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Antimicrobial activity of some ethno-medicinal plants used in Pakistan

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Medicinal plants have been used for their antimicrobial activity to discover new antimicrobial agents. Antimicrobial activity of aqueous and methanolic extracts of four ethnomedicinal plants such as *Acacia modesta, Thymus serphylum, Syzygium cumuni* L *and Olea ferruginea* were evaluated against five bacterial strains such as *S. aureus, E. coli, P. aeruginosa, S. epidermidis and B. subtilis* by disc diffusion method. The results showed that *Acacia modesta* showed maximum activity against *E. coli* with zone of inhibition (16.2 mm). *Thymus serphylum* showed maximum activity against *B. subtilis* with zone of inhibition (13.3 mm) but no activity against *P. aeruginosa* and *E. coli*. *Syzygium cumuni* L showed maximum activity against *S.epidermidis* with zone of inhibition (14.5 mm) but no activity against *E. coli, P. aeruginosa* and *B. subtilis.* Similarly *Olea ferruginea* displayed maximum activity against *S. epidermidis* with zone of inhibition (16.8 mm). Antimicrobial activity of plants was concentration dependent. Methanolic extracts of plants showed better activity than aqueous extracts.

Keywords: Antimicrobial activity, Aqueous, Methanolic, Disc diffusion

In Asia use of herbal medicine represents a wide range of history of human interaction with the environment. The medicinal importance of plants depends upon chemical constituents that produce marked healing action on human body. The most important of these agents are alkaloids, flavanoids and tannins [1]. According to World Health Organization medicinal plants represent source for a variety of drugs. About 80% of individuals from developed countries use traditional medicine, having compounds derived from medicinal plants [2]. Therefore, such plants should be investigated to better understand their properties, safety and efficiency. Alternative therapies have been utilized by people in Pakistan who have faith in spiritual healers, clergymen, *hakeems*, homeopaths or even many quacks. These are used to treat problems such as infertility, epilepsy, psychosomatic troubles, depression and many other ailments. The traditional medicine sector has become an important source of health care, especially in rural and tribal areas of the country [3]. Antimicrobial activity of four medicinal plants was determined which were collected from different localities of Pakistan and are used locally in the treatment of skin diseases and abdominal complications [Table1]. Evidently, there

are not many scientific studies that confirm the antimicrobial properties for most of the plants collected for this study. This paper narrates the bioassay test for antimicrobial activity.

Materials and Methods

Ethnobotanical Survey

Plants were selected for this study based on their medicinal use. *Acacia modesta, Syzygium cumuni and Olea ferruginea* were collected from the local areas of Sargodha while *Thymus serphylum* was collected from district Gilgit of Himaliya region of Pakistan in April 2007. The ethnobotanical data (local name, mode of preparation, medicinal uses) were collected through questionnaire, interviews and discussions among the tribal practitioners. The voucher specimens in duplicate were deposited in the herbarium of University of Sargodha, Pakistan.

Table 1: Uses of ethno-medicinal plants collected for antimicrobial screening

Botanical name and Family	Local name	Parts used	Ethnomedicinal use
Acacia modestea Mimosaceae	Phulai	Bark	Its miswak is used as mouthwash
Thymus serphylum Lamiaceae	Wild thyme	Aerial parts	Used remedy for treatment of respiratory, genitourinary and digestive problems
Syzygium cumuni L Myrtaceae	Jaman	Unripen fruit	Used for treatment of diarrhea
Olea ferruginea Oleaceae	Wild olive	Fruit	Used in skin and kidney problems

Preparation of Plant Extracts

Methanolic extracts of *Acacia modesta*, *Thymus serphylum* and *Olea ferruginea were* prepared by hot soxlation method for 8 hours [4]. The extract was filtered and dried under reduced pressure by rotary evaporator and percentage yield of *Acacia modesta*, *Thymus serphylum* and *Olea ferruginea* was 3.2%, 3%and 2% respectively. Methanolic extract of *Syzygium cumuni* L was prepared by cold maceration [5] and the percentage yield was 2%. Aqueous extracts of *Acacia modesta*, *Thymus serphylum*, *Olea ferruginea* and *Syzygium cumuni* L. were prepared by cold maceration. After filtration these were freeze dried in Lyophilizer [6].

Preparation of Inoculum

Inoculum were prepared by transferring a large number of bacterial cells from bacterial cell culture to test tube having 10ml nutrient broth and incubate for 24 hours at 37^{o}C. The tubes were shaken periodically to accelerate growth [7].

Antimicrobial susceptibility testing

The methanolic and aqueous extracts of four plants were screened against five bacterial strains. *Staphylococcus aureus* ATCC 25923 *Escherichia coli* ATCC 25922 *Pseudomonas aeruginosa* ATCC 27853 *Bacillus subtilis* ATCC 6633 *Staphylococcus epidermidis* ATCC 441 obtained from Musaji Adam & sons Rawalpindi. The antimicrobial assay for plants extract(s) against different bacterial strains was conducted by disk diffusion method [8]. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) (Oxoid LTD Basingstoke, Hampshire, England). 0.1% inoculum was spread uniformly and the inoculum was allowed to dry for 5 minutes. The different concentrations of plant extracts were loaded on 6mm disc. The loaded

discs were placed on the surface of medium then plates were placed in incubator for 24 hrs at 37C. At the end of incubation period inhibition zones were measured in millimeters.

Table 2. Antimicrobial activity of the aqueous and methanolic extracts of collected ethnomedicinal plants

Plant name	Extract	Conc. (mg/disc)	Zone of inhibition (mm)				
			Sa	Ec	Bs	Pa	Se
Acacia modestea	M	1mg	8.6	15.8	13	15	12
		2mg	10	16.2	14	15	12
	A	1mg	9	13	13	11	-
		2mg	9.1	15	14	13	-
Thymus serphylum	M	1mg	7	-	-	7	12
		2mg	8	-	-	8	13
	A	1mg	7	-	-	7	12
		2mg	8	-	-	8	13
Syzygium cumuni	M	1mg	10	-	-	13	-
		2mg	14.1	-	-	14.5	-
	A	1mg	12.5	-	-	13	-
		2mg	13.2	-	-	14.2	-
Olea ferruginea	M	1mg	-	-	11.4	16.8	14
		2mg	-	-	14.3	15.2	16
	A	1mg	-	-	12	14	14
		2mg	-	-	13	14	14

A– Aqueous; M – Methanol-, No activity; Sa – *Staphylococcus aureus;* Ec – *Escherichia coli;* Bs – *Bacillus subtilis;* Pa – *Pseudomonas aeruginosa*; Se- *Staphylococcus epidermidis*

Results and Discussion

The results of the antimicrobial screening of the crude extracts of all species of plants are shown in Table 2. Plants screened for antimicrobial activity showed significant activity against tested microorganisms, as the inhibition zone of 14mm or more considered as high antibacterial activity [9]. The results showed that *Acacia modesta* showed maximum activity against *E. coli* with zone of inhibition (16.2 mm). *Thymus serphylum* showed maximum activity against *B. subtilis* with zone of inhibition (13.3 mm) but no activity against *P. aeruginosa* and *E. coli*. *Syzygium cumuni* showed maximum activity against *S. epidermidis* with zone inhibition (14.5 mm) but no activity against *E. coli, P. aeruginosa* and *B. subtilis*. *Olea ferruginea* display maximum activity against *S. epidermidis* with zone of inhibition (16.8 mm). All the plants have significant activity against *S. epidermidis.* The tested plant extracts were more active against gram-positive microorganisms than gram-negative microorganisms, which is in agreement with previous literature by the several workers [10]. Aqueous and methanolic extracts of tested plants were used methanolic extracts exhibited a higher degree of antimicrobial activity than aqueous extracts. The antibacterial activity shown by plants was concentration dependent, which is in agreement with literature [11].

Conclusion

The antibacterial activity shown by plants was concentration dependent as the zone of inhibition increased by increasing the extract concentration in disc. Methanolic extracts showed better activity than aqueous extracts. The results also indicate that scientific studies carried out on medicinal plants having traditional claims of effectiveness might warrant

fruitful results. These plants used by local inhabitant's exhibit some degree of antibacterial activity towards *S. aureus, E. coli, P. aeruginosa, S. epidermidis and B. subtilis*. These plants could serve as useful sources for new antimicrobial agents.

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