Antimicrobial activities of *Aerva javanica* and *Paeonia emodi* plants

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Abstract: *Aerva javanica* and *Paeonia emodi* plants extracts were studied for antibacterial activity against *Escherichia coli* (NCTC 10418), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis* (NCTC 11047) and *Methicillin Resistant Staphylococcus Aureus* (MRSA) (NCTC 13143) and antifungal activity against *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus niger* and *Fusarium solani*. Extracts were obtained by using methanol, n-hexane, chloroform, ethyl acetate and aqueous fraction. The extracts of *Paeonia emodi* and *Aerva javanica* showed significant antibacterial activity but only *Salmonella typhi* was resistant to *Aerva javanica*. Moreover, the antifungal activity of *Aerva javanica* was very poor but the fractions of *Paeonia emodi* showed sufficient inhibition against fungal strains.

Keywords: *Aerva javanica*, *Paeonia emod*i, antibacterial, antifungal.

INTRODUCTION

Many ancient nations have awoken to the importance of herbal medicine (Ashur, 1986). Many countries of the world have traditional medicines as a source of first aid treatment. The existence and use of plants to treat human diseases, is as old as man (Sangawan & Alhaji Sangawan, 2010).

The plant *Aerva javanica* belongs to family Amaranthaceae. It is a perennial herb, native to Africa, Asia and extensively scattered in the far away areas of the world (Judd *et al*., 2008). This plant has got lot of application for example, this herb is used as diabetic demulcent, diuretic, the resultant liquid of plant is used to get rid of swellings. Powder of the plant is used to cure ulcers of domestic animals. Furthermore the seeds are used to mitigate headache and also used in rheumatism. Srinivas Reddy *et al* in 2009 had analyzed the presence of carbohydrates, flavonoids steroids and triterpenoids. Another plant investigated was the *Paeonia emodi*, of family Paeoniaceae, it is largely scattered in Northern Pakistan, North West India, China and West Nepal (Deyuan, 2004). It is a vertical perennial herb having long, glabrous, biternate or ternate leaves, pale lamina, solitary flowers of axillary (Kirtikar, 1918). Monoterpene glycosides, paeoniflorin, lactiflorin and oxypaeoflorine are among the compounds that have been reported in this plant (Muhammad *et al*., 1999). *Paeonia emodi* is applied in indigenous medical system. The rhizomes and roots are curative agents in backache, headache, dizziness, vomiting, oedema and epilepsy, also as an energizer, emetic, therapeutic, blood cleanser, helps in pregnancy and bellyache while the seeds are used as laxative (Shinwari *et al*., 2003, Ahmad & Sher, 2004).

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The current study was undertaken to authenticate antimicrobial activities of methanol extracts of both plants.

MATERIALS AND METHODS

The study was conducted in the Institute of Pharmaceutical Sciences (IPS) and Department of Biotechnology & Genetic Engineering, Kohat University of Science and Technology, Kohat, from October 2010 to December 2010.

Collection and identification of plants

The plants materials were collected from hills of Village Teri, Kashmir and from the backside mountains of Kohat University and were identified by Dr. Zafar Iqbal in the department of Plant Sciences, Kohat University of Science and Technology, Kohat.

Preparation of crude extract and fractions

Plants were dried by keeping in between old newspapers in the room. The shade dried plants were crushed into grinded coarse powder with the help of grinder. Obtained powder (15 Kg) of each plant were macerated in methanol by shaking at intervals at room temperature (Allen and Ansel, 2006) for 14 days for extraction. After 14 days maceration, methanol soluble fractions were filtered off. Filtrates were concentrated under vacuum at low temperature (37^{o}C) with the help of a rotary evaporator. A crude extract (150 gm) of each plant was extracted from both filtrates. The crude extract (130 gm) of each plant was suspended in distilled water (500 ml) and consecutively portioned with n- hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml), to give the n-hexane (40 gm), chloroform (30 gm), and ethyl acetate (25 gm) and aqueous fractions (35 gm)

respectively. These crude extracts and fractions obtained were firmly packed and kept in refrigerator at 4°C.

Antibacterial assay

Antibacterial activity of both plant extracts were determined by using well assay method of Perez *et al*. (1990). Seven bacterial strains *Escherichia coli* (NCTC 10418), *Klebsiella pneumoniae* (ATCC 700603), *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis* (NCTC 11047) and *Methicillin Resistant Staphylococcus Aureus* (MRSA) (NCTC 13143) were used in this assay. Muller Hinton agar (Oxoid, UK) media was prepared in conical flask. Properly sterilized media and equipments were used to carry out the experiment.

The culture of bacterial strains equivalent to 10^6 colony forming unit was inoculated upon the solidified media. Then, 6 mm wells were dug in the medium with the help of sterilized metallic borer. Stock solutions of crude extract and fractions dissolved in Dimethylsulfoxide (DMSO) at concentration of 10 mg/ml were prepared and 200 ul from each stock solution was supplemented into corresponding wells. The zones of inhibition were measured after incubating at 37°C for 24 hours.

Antifungal assay

Antifungal activity of the extracts was evaluated by the method of agar tube dilution. Four fungal strains *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger* and *Fusarium solani* were used in this assay. Each corresponding fraction was prepared in 5 ml DMSO. These were incorporated into the sabourad dextrose agar media at 45°C and poured in sterilized test tubes. Very small part of already refresh grown fungus was placed in each test tube and kept at 25°C for 5 days in incubator. After 5 days, each tube was observed for presence or absence of fungal growth and results were recorded. A negative control (without any fungi) and a positive control (with only fungal) strain were used in the assay to correlate the results.

RESULTS

Antibacterial activity

In the present work, the zones of inhibition of bacterial growth on agar plate were determined after treatment with different fractions of *Aerva javanica* and *Paeonia emodi*. All the plant fractions showed good antibacterial activity against the pathogenic bacteria. The zones of inhibition formed by the different fractions of these plants are listed in the following table 1 and 2.

Every fraction of *Aerva javanica* showed good activity against the tested pathogens except the *S. typhi* which resisted all the fractions significantly. While *Paeonia emodi* was almost equally active against all the seven pathogens included in this study. Thus, *Paeonia emodi* exhibits excellent antibacterial activity as observed upon treated microbes.

Antifungal activity

The results shown by different fractions of plants i.e. *Aerva javanica* and *Paeonia emodi* are given in table 3

Table 1: Antibacterial activity of *Aerva javanica*

Microorganisms	Zone of inhibition (mm)				
	Ethyl acetate	Chloroform fraction	Aqueous fraction	Crude fraction	n-Hexane
E. coli	10	12	10	18	8
P. Aeruginosa	14	14	10	14	18
K. Pneumoniae	10	12	8	10	6
MRSA	12	10	18	14	12
S. Aureus	16	14	10	18	10
S. Epidermidis	8	12	6	8	6
S. Typhi	6	6	6	6	6

Table 2: Antibacterial activity of *Paeonia emodi*

Microorganism	Zone of inhibition (mm)			
	n-Hexane	Chloroform	Ethyl acetate	Crude
E. coli	14	14	12	16
P. aeruginosa	20	18	20	16
K. pneumoniae	10	16	20	14
MRSA	12	14	18	14
S. Aureus	12	12	18	14
S. Epidermidis	10	18	16	16
S. Typhi	8	18	20	16

Table 3: Antifungal activity of *Aerva javanica*

Microorganism	Fractions treated				
	Ethyl acetate Fraction	Chloroform Fraction	Aqueous Fraction	Methanol Fraction	n-Hexane Fraction
A. niger	NA*	NA*	NA*	NA*	NA*
A. fumigatus	NA*	NA*	NA*	NA*	NA*
A. flavus	NA*	A**	NA*	A**	A**
F. solani	NA*	NA*	NA*	NA*	NA*

Table 4: Antifungal activity of *Paeonia emodi*

Microorganism	Fractions treated				
	Ethyl acetate	Chloroform Fraction	Aqueous Fraction	Methanol Fraction	n-Hexane Fraction
A. niger	NA*	A**	NA*	NA*	NA*
A. fumigatus	NA*	NA*	NA*	NA*	NA*
A. flavus	NA*	NA*	NA*	NA*	A**
F. solani	NA*	NA*	NA*	NA*	NA*

NA* Not Active, A** Active

and 4. None of the plant extract fraction showed promising results. However, methanol, n-hexane and chloroform fractions of *Aerva javanica* were active against *A. flavus* while methanol and n-hexane fractions of *Paeonia emodi* showed activity against *A. niger* and *A. flavus* respectively.

DISCUSSION

Biochemical components present in plants are used as curative agents for different diseases. These components are checked for there various activities like antibacterial, antifungal and other activities of beneficial interests throughout the world which play an important role in finding innovative compounds as herbal remedies designed for diagnosis and treatment of a range of diseases (Urzua *et al*., 2008). The local people of Teri, Karak, Kohat and Kashmir have good knowledge of plants and they use plants for curing various ailments.

Majority of microbes have developed resistance to the present day antibiotics and hence pharmaceutical companies are using different plants to get effective antimicrobial agents and come over the problem of resistance breaking strains of microorganisms (Ullah *et al*., 2009; Rafie *et al*., 2010; Angeh *et al*., 2007). Our study showed that *Aerva javnanica* and *Paeonia emodi* possesses antibacterial and antifungal activities. The *MRSA* is referred as superbug usually in the media and in general has multi drug resistance (Dettenkofer *et al*., 2008). Such disease causing agents produces infections of skin and soft tissues, ear, respiratory tract, blood and urinary tract (Furukawa *et al*., 2008; Gould, 2009; Hawser, 2009; Pereira *et al*., 2009).

Our outcomes are consistent with the antibacterial activity shown by other species of the genus Aerva. Vijayan *et al.* (2010) enclosed report of antibacterial and antifungal activity of *Aerva lanata*. It was effective against all bacterial species except *Klebsiella*. Earlier researches showed that ethyl acetate and methanol extracts of *Aerva lanata* have some interesting antimicrobial properties (Choudhary, 2004).

In our present study both plants showed significant activity against *S. epidermidis*. The crude and other fractions exhibited good activity next to *P. aeruginosa*. This is the initial work assuring antibacterial activity of *Aerva javanica* against S. epidermidis and *P.aeruginosa*. The plant *Aerva javanica* also have activity against *Staphylococcus aureus* and plant *Paeonia emodi* inhibited growth of *Klebsiella pneumoniae, Salmonella typhi* and *Pseudomonas auroginosa*.

Fungi cause multiples infections e.g. infection of blood, liver, lungs and mouth etc (Fung, 2002; Ker *et al*., 2002; Danziger-Isakov *et al*., 2008). In most of the cases they cause skin problems in humans and other animals (Sogair *et al*., 1991). *Aspergillus niger* typically cause infectivity in lungs and has also been detected on skin of burnt injuries (Singhal *et al*., 2005). *Aspergillus fumigatus, Aspergillus flavus* and *Fusarium solani* have been found to be engaged in lungs and eye infections (Kang *et al*., 2008). Our observations showed that both plants are inactive against fungal strains and thus exhibit very poor activity.

CONCLUSION

Both plants *Aerva javanica* and *Paeonia emodi* showed significant antibacterial activity and thus they are capable to be used in cure of infectious disea*ses* caused by *E. coli, P. aeruginosa, S. aureus, K. pneumonia, S. epidermidis, S. typhi, and MRSA* bacteria. On other hand the plants fractions did not show any significant antifungal activity

against *F. solani, A. niger, A. flavus and A. fumigatus*. The presence of phytoconstituents like steroids, terpenoids, flavonoids and phenolics are likely to be responsible for the observed antimicrobial activity.

Future prospects

1. Identification of antibacterial and antifungal compounds.
2. Screening of medicinal plants of surrounding area for antimicrobial activities.
3. Enhancement of activities of antibiotics by combining with plant extracts.
4. Search for new drugs effective against severe diseases.

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