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Antimicrobial and Phytochemical Evaluation of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

Ambrin[1]*, Ghulam Dastagir[1], Jehan Bakht[2] and Muhammad Adil[1]

[1]*Department of Botany, University of Peshawar, Peshawar, KPK, Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, KPK, Pakistan*

ABSTRACT

The present study investigates antimicrobial activities of different solvent extracted samples of *Ziziphus mauritiana* var. *spontanea* and *Oenothera biennis* against different microbes. Ethyl acetate and ethanolic extracts of *Z. mauritiana* var. *spontanea* measured the highest zone of inhibition against *Staphylococcus epidermidis, Escherichia coli* and *Salmonella typhi*. The MIC values of ethyl acetate and ethanolic extracts of *Z. mauritiana* var. *spontanea* were the lowest for *Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumonia, Shigella flexneri* and *Staphylococcus aureus*. Highest zone of inhibition against *Klebsiella pneumonia and Pseudomonas aeruginosa* was shown by ethyl acetate and ethanolic extracts of *O. biennis*. Lowest MIC value was noted for ethyl acetate and ethanolic extracts of *O. biennis* against *Staphylococcus epidermidis, Staphylococcus aureus, Salmonella typhi* and *Shigella flexneri*. The ethyl acetate and ethanolic extracts of *Z. mauritiana* var. *spontanea* showed good antifungal activity against *Aspergillus niger, Penicillium notatum*. Minimum MIC value was shown by ethyl acetate and ethanolic extracts of *Z. mauritiana* against *Aspergillus fumigatus* and *Aspergillus niger*. The ethyl acetate and ethanolic extract of *O. biennis* revealed maximum antifungal activity against *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. The MIC value of ethyl acetate and ethanolic extracts of *O. biennis* was lowest for *Fusarium solani* and *Penicillium notatum*. Phytochemical screening of *Z. mauritiana* var. *spontanea* and *O. biennis* revealed the presence of amino acids, reducing sugar, proteins, triterpenoids, gums and mucilages, fats, steroids, phenols and phytosterol.

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Authors' Contribution
Ambrin conducted the experiments and collected the data. GD designed the study and provided lab facilities. JB wrote the manuscript. MD did statistical analysis.

Key words
***Z. mauritiana, O. biennis*, Antibacterial, Antifungal, Phytochemical screening**

INTRODUCTION

Plants used in traditional medicine contain a wide variety of compounds which can be used to treat chronic and infectious diseases. It is estimated that about 35,000 to 70,000 plants species are used as medicinal plants worldwide (Bibi *et al*., 2011). Approximately 80% of the population in Pakistan use traditional medicines for several ailments (Haq, 1997; Munir *et al*., 2013). The antimicrobial potential of plant extracts may be due to the presence of secondary metabolites (Ganesh *et al*., 2014). Medicinal plants can be used to cure different microbial infections (Bakht *et al*., 2018; Bilal *et al*., 2018; Ayaz *et al*., 2017, 2018; Wajid *et al*., 2017). Contagious diseases are common in most of the developing countries, including Pakistan, therefore, it is important to search out and promote plant-based medication as natural medicines cost effective with fewer side effects as compared to the synthetic medicines. Synthetic antimicrobial agents are expensive and beyond the reach of common people.

Ziziphus mauritiana var. *spontanea* Edgew. commonly known as Beri, Jand, Mada bera, Ashar in Pakistan belongs to the family Rhamnaceae (Hussain *et al*., 2011; Ahmad *et al*., 2011; Ahmad *et al*., 2012). Members of this family are found in tropical and subtropical regions of the world including India, Sri Lanka, Pakistan, Iran, Greece and Afghanistan. Generally, *Ziziphus* reported to have anxiolytic, sedative, haemolytic, antidiabetic, analgesic, antiplasmodial, diuretic, anticonvulsant, hypoglycemic and anti-inflammatory activities (Goyal *et al*., 2012). It occurs commonly in Pakistan, China, Australia, India, Afghanistan and Ceylon. Several species of *Ziziphus* have shown medicinal value such as *Z. oxyphylla* is used in Pakistan for Obesity, urinary troubles, digestive disorders, skin infections, weakness, diabetes, diarrhea, liver complaints, fever and insomnia (Kaleem *et al*., 2014). *Z. nummularia* can be used as cooling, laxative, astringent, stomachic, cures mucous and for treating scabies. *Z. spina-christi* leaves are effective as anti-inflammatory, antiseptic and anti-fungal and used to treat obesity, digestive disorders, urinary troubles and can be used as anti-microbial agent. Fruit of *Z. mauritiana* var. *spontanea*

* Corresponding author: Ambreenkhan727@gmail.com
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is used as blood purifier and digestive stimulant. Stem bark is used for dysentery and diarrhea when mixed with honey or milk (Amjad *et al*., 2017). *Oenothera biennis* L. commonly known as evening primrose, evening star, or sun drop belongs to the family Onagraceae. It is found in eastern and central North America. Its seeds contain high quantities of Gamma Linolenic Acid (up to 20-25% GLA) and can be used for curing several ailments (Anwar *et al*., 1998). *Oenothera biennis* possesses antiviral, antibacterial, anti-inflammatory, anti-thrombolytic, anti-hyperlipidaemic, antioxidant and cytotoxic activities (Singh *et al*., 2012).

MATERIALS AND METHODS

Collection and identification of plant materials

The fresh plants of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L. were collected from Palosai area of district Peshawar KPK Pakistan and identified at the Department of Botany, University of Peshawar, KPK Pakistan. The shade dried plant materials were ground to get powder. Fifty grams of powdered plant materials were soaked in 250 ml each of ethanol and ethyl acetate in separate flat-bottomed flasks. The mixture was filtered by Whatman filter paper No. 1823 and the same procedures were followed three times. The pooled filtrates were concentrated by rotary evaporator (Heidolph Collegiate, LV28798826, New Jersey, USA) and stored at 4°C until used.

Antimicrobial activity

The antimicrobial activity of ethanolic and ethyl acetate extracts of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L. was studied using well diffusion agar method as described by Atta-ur-Rehman *et al*. (1991) against *Staphylococcus aureus*, *Staphylococcus epidermidis* and five Gram negative bacterial strains namely *Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeraginosa, Shigella flexneri, Salmonella typhi, Aspergillus niger*, *Aspergillus flavus, Fusarium solani, Aspergillus fumigatus* and *Penicillium notatum*.

Phytochemical screening

Steroids and reducing sugars were determined according to the methods of Edeoga *et al*. (2005) and Evans (2002) respectively. Protein was estimated by Kumar and Kiladi (2009). The method of Harborne (1998) was used for the determination of tri-terpenoids and phytosterol. Phenolic compounds, gum and mucilage was estimated by the procedures of Dahiru *et al*. (2006) and Ansari (2006), respectively.

Statistical analysis

Data are presented as mean values of three replications with standard deviation. MSTATC computer software was used for to carry out statistical analysis (Russel and Eisensmith, 1983).

RESULTS

Antibacterial activity

The antibacterial activity of ethyl acetate extract of *Ziziphus mauritiana* var *spontanea* showed the highest zone of inhibition (13 mm) against *Staphylococcus epidermidis* followed by *Staphylococcus aureus*, *Shigella flexneri* (10 mm) and the lowest zone of inhibition (6 mm) was shown by *E. coli*. Similarly, the ethanolic extract of *Ziziphus mauritiana* showed the highest antibacterial potential (14mm) against *Escherichia coli* and *Salmonella typhi* followed by *Staphylococcus epidermidis* and *Staphylococcus aureus* (10mm) and the lowest zone of inhibition (6 mm) was shown against *Klebsiella pneumoniaee* (Table I). The MIC values of ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea* were the lowest (2µg/ml) for *Staphylococcus aureus, Staphylococcus epidermidis* and *Klebsiella pneumoniaee* (Table II). Likewise, the MIC values of ethanolic extract were the lowest (2 µg/ml) for *Shigella flexneri* and *Staphylococcus aureus* (Table II).

The antibacterial activity of ethyl acetate extract of *Oenothera biennis* showed the highest zone of inhibition (17mm) against *Klebsiella pneumoniaee* followed by *E. coli, Pseudomonas aeruginosa* and the lowest zone of inhibition (6 mm) was shown by *Salmonella typhi*. Similarly, the antibacterial activity of ethanolic extract of *Oenothera biennis* revealed the highest zone of inhibition (15mm) against *Pseudomonas aeruginosa* followed by *Escherichia coli* and *Salmonella typhi* (12mm) and the lowest zone of inhibition (8 mm) was shown by *Klebsiella pneumoniaee* (Table I). The MIC values of ethyl acetate extract of *Oenothera biennis* were the lowest (2 µg/ml) for *Staphylococcus epidermidis, Staphylococcus aureus* and *Salmonella typhi* (Table II). The MIC values of ethanolic extract were lowest (2 µg/ml) for *Staphylococcus epidermidis* and *Shigella flexneri* (Table II).

Antifungal activity

The ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea* showed good antifungal activity (16mm) against *Aspergillus niger* followed by *Fusarium solani* (12 mm) and lowest antifungal activity against *Aspergillus flavus* (8mm). Similarly, the ethanolic extract of *Ziziphus mauritiana* var. spontanea measured the highest antifungal activity (14 mm) against *Penicillium notatum* followed by *Aspergillus fumigatus* (12mm) and the lowest antifungal activity

Table I. Antibacterial activity as demonsrated by zones of inhibition (mm) of organic solvent extracts of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

Bacteria	Zone of inhibition (mm)																
	Control		*Ziziphus mauritiana* var. *spontanea* Edgew.								*Oenothera biennis* L.							
	Positive	Negative	Ethyl acetate extract (µg/ml)				Ethanol extract (µg/ml)				Ethyl acetate extract (µg/ml)				Ethanol extract (µg/ml)			
Concentrations			**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**
Pseudomonas aeruginosa	18±0.43	0	4±0.43	4±0.83	6±0.45	8±0.73	6±0.20	8±0.10	8±0.25	8±0.15	6±0.26	10±0.26	10±0.40	12±0.15	6±0.15	8±0.36	10±0.41	15±0.47
Escherichia coli	15±0.78	0	2±0.25	4±0.20	6±0.43	6±0.55	6±0.15	9±0.25	10±0.49	14±0.20	4±0.20	8±0.52	8±0.43	12±0.55	2±0.50	2±0.20	8±0.60	12±0.45
Staphylococcus epidermidis	15±0.20	0	4±0.40	6±0.62	12±0.80	13±0.51	4±0.64	6±0.55	8±0.95	10±0.47	2±0.56	4±0.80	8±0.36	8±0.37	4±0.79	8±0.56	8±0.60	10±0.65
Staphylococcus aureus	18±0.58	0	3±0.49	6±0.65	6±0.70	10±0.35	2±0.80	4±0.77	4±0.65	10±0.85	8±0.86	6±0.83	6±0.45	10±0.70	6±0.25	8±0.66	10±0.75	10±0.30
Klebsiella pneumoniae	19±0.20	0	4±0.81	6±0.64	8±0.20	8±0.96	2±0.40	2±0.10	4±0.55	6±0.26	8±0.40	10±0.45	12±0.72	17±0.40	6±0.30	8±0.26	8±0.76	8±0.60
Salmonella typhi	16±0.73	0	2±0.20	4±0.65	4±0.26	8±0.26	4±0.36	8±0.52	10±0.70	14±0.25	2±0.20	4±0.60	6±0.47	6±0.40	4±0.50	4±0.66	8±0.20	12±0.20
Shigella flexneri	20±0.58	0	6±0.65	6±0.40	8±0.55	10±0.25	2±0.15	4±0.45	6±0.65	8±0.26	4±0.25	4±0.40	6±0.41	10±0.20	3±0.41	6±0.66	6±0.36	11±0.17

±, Standard deviation of the triplicated data

Table II. MIC values of ethyl acetate and ethanolic extract of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

MIC values (µg/ml)	*Ziziphus mauritiana.* var. *spontanea*		*Oenothera biennis.* L.	
Bacteria	**Ethyl acetate extract**	**Ethanol extract**	**Ethyl acetate extract**	**Ethanol extract**
Pseudomonas aeraginosa	8±0.55	5±0.36	6±0.15	6±0.45
Escherichia coli	5±0.25	8±0.73	5±0.76	8±0.25
Staphylococcus epidermidis	2±0.20	8±0.87	2±0.70	2±0.25
Staphylococcus aureus	2±0.52	2±0.30	2±0.75	8±0.50
Klebsiella pneumoniae	2±0.58	6±0.55	8±0.40	6±0.05
Salmonella typhi	6±0.66	5±0.49	2±0.20	8±0.41
Shigella flexneri	6±0.30	2±0.15	8±0.20	2±0.15

±, Standard deviation of the triplicated data

Table III. Antifungal activity of *Ziziphus mauritiana.* var. *spontanea* Edgew. and *Oenothera biennis.* L.

Fungi	*Ziziphus mauritiana* var. *spontanea* Lam.								*Oenothera biennis* L.							
	Positive control	Negative control	Ethyl acetate extract (µg/ml)				Ethanol extract (µg/ml)				Ethyl acetate extract (µg/ml)				Ethanol extract (µg/ml)			
Concentrations			**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**
Aspergillus flavus	18±0.25	0	6±0.55	8±0.43	10±0.25	8±0.75	4±0.10	4±0.75	8±0.25	10±0.62	3±0.15	6±0.20	10±0.25	10±0.41	7±0.26	10±0.47	10±0.10	16±0.70
Aspergillus fumigatus	19±0.55	0	0±0.00	5±0.43	8±0.52	10±0.49	2±0.20	4±0.70	10±0.51	12±0.45	5±0.62	7±0.64	9±0.20	10±0.55	5±0.62	7±0.65	10±0.40	16±0.40
Fusarium solani	24±0.40	0	3±0.20	7±0.15	10±0.25	12±0.45	0	0	6±0.20	6±0.17	0	3±0.20	7±0.20	12±0.20	4±0.45	6±0.34	6±0.49	8±0.73
Aspergillus niger	18±0.41	0	2±0.15	5±0.56	7±0.66	16±0.15	0	6±0.32	9±0.15	11±0.20	0	0	2±0.56	15±0.30	5±0.56	8±0.25	8±0.51	10±0.20
Penicillium notatum	22±0.37	0	6±0.15	8±0.73	8±0.25	10±0.20	8±0.41	10±0.26	12±0.55	14±0.45	0	4±0.20	8±0.40	2±0.40	8±0.25	12±0.61	16±0.32	12±0.83

±, Standard deviation of the triplicated data.

(6mm) against *Fusarium solani* at 40 µg/ml extract concentration (Table III). The MIC value of ethyl acetate extract of *Z. mauritiana* was lowest for *Aspergillus fumigatus* whereas the MIC value of ethanolic extract of *Z. mauritiana* was lowest for *Aspergillus niger* (Table IV).

Table IV. MIC values of ethyl acetate and ethanolic extract of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

***Ziziphus mauritiana.* var. *spontanea* Edgew.**		***Oenothera biennis* L.**	
MIC values (µg/ml)			
Fungi	**Ethyl acetate extract**	**Ethanol extract**	**Ethyl acetate extract**	**Ethanol extract**
Aspergillus flavus	7±0.56	8±0.52	7±0.49	6±0.15
Aspergillus fumigatus	3±0.37	6±0.58	6±0.30	5±0.15
Fusarium solani	5±0.76	6±0.76	2±0.23	6±0.30
Aspergillus niger	7±0.26	2±0.15	4±0.65	4±0.55
Penicillium notatum	5±0.26	5±0.45	8±0.35	2±0.60

Table V. Phytochemical analysis of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

	***Ziziphus mauritiana* var. *Spontanea* Edgew.**		***Oenothera biennis* L.**	
Chemical constituents	Ethyl acetate extract	Ethanol extract	Ethyl acetate extract	Ethanol extract
Amino acids	+	+	+	+
Reducing sugar	+	+	+	-
Gum and mucilage	-	+	+	+
Phenols	+	+	-	+
Phytosterol	+	-	-	+
Proteins	+	+	+	+
fats	-	+	+	+
Triterpenoids	+	+	+	-
Steriods	+	-	+	+

The ethyl acetate extract of *Oenothra biennis* revealed maximum antifungal activity (15mm) against *Aspergillus niger* followed by *Fusarium solani* (12 mm) and minimum (2mm) against *Penicillium notatum*. Similarly, the ethanolic extract of *Oenothera biennis* showed highest antifungal activity (16 mm) against *Aspergillus flavus, Aspergillus fumigatus* followed by *Penicillium notatum* (12mm) and the lowest antifungal activity (8mm) against *Fusarium solani* at the maximum 40 µg/ml extract concentration (Table III). The MIC value of ethyl acetate extract of *O. biennis* was lowest for *Fusarium solani* whereas the MIC value of ethanolic extract of *O. biennis* was lowest for *Penicillium notatum* (Table IV).

Phytochemical analysis of different solvent extracted samples of *Ziziphus mauritiana* var. *spontanea* revealed the presence of amino acids, reducing sugar, phenols, phytosterol, proteins, steroids, terpenoids and gum and mucilages. Similarly, phytochemical screening of whole plant of *Oenothera biennis* indicated the presence of amino acids, fats, proteins, reducing sugar, steroids, phenols, phytosterol, gums and muclilages and terpenoids and (Table V).

DISCUSSION

The selection of the plants reported in this study was based on the medicinal uses reported by various workers in literature review. The antibacterial activity revealed that *E. coli* and *Salmonella typhi* were the most sensitive bacteria to ethanolic extract of *Ziziphus mauritiana* var. *spontanea*. These results agree with Okwulehie *et al.* (2013) who reported similar results for *Ficus experata* and *Camellia sinensis*. *Staphylococcus epidermidis* was the most sensitive to the ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea.* These results are in line with Prabhakaran *et al.* (2014) and Mills-Robertson *et al.* (2012) who reported similar findings for *Careya arborea* and *Cryptolepis sanguinolenta.* The MIC values revealed that *Staphylococcus aureus, Staphylococcus epidermidis* and *Klebsiella pneumoniae* were the most susceptible bacteria to the ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea* whereas *Shigella flexneri* and *Staphylococcus aureus* were the most susceptible bacteria to ethanolic extract of *Ziziphus mauritiana* var. *spontanea*. *Pseudomonas aeruginosa* was the most sensitive bacteria to ethanolic extract of *Oenothera biennis.* These results agree with Abalaka *et al.* (2017) and Awah *et al.* (2017) who reported similar antibacterial potential of ethanolic extract of *Garcinia kola* and *Carica papaya*. *Klebsiella pneumoniae* was the most sensitive bacteria to ethyl acetate extract of *Oenothera biennis.* Idris *et al.* (2016) reported similar antibacterial effect of the ethyl acetate extract of *Peperomia pellucida* and *Bridelia ferruginea*. The MIC values exhibited that *Staphylococcus epidermidis, Staphylococcus aureus* and *Salmonella typhi* were the most susceptible bacteria to the ethyl acetate extract of *Oenothera biennis* whereas *Staphylococcus epidermidis* and *Shigella flexneri* were the most susceptible bacteria to ethanolic extract of *Oenothera biennis*. These results

are in line with Bajpai *et al*. (2009) and Leela and Satirapipathkul (2011) who reported that susceptibility of Gram positive bacteria as compared to the Gram negative bacteria due to a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts (Biswas *et al*., 2013). Interaction of phenols, terpenoids and steroids with membrane proteins of bacteria can modify membrane permeability resulting in cell destruction. They can also penetrate into bacterial cells and coagulate cell content (Sengul *et al*., 2009; Shihabudeen *et al*., 2010).

The ethyl acetate extract of *Ziziphus mauritiana* var. *spontanea* revealed maximum antifungal activity against *Aspergillus niger*. These results agree with Rashid *et al*. (2017) who reported significant antifungal activity against *Aspergillus niger* by the ethyl acetate extract of *Seidenfia rheedii* and *Hartmannia rosea.* Similarly, the ethanolic extract of *Ziziphus mauritiana* var. *spontanea* measured the highest antifungal activity against *Penicilium notatum*. Kumar *et al*. (2010) reported highest inhibition of *Penicilium notatum* due to ethanolic extract of *Murraya koenigii* and *Rhizophora mucronata.* The MIC value showed that *A. fumigatus* was susceptible to the ethyl acetate extract of *Z. mauritiana* and *A. niger* was susceptible to the ethanolic extract of *Z. mauritiana*.

The ethyl acetate extract of *Oenothera biennis* indicated good antifungal activity against *Aspergillus niger.* These results are in line with Bharathi *et al*. (2014) who reported highest antifungal against *Aspergillus niger* by the ethyl acetate extract of *Azadirachta indica.* Similarly, the ethanolic extract of *Oenothera biennis* revealed highest antifungal activity against *Aspergillus flavus, Aspergillus fumigatus.* Abbas *et al*. (2017) reported highest antifungal activity against *Aspergillus flavus* and *Aspergillus fumigatus* by the extracts of *Heterostemma tanjorense* and *Melia azedarach.* The MIC value showed that *F. solani* was susceptible to the ethyl acetate extract of *O. biennis* and *P. notatum* was susceptible to the ethanolic extract of *O. biennis*).

Phytochemical analysis showed the occurrence of various groups of compounds in both plants. Both plants revealed the presence of amino acids, reducing sugar, phenols, phytosterol, proteins, steroids, terpenoids and gum and mucilages (Khamis and Aly, 2017; Tikadar *et al*., 2017). Manchu *et al*. (2015) reported that phenols, terpenoids, steroids and phytosterols possess antimicrobial properties. The secondary metabolites present in plants reveal biological activities including antimicrobial activities (Yadav *et al*., 2014). These results showed that both plants are rich sources of secondary metabolites that can be used for the synthesis of new drugs for curing various diseases.

CONCLUSION

These results revealed the antimicrobial potentiality of ethyl acetate and ethanolic extracts of *Ziziphus mauritiana* and *Oenothera biennis*. The ethanolic extract of *Z. mauritiana* caused greater inhibition of bacteria as compared to ethyl acetate extract. Whereas ethyl acetate extract of *O. biennis* was more potent against different strains of bacteria as compared to ethanolic extract. Similarly, the ethyl acetate extract of *Z. mauritiana* showed highest antifungal activity and in case of *O. biennis* the ethanolic extract showed greater antifungal activity. Phytochemical screening revealed the the presence of phenols, terpenoids, amino acids, gums and mucilages, phytosterols, reducing sugar, steroids, proteins in the extracts of both plants.

Statement of conflict of interest

The authors have declared no conflict of interest.

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