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Evaluation of Antimicrobial Activities and Phytochemical Screening of Selected Wild Edible Plants of District Malakand, Khyber Pakhtunkhwa, Pakistan

Muhammmad Ibrahim[1*], Naveed Akhtar[1], Arshad Iqbal[1], Haji Bahadar[2], Sara[3]

[1]*Department of Botany, Islamia College Peshawar, Khyber Pakhtunkhwa-Pakistan*

[2]*Institute of Pharmaceutical Sciences, Khyber Medical University Peshawar, Peshawar-Pakistan*

[3]*Department of Zoology, University of Peshawar, Khyber Pakhtunkhwa-Pakistan*

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Abstract

The current research work was done to evaluate the antimicrobial and phytochemical perspective of methanolic extracts of five selected wild edible plants such as *Asparagus officinalis, Caralluma tuberculata Chenopodium album, Rumex dentatus* and *Solanum nigrum* of district Malakand, Pakistan. These selected wild edible plants are traditionally used as food and medicinal plants with several healing possessions. The strains of tested microbes were exposed to the methonolic extracts of selected plants by applying the agar well diffusion method. The bacterial strains used were *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus*. The fungal strains used were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporium* and *Alternaria alternata*. The phytochemical investigation was achieved by using standard procedures. The enhancement in the antibacterial activity was detected by increasing the concentration of methanolic extracts. Methanolic extract of *Rumex dentatus* revealed maximum antibacterial activity against *Staphylococcus aureus* with inhibition zone (21 ± 1.2 mm), followed by *Solanum nigrum* against *Escherichia coli* with inhibition zone (20 ± 0.5 mm) at the concentration (9µl). The maximum antifungal action was shown by the methanolic extract of *Solanum nigrum* against *Fusarium oxysporium* with the zone of inhibition (27 ± 0.3 mm), followed by *Rumex dentatus against Aspergillus flavus* having inhibition zone (26 ± 0.3 mm) at the concentration (9µl). The phytochemical analysis exposed that all these selected wild edible plants were rich in different secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, tannins and steroids. The study concludes that these plants have new substances with notable antimicrobial possessions.

* **Corresponding Author:** Muhammmad Ibrahim ✉ ibrahim.bot@gmail.com

Introduction

Plants play a vital and significant role in the health of individuals and populations (Thakur and Rays, 2014). Plants have been used as a first-line defense system to cure microbial infections. When at the start of the 20th century the antibiotics were discovered, the scientists started to develop new synthetic antibiotics of microbial origin. Excessive use of these antibiotics by human beings and other animals, give rise to the issue of antimicrobial opposition (Moshirfar *et al.*, 2006). Now at present, antimicrobial resistance is the most important health issue that has made the use of several antibiotics ineffective. The frequent use of so-called "miracle drugs" for a longer period has made these synthetic drugs less effective than they were before. The number of antibiotic-resistant strains has been increased to an alarming situation. Bacterial and fungal infections are becoming resistant to antibiotics throughout the world (Johnson *et al.*, 2006; Woodford and Ellington, 2007). The synthesis of novel antibiotics has been extremely decreased nowadays. (Davis, 1994). Moreover, these synthetic drugs are causing adversative effects by initiating hypersensitivity and suppression of the immune system in the host (Ahmad *et al.*, 1996). Hence it is needful to discover and improve alternate medicines to cure different contagious diseases from plant sources (Cordell, 2000).

Plants are the natural paragon of useful prospective medicines. It is thought that about 250,000 thousand to 500,000 thousand plants are present on the earth's surface (Borris, 1996). A very insignificant number of these plants have been exposed so far for their antimicrobial and therapeutic properties.

It is said that only 1% higher plants out of 250,000 species have been analysed for their photochemical or pharmacological activities (Petlevski *et al.*, 2001). Plants are the best springs of antimicrobial and phytochemical constituents especially phenols, tannins, alkaloids, flavonoids, saponins, terpenes and terpenoids (Tiwari and Singh, 2004; Lewis and Ausubel, 2006). Research-based studies indicated that plant extracts showed antimicrobial latent against many disease-causing microorganisms. It is important to comment that plant-based phytochemicals are the best emerging antimicrobial agents against infectious microbes (Hussain *et al.*, 2015; Sharvani *et al.*, 2015).

Disease-causing microorganisms constantly postured stern issues to the health of human beings and other animals. Infectious agents like viruses, bacteria, fungi and protozoans are the causal agents of many contagious diseases such as AIDS, tuberculosis, syphilis, candidiasis, aspergillosis, etc.

Infectious ailments are the second prominent reason for worldwide deaths (WHO, 2002). From times immemorial humans are being infected by these microbes till the present day. Microbes like *Bacillus cereus*, *Klebsiella pneumonia*, and *Escherichia coli* causes gastrointestinal diseases, vomiting, pneumonia, skin diseases, and respiratory tract infections. *E. coli* also cause gynecological infections. *Staphylococcus aureus* causes gastrointestinal disorders, lower respiratory tract infections and skin infections (Eley, 1992; Karch and Karch, 2014). Fungal pathogens such as *Aspergillus niger* cause aspergillosis, fungal ear infections (Otomycosis) (Gautam *et al.*, 2011), *Aspergillus flavus* causes Sinusitis, cutaneous aspergillosis and wound infections (Hedayati *et al.*, 2007). *Fusarium oxysporium* causes sinusitis and mycotoxicosis in severely immunocompromised patients (Jain *et al.*, 2011) whereas, *Alternaria altenata* causes asthma and allergic sinusitis (Pastor and Guarro, 2008).

Materials and methods

Collection and identification of plant materials

Samples of *Asparagus officinalis, Caralluma tuberculata, Chenopodium album, Rumex dentatus* and *Solanum nigrum* were gathered in field visits from study sites of District Malakand, Khyber Pakhtunkhwa, Pakistan during spring 2020.

The plants were then recognized with the support of relevant literature and the flora of Pakistan (Ali and Qaiser, 1993-2015).

Preparation of plant extracts

The plants collected were first washed and then dried in shade at normal room temperature. The samples when completely dried were ground into fine powder in a mixer. The powder was extracted with help of methanol solvent, with the help of Soxhlet extractor at a temperature between 60- 80°C. The methanolic plant extracts were made concentrated by using a rotary evaporator under low pressure to get solid plant extracts. Preparation of methanolic plant extracts was done by dissolving solid extracts in 10% Dimethyl sulfoxide. The extracts of plants thus made were kept at 4°C in a refrigerator till use.

Tested micro-organisms

Four types of bacteria consisting of two Gram-negative bacteria (*Klebsiella pneumoniae* and *Escherichia coli*) while two Gram-positive bacteria (*Bacillus cereus* and *Staphylococcus aureus*) were tested.

The four fungal strains used were *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *Alternaria alternata*. All the clinical isolates were obtained from the Institute of Biotechnology and Genetic Engineering, University of Agriculture Peshawar, Pakistan. The strains of Bacteria and fungi were retained by keeping them on Agar and Sabouraud Dextrose agar (Himedia) separately. Chloramphenicol and Fluconazole discs were taken as positive controls for antibacterial and antifungal tests. For negative control, Dimethyl sulfoxide (DMSO) 10 % was used.

Antibacterial assay

Agar well diffusion method was used for antibacterial assays against methanolic plant extracts as previously described (Irshad *et al.*, 2012). The standardized inoculum (100 µl) of each bacteria was inoculated on molten Mueller Hinton Agar medium, make uninformed and transferred into sterile Petri dishes. The Petri dishes get solidified inside the laminar hood flow. A sterile cork borer of 6 mm in diameter was used to make 3 uniform wells at the periphery in each petri dish. Plant extracts, prepared in 10% Dimethyl sulfoxide (DMSO) of 3µl, 6µl and 9µl were poured into each of the 3 peripheral wells. Chloramphenicol disc was taken as a positive control whereas, Dimethyl sulfoxide (DMSO) was used as a negative control in separate Petri dishes. The Petri dishes were kept for 18 - 24 hours at 37°C in an incubator. Petri dishes were seen for the antibacterial inhibition zones. The antibacterial potential of different plant extracts was assessed by measuring the diameters of zones of inhibition and were expressed in millimeters (mm).

Antifungal assay

Agar well diffusion methods were used for Antifungal assays against methanolic plant extracts (Ahmad *et al.*, 2012). Standardized inoculum (100 µl) for each fungus was inoculated on sterile molten Sabouraud Dextrose Agar (Himedia), homogenised and transferred into sterilized Petri dishes. Petri dishes were permitted to solidify in the laminar hood flow. Sterile cork borers of 6 mm diameter were used to make 3 uniform wells at the periphery of each petri dish. Plant extracts, made in 10% dimethyl sulfoxide (DMSO) of 3µl, 6µl and 9µl were laden into 3 peripheral wells. Fluconazole disc was taken as positive control while 10% dimethyl sulfoxide was used as a negative control. The Petri dishes were kept in an incubator for 24 - 36 hours at 32° C. Petri dishes were seen for the inhibition zones. Antifungal activity of different plant extracts was assessed by calculating zone of inhibition diameters and expressed in millimeters (mm).

Phytochemical analysis

Qualitative analysis of important phytochemicals in the methanolic plant extracts of *Asparagus officinalis, Caralluma tuberculata, Chenopodium album, Rumex dentatus* and *Solanum nigrum* were performed by methods stated by Harborne (1998).

Statistical analysis

Descriptive statistical analysis was made by using standard statistical procedures (Steel *et al.*, 1997). All mathematical and statistical computations were made using Microsoft Excel (version 2016).

Table 1. Antibacterial activity of Wild edible Plants of District Malakand, Pakistan.

S.No	Name of Plant extract	Concentration of extract (µl)	Inhibition Zone (mm)			
			K.P	E.C	B.C	S.A
1	*Asparagus officinalis*	3 µl	5 ± 0.5	7 ± 0.1	8 ± 0.2	9 ± 0.3
		6 µl	11 ± 0.3	13 ± 0.5	12 ± 0.5	13 ± 0.2
		9 µl	16 ± 0.5	17 ± 0.8	18 ± 0.3	19 ± 0.3
2	*Caralluma tuberculata*	3 µl	6 ± 0.3	6 ± 0.5	7 ± 0.3	9 ± 0.5
		6 µl	11 ± 0.5	12 ± 0.1	13 ± 0.3	13 ± 0.6
		9 µl	17 ± 1.1	18 ± 0.3	20 ± 1.2	19 ± 1.1
3	*Chenopodium album*	3 µl	7 ± 0.2	6 ± 0.8	8 ± 1.1	8 ± 1.2
		6 µl	13 ± 0.4	13 ± 1.3	15± 1.2	16 ± 0.4
		9 µl	18 ± 0.3	17 ± 1.4	21 ± 1.2	19 ± 1
4	*Rumex dentatus*	3 µl	7 ± 0.6	6 ± 1.2	7 ± 0.6	8 ± 1.1
		6 µl	12 ± 0.2	11 ± 0.3	15 ± 1.1	14 ± 0.5
		9 µl	18 ± 1.0	17 ± 0.1	19 ± 0.3	21 ± 1.2
5	*Solanum nigrum* Leaves	3 µl	7 ± 0.6	8 ± 0.4	8 ± 0.3	8 ± 0.5
		6 µl	12 ± 0.6	13 ± 0.7	14 ± 0.5	15 ± 0.3
		9 µl	19 ± 0.5	20 ± 0.5	19± 0.3	18 ± 0.3
6	Positive Control (Chloramphenicol)	3 µl	15 ± 0.4	16 ± 0.3	18 ± 0.5	20 ± 0.5
		6 µl	22 ± 0.6	23 ± 0.5	24 ± 0.5	26 ± 0.6
		9 µl	29 ± 0.3	30 ± 0.2	31 ± 0.5	33 ± 0.5
7	Negative Control (DMSO)	3 µl	-	-	-	-
		6 µl	-	-	-	-
		9 µl	-	-	-	-

Key: K.P (*Klebsiella pneumoniae*), E.C (*Escherichia coli*), B.S (*Bacillus cereus*), S.A (*Staphylococcus aureus)*. The data were expressed as mean standard deviation (± SD). The zone of inhibition was measured in milli meter (mm).

The experiments were performed in triplicates and inhibition zones were presented as mean ± standard deviation (SD).

Results and discussion

Antibacterial activity

The findings of the antibacterial activity of different methanolic plant extracts again tested bacterial strains are shown in Table 1. Plant extract of *Asparagus officinalis* exhibited maximum inhibition zone against *Staphylococcus aureus* (19 ± 0.3 mm at conc. 9 µl) whereas, minimum inhibition zone was shown against *Klebsiella pneumonia*e (16 ± 0.5 mm at conc.9 µl). The findings of the antibacterial activity of *Asparagus officinalis* are compatible to the previous studies such as Uddin *et al.*, (2012). The methanolic extract of *Caralluma tuberculata* showed maximum inhibition zone against *Bacillus cereus* (20 ± 1.2 mm at conc.9 µl) while the minimum zone of inhibition was shown against *Klebsiella pneumonia*e (17 ± 1.1 mm at conc. 9 µl). The results of antimicrobial activity of *Caralluma tuberculata* are in agreement with the earlier work of Adnan *et al.*, (2014). The *Chenopodium album* plant extract showed the highest antibacterial activity against *Bacillus cereus* (21 ± 1.2mm at conc. 9 µl) whereas, lowest antibacterial activity was shown against *Escherichia coli* (17 ± 1.4 mm at conc. 9 µl).

The results of *Chenopdium album* antibacterial activity are comparable with the results of Amjad and Alizard (2012). The plant extract of *Rumex dentatus* showed maximum antibacterial potential against *Staphylococcus aureus* (21 ± 1.2 mm at conc. 9 µl) whereas, the minimum antibacterial potential was shown against *Escherichia coli* (17 ± 0.1 mm at conc. 9 µl). These results are alike to the results of Fatima *et al.*, (2009).

Table 2. Antifungal activity of Wild edible Plants of District Malakand, Pakistan.

S.No	Name of Plant extract	Concentration of extract (µl)	Inhibition Zone (mm)			
			A.N	A.F	F.O	A.A
1	*Asparagus officinalis*	3 µl	11 ± 0.4	12 ± 0.3	11 ± 0.2	9 ± 0.3
		6 µl	15 ± 0.5	17 ± 1.2	15 ± 0.5	14 ± 0.3
		9 µl	20 ± 1.0	22 ± 1.1	21 ± 1.0	19 ± 0.4
2	*Caralluma tuberculata*	3 µl	9 ± 0.5	10 ± 0.8	10 ± 0.5	9 ± 0.6
		6 µl	14 ± 1.2	16 ± 0.3	16 ± 0.6	14 ± 0.5
		9 µl	19 ± 0.3	23 ± 0.2	24 ± 0.4	20 ± 0.4
3	*Chenopodium album*	3 µl	13 ± 0.5	13 ± 0.1	12 ± 0.2	11 ± 0.1
		6 µl	19 ± 0.5	18 ± 0.5	18 ± 1.0	17 ± 0.4
		9 µl	25 ± 0.2	24 ± 0.2	24 ± 0.7	22 ± 0.6
4	*Rumex dentatus*	3 µl	12 ± 0.2	11 ± 0.2	13 ± 0.4	12 ± 0.3
		6 µl	17 ± 0.3	18 ± 0.1	20 ± 0.6	18 ± 0.5
		9 µl	22 ± 0.5	26 ± 0.5	25 ± 0.8	24 ± 0.4
5	*Solanum nigrum*	3 µl	11 ± 0.2	13 ± 0.5	13 ± 0.5	12 ± 0.7
		6 µl	16 ± 0.6	19 ± 0.2	21 ± 0.4	18 ± 0.4
		9 µl	23 ± 0.5	25 ± 0.7	27 ± 0.3	22 ± 0.5
6	Positive Control (Fluconazole)	3 µl	22 ± 0.2	22 ± 0.2	23 ± 0.2	23 ± 0.7
		6 µl	29 ± 0.6	30 ± 0.3	29 ± 0.5	28 ± 0.5
		9 µl	35 ± 0.2	35 ± 0.5	36 ± 0.4	34 ± 0.3
7	Negative Control (DMSO)	3 µl	-	-	-	-
		6 µl	-	-	-	-
		9 µl	-	-	-	-

Key: A.N (*Aspergillus niger*), A.F (*Aspergillus flavus*), F.O (*Fusarium oxysporium*), A.A (*Alternaria alternata*).
The data were expressed as mean standard deviation (± SD). The zone of inhibition was measured in milli meter (mm).

The *Solanum nigrum* methanolic extract exhibited the highest antibacterial activity against *Bacillus cereus* (20± 0.5 mm at conc. 9 µl) whereas, lowest antibacterial activity was shown against *Klebsiella pneumonia*e (19 ± 0.5 mm at conc. 9 µl). Similar conclusions about *Solanum nigrum* plant antibacterial activity have been reported by Dar *et al*., (2017).

The Positive Control (Chloramphenicol) showed maximum inhibition zone against *Staphylococcus aureus* (33 ± 0.5 mm at conc. 9 µl) whereas, minimum inhibition zone was shown against *Klebsiella pneumonia*e (29 ± 0.3 mm at conc. 9 µl). The negative control (DMSO) indicated no antibacterial action against any bacteria.

Antifungal activity

Results of the antifungal activity of methanolic plant extract against all tested fungal strains are shown in Table 2. The *Asparagus officinalis* plant extract revealed the highest antifungal action against *Asparagus flavus* (22 ± 1.1 mm at conc. 9 µl) whereas, minimum antifungal activity was shown against *Alternaria alternata* (19 ± 0.4 mm at conc. 9 µl). These results are in line with the result of the Khorasani *et al.,* (2010). The *Caralluma tuberculata* extract revealed maximum antifungal activity against *Fusarium oxysporium* (24 ± 0.4 mm at conc. 9 µl) while the lowest antifungal activity was shown against *Aspergillus niger* (19 ± 0.3 mm at conc. 9 µl). The results of the antifungal activity of *Caralluma tuberculata* are compatible with the results of Ahmad

et al., (2014). The *Chenopodium album* plant extract showed maximum antifungal activity against *Aspergillus niger* (25 ± 0.2 mm at conc. 9 µl), whereas, the minimum antifungal activity was showed against *Alternaria alternata* (22 ± 0.6 mm at conc. 9 µl). The results of *Chenopdium album* antifungal activity are in line with the results of Singh *et al*., (2010). The methanolic extract of *Rumex dentatus* showed maximum antifungal potential against *Aspergillus flavus* (26 ± 0.3 mm at conc. 9 µl), whereas, the minimum antifungal potential was observed against *Aspergillus niger* (22 ± 0.5 mm at conc. 9 µl). These results are in agreement to the results of Hussain *et al*., (2010).

Table 3. Qualitative Phytochemical Analysis of Methanolic extracts of Selected Wild Edible Plants of Malakand.

Plant source	Alkaloids	Flavonoids	Glycosides	Phenols	Saponins	Tannins	Steroids
Asparagus officinalis	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Caralluma tuberculata	+ +	+ +	+ +	+ +	+ +	+ +	+ +
Chenopodium album	+ +	+ +	_ _	+ +	+ +	+ +	+ +
Rumex dentatus	+ +	+ +	+ +	+ +	+ +	+ +	_ _
Solanum nigrum	+ +	+ +	+ +	+ +	+ +	+ +	+ +

Key: **(++)** = Presence, (_ _) Absence.

The *Solanum nigrum* plant extract showed the highest antifungal activity against *Fusarium oxysporium* (27 ± 0.3 mm at conc. 9 µl) whereas, the lowest antifungal activity was observed against *Alternaria alternata* (22 ± 0.5 mm at conc. 9 µl). Similar conclusions about *Solanum nigrum* plant antifungal activity have been described by Modilal *et al*., (2015). The Positive control (Fluconazole disc) showed the highest inhibition zone against *Fusarium oxysporium* (36 ± 0.4 mm at conc. 9 µl) whereas, the minimum zone of inhibition was shown against *Alternaria alternata* (34 ± 0.3 mm at conc. 9 µl). The negative control (DMSO) revealed no results against any tested fungal strains.

Phytochemical analysis

The phytochemical screening of the methanolic extracts of the selected wild edible plants revealed that *Asparagus officinalis*, *Caralluma tuberculata*, and *Solanum nigrum* showed the occurrence of important phytochemicals such as glycosides, phenols, alkaloids, flavonoids, saponins, tannins and steroids. The study also showed that *Chenopodium album* does not contain glycosides while *Rumex dentatus* don't contain steroids in their methanolic extracts.

In the current research work, the methanolic extracts of selected wild edible plants showed effective antimicrobial actions against all tested bacterial and fungal strains. It is because these plants contain significant phytochemicals in their methanolic extracts due to their solubilities in this solvent as stated by Al-Zubaydi *et al*., 2009; Boklari, 2009; Bakht *et al*., 2011). Earlier research studies have shown that extracting solvent is important for the true evaluation of the therapeutic activity of any plant species (Parekh *et al*., 2005). So far as the microbial susceptibility is considered, *Escherichia coli* and *Staphylococcus aureus* were observed to be the utmost vulnerable bacteria, whereas, *Fusarium oxysporium* and *Aspergillus flavus* were the best prone fungal strains. Such findings were described and stated by Antara and Amla (2012).

Phytochemical studies indicate that these wild plants are also a good source of important secondary metabolites such as phenols, saponins, tannins, alkaloids, flavonoids, glycosides, and steroids. Our findings are in respectable accord with the findings of Belkacem *et al*., (2014). Due to the presence of different phytochemicals in plants, they show

excellent antimicrobial activities. These phytochemical substances control various metabolic activities inside the bodies of living organisms which includes the breaking of the cell membrane, stopping cell wall formation, enzyme inactivation and inhibition of proteins and nucleic acid formation, etc. (Cowan, 1999). Research studies have also shown that these phytochemicals may also disturb the activities of multiple drug-resistant (MDR) microbes by manipulating several factors like bacterial gene transposition and resistance plasmids (Mahindra and Kateryna, 2013).

Conclusion

The present research study has verified the claim that these wild edible plants are not only a good source of food but are also a potential source of antimicrobial and phytochemical agents and may be used in local medications to cure different microbial ailments. The plants under investigation also contain effective antimicrobial substances, operative in the cure of several microbial diseases. Though, more research is required in the course antimicrobial activities, phytochemical screening and compounds isolation to find and isolate favorable substances active and operative against infectious microbes.

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