
Chloroform	30.5 ± 2.7	250.6
Ethyl acetate	79.9 ± 3.1	42.5
Methanol	80.9 ± 2.4	5.5
Querceitin	98.6 ± 2.7	4.12

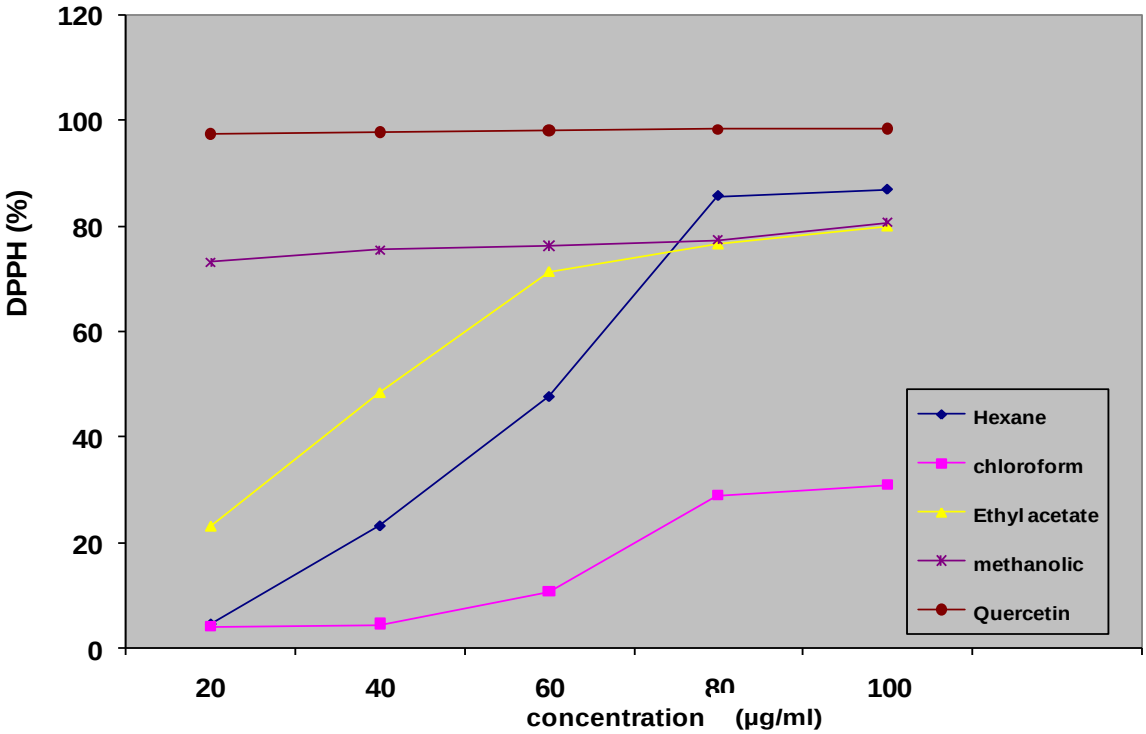

Figure 2. DPPH radical scavenging activities of the crude extract and fractions of *E. umbellate.*

antibacterial activities (Shashi and Sucharita, 1997). Alkaloids presence in the chloroform, ethyl acetate fractions and methanolic extract exhibited activities; anthelmintic activity (Bever, 1986), aphrodisiac activity (Maggi et al., 2000), treatment of venereal diseases (Boustie et al., 1989) and anti-malarial activity (Chawira et al., 1987).

Our findings correlate with the observations of previous screened medicinal plants for antimicrobial activity, where most of the active plants showed activity against Gram-positive strains while few of them are active against Gram-negative bacteria (Arias et al., 2002).

The pharmacological activity of *E. umbellata* was confirmed from the antimicrobial assay of various fractions and methanolic extract. The ethyl acetate fraction possessed activity against *S. aureus, S. epidermis and B. subtilis* with a zone of inhibition ranging from 18 to 20 mm, showing its medicinal importance in the treatment of gastroenteritis, and pneumonia while the methanolic extract showed active against *S. aureus, S. epidermis and B. subtilis* with a zone of inhibition ranging from 14 to 18 mm. The *n*-hexane fraction showed activity against, *S. epidermis and B. subtilis* with a zone of inhibition ranging from 8 to 16 mm while chloroform fraction showed significant activity against, *S. aureus, S. epidermis and B. subtilis* with a zone of inhibition ranging from 10 to 14 mm. The antimicrobial activity in other species of this genus has been found to be closely related to the levels of alkaloids and alkaloids derivatives (Arias et al., 2002), which are responsible for the antimicrobial activities. Our results suggested that further work is needed to locate the active principles from the various extracts or fractions and such efforts could result in the discovery of new compounds possessing a wide range of bioactivity for the treatment of infectious disease.

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