Research Article

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ANTIMICROBIAL ACTIVITY OF *MALVA NEGLECTA* AND *NASTURTIUM MICROPHYLLUM*

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ABSTRACT
The most commonly used plants namely *Malva neglecta* and *Nasturtium microphyllum* were brought under study after observing their medicinal values. In this research it was aimed to investigate the antimicrobial activity of *Malva neglecta* and *Nasturtium microphyllum.* The different fractions of these two plants were obtained by using different organic solvents and these were subjected to antibacterial and antifungal activity. Bacterial strains, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Salmonella typhi* and *Bacillus subtilis* were used for the antibacterial activity while the fungal strains, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger and Fusarium solani* were used for antifungal activity in this research. As both of the plants showed best activity against most of the bacterial and fungal strains, therefore these plants can be used to treat different microbial diseases and infections.
Keywords: *Malva neglecta*, *Nasturtium microphyllum*, antibacterial and antifungal activity

INTRODUCTION

Food spoilage is one of the big problems faced by the food industry. In fact, food-borne illnesses are a global problem, even in developed and developing countries. Food spoilage or deterioration is predominantly caused by the growth of microorganisms. Many pathogenic microorganisms, including *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis*, *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger, Fusarium solani* and *Salmonella* spp. have been identified as the causal agents of food-borne diseases or food spoilage[1]. Most of the bacterial strains got resistant to certain antibiotics due to mutations in genes, changes in their structures and due to indiscriminate use of antibiotics to treat infectious diseases which led towards the ineffectiveness of most antibiotics. This generated a renewed interest in herbal medicines[2].

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many powerful and potent drugs. A wide range of medicinal plant extracts are used as raw drugs and possess varied medicinal properties. Although hundreds of plants species have been tested for antimicrobial properties but vast majority of which have not been adequately evaluated[3].

Experts turned their concentration back towards obtaining benefits from medicinal plants after observing the more harsh effects of synthetic drugs as compared to their benefits which led towards new discoveries[4]. Human depends fully or partially on herbal medicine because of their effectiveness, affordability, availability, low toxicity and acceptability[5]. Globally plant extracts are employed for their antibacterial, antifungal and antiviral activities because biologically active compounds from natural sources are of great interest for scientists working on infectious diseases[6]. The experiment was carried out with the crude extract (methanol) and other solvent soluble fractions (*n*-hexane, chloroform, ethyl acetate, *n*-butanol and water) with a view to find out the potentiality of traditional medicinal plants *Malva neglecta* and *Nasturtium microphyllum* belong to the families *Malveceae* and *Brassicaceae* respectively. These plants are used in the treatment of microbial infections by local physicians. The *Malva neglecta* is used to treat acne, broken bones, help in abdominal pain and to treat swelling, dermatitis, burns and throat infection[7] while *Nasturtium microphyllum* can be used to treat lung cancer, chest problems and in the cure of baldness and freckles[8].

MATERIALS AND METHODS

Plants Collection and Identification

Both of the plants i.e. *Malva neglecta* (sample 1) and *nasturtium microphyllum* (sample 2) were collected from swat in March 2010, and were identified by Mr. Nisar Ahmad, Chairman, Department of Botany , University of Science & Technology, Kohat, Pakistan. Both of plants were washed to step for experiment.

The Extraction and Fractionation

Malva neglecta (sample 1) and *Nasturtium microphyllium* (sample 2) were washed under running tape water and dried in air under shade. Then these were subjected to size reduction process by chopping and grinding them to

coarse powder. Each sample was soaked separately in methanol for 10 days, extracted three times in the same solvent and filtered. The filtrate was evaporated under reduced pressure leaving behind blackish residues. The methanolic extract of *Malva neglecta* (sample 1) was fractionated to *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water fractions while the methanol extract of *Nasturtium microphyllium* (sample 2) was partitioned into *n*-Hexane, chloroform, ethyl acetate and water fractions[9].

The Antibacterial Activity

The crude extracts and their respective solvents soluble fractions were subjected to antibacterial evaluation against five bacterial strains *E. coli*, *K. pneumonia, B. subtilis*, Methicillin resistant *S. aureus* (MRSA) and *S. typhi* and were tested by using Well assay method[10].

The media (Nutrient agar) 14g was prepared in 500ml conical flask and it was sterilized along with Petri dishes, cork borer and pipette in autoclave for 15 minutes at 121^0C at 15 psi pressure.

The media was poured into Petri dishes under laminar flow hood to avoid bacteria from environment. The stock solutions of corresponding fractions and the crude extracts were prepared in Dimethyl sulfoxide (DMSO) i.e. 4 mg/ml. The modified method of Perez et al, 1990, was followed. The bacterial culture of 104 to 106 CFU was inoculated on the solidified media through sterilized loops. Wells were dug in solidified media with the help of sterile cork borer then the dilutions of stock solutions (0.1ul in 0.9ul DMSO) were added to the respective wells. These Petri dishes were incubated for 24 hours at 37^0C. The augmentine was used as standard. The diameter of transparent zones of inhibition was measured in mm[10].

The Antifungal Activity

Four strains of fungus i.e. *A. niger, A. flavus, A. fumigatus*, and *F. solani*, were used for the antifungal activity. The stock solutions of crude and other fractions were prepared in DMSO i.e. 4mg/ 1ml. By using Agar tube dilution method[11]. One ml from each dilution was added to sterilized test tubes, then 9 ml sterilized media (Nutrient agar) was added to each tube. In each tube small piece of previously grown fungus was placed with the help of sterilized loops, then tubes including positive (fungus containing) and negative control (without fungi) were incubated for seven days at 25^0C. After seven days the fungal growth was observed in each tube with reference to the (positive) fungal containing test tubes.

Table 1: Zones of inhibition in mm by sample 1 and sample 2 against bacteria

Plant Sample	Fractions	*E. coli*	*MRSA*	*K.pneumoniae*	*S. typhi*	*B. subtilis*
Malva neglecta	Crude	0	0	12.5	11	15
	n-Hexane	0	0	19	14.5	18
	Chloroform	12	0	14.5	13	0
	Et.acetate	0	0	14	14	14
	water	10	0	14.5	12	12
Nasturtium microphyllum	Crude	13	7	11	16	10
	n-Hexane	13.5	15.5	0	0	15
	Chloroform	14	16	14	16.5	11
	Et.Acetate	0	10	0	0	0
	n -butanol	0	0	17	0	17
	Water	10	12	0	10	0

Table 2: Activity against fungi by sample 1 and sample 2

Plant sample	Fractions	*A.Flavus*	*A.Fumi*	*A. Niger*	*F. Solani*
Malva neglecta	Crude	+	+	+	+
	n-hexane	+	-	-	-
	Chloroform	+	-	+	+
	Et.Acetate	+	-	+	+
	Water	+	+	+	-
Nasturtium microphyllum	Crude	+	+	-	+
	n-hexane	-	+	+	-
	Chloroform	+	+	-	-
	Et.acetate	-	+	+	+
	n-butanol	+	+	-	+
	Water	-	+	+	+

RESULTS AND DISCUSSION

Most publications provide generalizations about whether or not a plant extract and fractions possesses activity against Gram-positive and Gram-negative bacteria and fungi. However, not all provide details about the extent or spectrum of this activity. The results of the study reveals that the non-polar and polar solvent fractions of *Malva neglecta* (sample 1) and *Nasturtium microphyllum* (sample 2) were active against the strains of the bacteria that are common cause of infections. The antibacterial activities of samples 1 and sample 2 are appended in table 1, while the antifungal activities have been shown in table 2.

Bacterial strains, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Bacillus subtilis* were used for the antibacterial activity while fungal strains *Aspergillus fumigatus, Aspergillus flavus, Aspergillus niger* and *Fusarium solani* were used for antifungal activity in this research.

All the solvents soluble fractions of *Malva neglecta* (sample 1) showed best activity against *Klebsiella pneumonia, Salmonella typhi* and *Bacillus subtilis* but no activity was recorded against *MRSA* while in case of

Nasturtium microphyllum (sample 2), the activity of crude and chloroform fraction were highest against all the five strains of becteria. Similarly the crude extract of sample 1 showed complete antifungal activity for all fungal strains. Chloroform and Ethyl acetate fractions were active in all three fungal strains except *Aspergillus fumigatus*. The water and *n*-butanol fractions showed best Antifungal activity. The crude extract, Ethyl acetate, water and *n* - hexane fractions of *Nasturtium microphyllum* also showed good anti-fungal activity. Antimicrobial efficacy shown by *Malva neglecta and Nasturtium microphylium* provides scientific basis and thus validates their traditional uses as homemade remedies.

There are so many reasons to the question that why these plants shows antibacterial and antifungal activities. The most effective reason for both of the plants is the presence of certain phytochemicals like glucosinoltes, alkaloids, flavonoids, rhamnose, galactose, galacturonic acid, glucuronic acid, phenolic acids, tannins, and volatile oils. Both of the plants i.e. *Malva neglecta* and *Nasturtium microphyllum* showed best antibacterial activity against such bacterial strains that are found in our environment and also the fungal strains that cause infections easily and are found around us particularly in food. Both of the plants, *Malva neglecta* and *Nasturtium microphyllum* can be used as antimicrobial, to treat diseases and infections.

CONCLUSION

The findings suggest that these native plants have good antibacterial and antifungal properties and can be used for infection control and treatment. It can also be as new source for antibiotics discovery and infection treatment. Further studies are necessary to isolate and characterize the active components of the extracts\fractions and also to elucidate their mechanisms of action for this activity.

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