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Antimicrobial Activity of Cold and Hot Successive Pseudobulb Extracts of *Flickingeria nodosa* (Dalz.) Seidenf

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Abstract: *Flickingeria nodosa* (Dalz.) Seidenf is a medicinally important orchid plant. It is used for the treatment of asthma, bronchitis, throat infections, dermatological infections and also used as blood purifier. Based on its importance the present study was designed to evaluate its antibacterial and antifungal activity against human pathogens with cold and hot successive extracts. The antimicrobial activities of the plant extracts were evaluated against 7 bacterial and 6 fungal strains using well diffusion method on Mueller Hinton agar medium. The cold water extract has antibacterial activity against *S. aureus* and *S. citreus* with maximum zone of inhibition. The cold chloroform extract has good antifungal activity against *T. mentagrophytes*. The plant can be a source material to herbal drug industry since it has some important antimicrobial components in the extracts that can be used for the development of therapeutic phytomedicine.

Key words: *Flickingeria nodosa* (Dalz.) Seidenf., antibacterial, antifungal, human pathogen, extracts

INTRODUCTION

Orchids are the largest and most diverse group among the angiosperms. They are cultivated for beautiful flowers. They are widely known for their economic importance but less for their medicinal value. Some orchids have been documented for their medicinal value. In India, orchids have been in use in indigenous system of medicines since vedic period. They have a variety of therapeutic use in different systems of traditional medicine like Ayurveda, Siddha and Unani (Rao, 1998). Traditional therapeutic uses of a number of orchids have been documented in ethanobotanical literatures (Singh and Duggal, 2009). In Ayurvedic system, "Ashtawarga", a group of eight drugs, is used for preparation of tonics such as "Chyavanprash" and consists of four orchid species out of which *Flickingeria nodosa* is also one among them (Kaushik, 1983). They are called as "Purusharathna" in Kannada. In Charak Samhita, it is known as Jeewanti (Rao, 1998). In folk medicine locals prepare a halva which is used as astringent, aphrodisiac and expectorant. Further, it is used for the treatment of asthma, bronchitis, throat infections, dermatological infections and is also used as blood purifier. In ayurvedic and traditional medicines it is believed that it can cure tridosha (Rao, 1998).

So the present investigation was under taken with an objective to study the comparative effect of cold and hot successive pseudo bulb extracts of *Flickingeria nodosa* against different bacterial and fungal pathogens.

MATERIALS AND METHODS

Preparation of the plant material: The pseudobulb stem plant material was collected from the natural habitat and rinsed with distilled water to remove the runaway dust material. The water was removed by blotting over a filter paper. The plant materials were shade dried and powdered. Ten grams of powdered plant materials was weighed, taken in a muslin cloth and made into packets. The packets were used for the successive extraction by using 5 solvents namely petroleum ether, chloroform, acetone, ethanol and water.

Cold successive extraction: Ten grams of powdered plant material made into packet was soaked in petroleum ether at 25°C on an orbital shaker at 100 rpm for 24 h. The solvent was decanted into collection bottle and the fresh solvent was added repeatedly till all the plant metabolites were leached out. Then the packet containing the plant material was dried and the extraction was carried out with next successive solvents. The successive extracts were

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dried by using rotary vacuum evaporator. The dried extracts were dissolved in DMSO (100 μg mL^{-1}) and used to check its antimicrobial activity.

Hot successive extraction: Ten grams of powdered plant material made into packet was placed in the soxhlet extraction apparatus basket which is a vessel with perforated sides and bottom so that liquid can fall through it. When gentle heat is applied to the main flask, the solvent begins to evaporate and the solvent vapors reach the cold condenser at the top of the flask and begin to liquefy on the condenser. The re-condensed solvent on the sides of the condenser begins flowing down the sides of the condenser and begins dripping off of drip points on the end of the condenser. This solvent drips into the top of the soxhlet basket. The solvent flows through the basket and out of the holes in the bottom of the basket carrying the extract with it into the bottom of the flask. The extract laden solvent falling from the soxhlet basket is dark in color and as it becomes clearer, one can know that the plant material is leached out and the process is finished. Then the packet containing the plant material was dried and the extraction was carried out with next successive solvents. The successive extracts were dried by using rotary vacuum evaporator. The dried extracts were dissolved in DMSO (100 μg mL^{-1}) and used to check its antimicrobial activity.

Source of microorganisms: The bacterial and fungal pathogenic clinical isolates maintained in Genohelix Biobabs, A Division of CASB, Jain University, Bangalore were used as a source for anti microbial activity studies. The test bacterial pathogens included *Staphylococcus aureus*, *Staphylococcus citreus*, *Bacillus cereus*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi* and *Klebsiella pneumoniae*.

The test fungal pathogens comprised of *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton rubrum*, *Trichophyton mentagrophytes*, *Candida albican* and *Candida parapsilosis*.

Assay of antibacterial activity: Antibacterial activities of all the 10 extracts were studied by agar well diffusion method (Bauer *et al*., 1966). Test cultures of the bacterial pathogens were prepared by transferring a loop full of bacteria from nutrient agar slants into Mueller Hinton broth and incubated at 37±1°C for 2 h. Lawn cultures of the test pathogens were prepared by swabbing sterile Mueller Hinton agar plates with 2 h old bacterial broth. Wells were punched with a sterile cork borer (6 mm internal diameter) and 100 μL of the extracts was added to each well. Controls were maintained with DMSO. Streptomycin (100 μg mL^{-1}) dissolved in DMSO was used as standard antibiotic. Following incubation at 37°C for 24 h, diameters of the inhibitory zones were measured to the nearest millimeter.

Assay of antifungal activity: Antifungal activities of the extracts were studied by agar well diffusion method. Suspensions of fungal pathogens were prepared by transferring a loop full of fungi from Sabouraud Dextrose agar slants into Mueller Hinton broth. Lawn cultures of the test pathogens were prepared by swabbing sterile Mueller Hinton agar plates with the fungal suspensions. Wells were punched with a sterile cork borer (6 mm internal diameter) and 100 μL of the extracts was added to each well. Controls were maintained with DMSO. Fluconazole (100 μg mL^{-1}) dissolved in DMSO was used as the standard antifungal. Following incubation at 27°C for 48 h, diameters of the inhibitory zones were measured to the nearest millimeter.

RESULTS

Antibacterial activity: Among the gram-positive bacteria *S. citreus* (28.00±0.000 mm) and *S. aureus* (26.0±0.0 mm) were found to be more sensitive showing maximum zone of inhibition in cold water extract. Moderate inhibition was recorded with *B. cereus* (17.33±0.333 mm) in cold water extract (Table 1, 2; Fig. 1). Among gram-negative bacteria, *E. coli* (5.33±0.333 mm) showed maximum zone of inhibition with cold acetone extract. *Salmonella typhi* (13.0±0.0 mm) was moderately inhibited with hot water extract whereas *K. pneumoniae* and *P. mirabilis* showed no zone of inhibition indicating their resistance to extracts (Table 1, 2, Fig. 1).

Table 1: *In vitro* antibacterial activities of 5 cold successive extracts on 7 bacterial clinical isolates showing diameters of the inhibitory zones (mm)

	Zone of inhibition (mm) X*±SE					
	Extracts					
Bacterial pathogens	CPE (1)	CC (2)	CA (3)	CE (4)	CW (5)	STD (11)
B. cereus	11.33±0.333	13.66±0.333	16.00±0.577	-	17.33±0.333	32.66±0.333
E. coli	2.66±0.333	4.00±0.000	5.33±0.333	5.00±0.000	3.00±0.000	8.660±0.333
S. aureus	-	12.00±0.000	16.00±0.000	-	26.00±0.000	32.66±0.333
S. citreus	10.00±0.577	13.66±0.333	16.00±0.000	9.66±0.333	28.00±0.000	31.00±0.000
K. pneumoniae	-	-	-	-	-	21.00±0.000
P. mirabilis	-	-	-	-	-	20.00±0.000
S. typhi	-	-	-	-	-	21.00±0.000

*Mean of 3 replications, SE: Standard error, C: Cold extract, PE: Petroleum ether, C: Chloroform, A: Acetone, E: Ethanol and W: Aqueous

Table 2: *In vitro* antibacterial activities of 5 soxhlet successive extracts on 7 bacterial clinical isolates showing diameters of the inhibitory zones (mm)

	Zone of inhibition (mm) X*±SE					
	Extracts					
Bacterial pathogens	SPE (6)	SC (7)	SA (8)	SE (9)	SW (10)	STD (11)
B. cereus	-	13.00±0.000	12.00±0.000	-	-	32.66±0.333
E. coli	-	-	-	-	-	8.66±0.333
S. aureus	-	14.66±0.333	13.66±0.333	-	-	32.66±0.333
S. citreus	-	13.33±0.333	-	-	-	31.00±0.000
K. pneumonia	-	-	-	-	-	21.00±0.000
P. mirabilis	-	-	-	-	-	20.00±0.000
S. typhi	-	-	11.00±0.000	12.66±0.666	13.00±0.000	21.00±0.000

*Mean of 3 replications, SE: Standard error, Hot extract (S), PE: Petroleum ether, C: Chloroform, A: Acetone, E: Ethanol and W: Aqueous

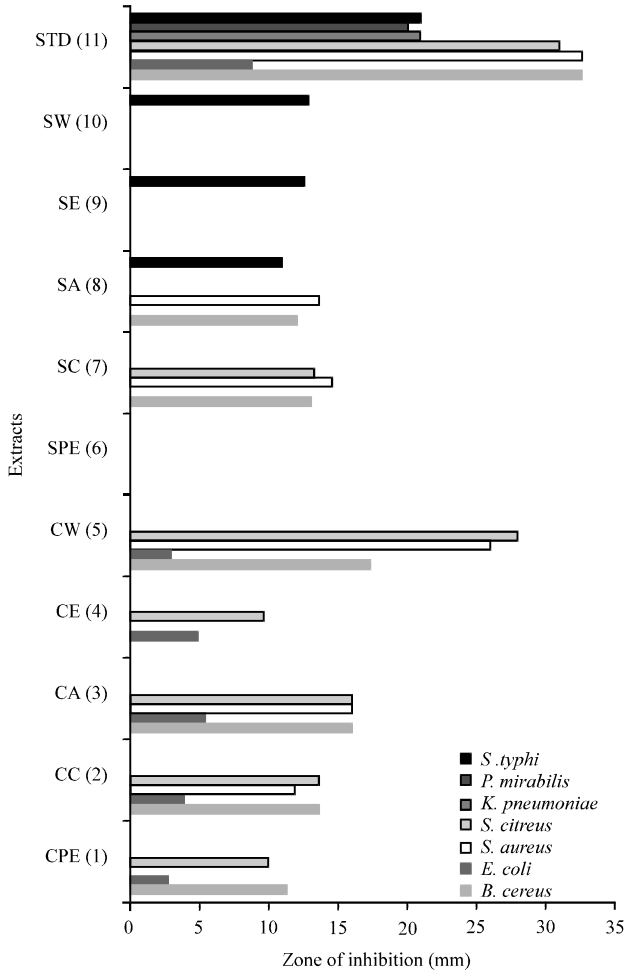

Fig. 1: *In vitro* antibacterial activities 10 extracts on 7 bacterial clinical isolates showing diameters of the inhibitory zones (mm)

Among the cold successive extracts and hot successive extracts, the cold successive extracts have given better antibacterial activity against gram-positive bacteria (*S. aureus*, *S. citreus* and *B. cereus)* than gram-negative bacteria (*E. coli*). *Salmonella typhi* showed its zone of inhibition with only hot successive extracts.

Antifungal activity: In cold chloroform extract the fungal form *T. mentagrophytes* (14.00±0.577 mm) was found to be more sensitive showing maximum zone of inhibition. *A. flavus* (15.00±0.000 mm) was found to be more sensitive showing maximum zone of inhibition in hot acetone extract. Moderate inhibition was recorded in *A. niger* (6.00±0.000 mm) with cold acetone extract.

Table 3: *In vitro* antifungal activities of 5 cold successive extracts on 6 fungi clinical isolates showing diameters of the inhibitory zones (mm)

	Zone of inhibition (mm) X*±SE					
	Extracts					
Fungal pathogens	CPE (1)	CC (2)	CA (3)	CE (4)	CW (5)	STD (11)
C. albicans	-	13.00±0.577	15.00±0.577	12.00±0.577	-	40.00±1.527
C. parapsilosis	-	-	-	-	-	44.66±0.0881
T. rubrum	4.66±0.881	5.00±0.577	7.33±0.333	1.00±0.000	7.00±0.00	-
T. mentagrophytes	13.33±0.881	14.00±0.577	13.00±0.000	11.33±0.333	13.33±0.333	14.00±0.000
A. niger	3.66±0.333	4.33±0.333	6.00±0.000	-	-	6.00±0.000
A. flavus	-	-	-	-	-	17.00±0.000

*Mean of 3 replications, SE: Standard error, C: Cold extract, PE: Petroleum ether, C: Chloroform, A: Acetone, E: Ethanol and W: Aqueous

Table 4: *In vitro* antifungal activities of 5 soxhlet successive extracts on 6 fungi clinical isolates showing diameters of the inhibitory zones (mm)

	Zone of inhibition (mm) X*±SE					
	Extracts					
Fungal pathogens	SPE (6)	SC (7)	SA (8)	SE (9)	SW (10)	STD (11)
C. albicans	-	-	-	-	-	40.00±1.5270
C. parapsilosis	-	14.00±0.577	11.33±0.881	12.66±0.666	-	44.66±0.0881
T. rubrum	-	-	-	-	-	-
T. mentagrophytes	13.00±0.000	11.00±0.577	12.00±0.000	12.00±0.000	12.33±0.333	14.00±0.0000
A. niger	-	-	-	2.33±0.333	2.00±0.000	6.00±0.0000
A. flavus	12.00±0.577	12.00±0.000	15.00±0.000	13.33±0.333	-	17.00±0.0000

*Mean of 3 replications, SE: Standard error, Hot extract (S), PE: Petroleum ether, C: Chloroform, A: Acetone, E: Ethanol and W: Aqueous

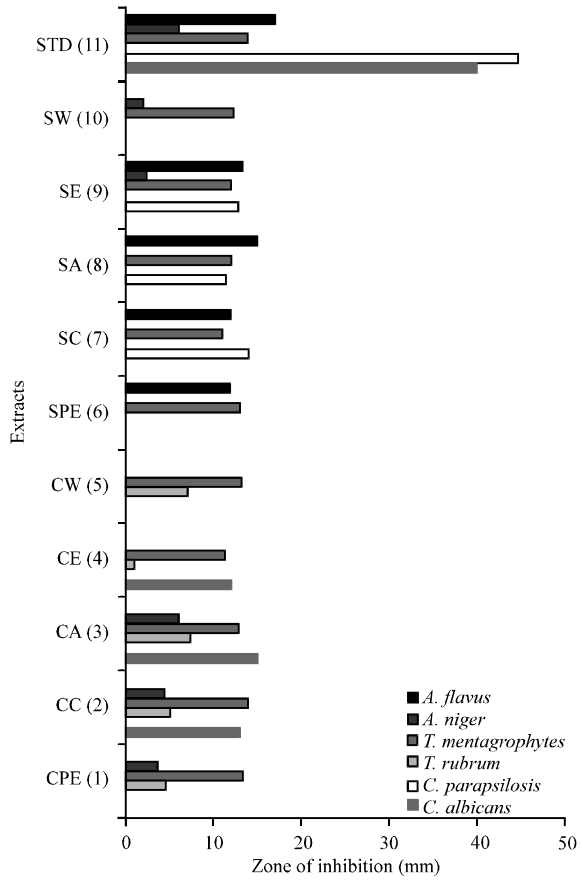

Fig. 2: *In vitro* antifungal activities of 10 extracts on 6 fungal clinical isolates showing diameters of the inhibitory zones (mm)

For *A. flavus* and *C. parapsilosis* showed zone of inhibition with only hot successive extracts. *C. albicans*, *T. rubrum*, *T. mentagrophytes* and *A. niger* showed good zone of inhibition with cold successive extracts (Table 3, 4, Fig. 2).

DISCUSSION

The traditional knowledge of Ayurvedic medicine is being explored and widely accepted. Presently, the principal focus of pharmaceutical research is on the ethnobotanical approach for the discovery of new drugs. The medicinal plants have the ability to synthesise a variety of secondary metabolites, of which at least some have been isolated (Schultes, 1978). Plants are rich in secondary metabolites with antimicrobial properties, such as phenols, tannins, terpenoids, alkaloids and flavonoids (Obeidat *et al*., 2012; Bouzada *et al*., 2009). *Flickingeria nodosa* has high medicinal importance to cure bronchitis, throat infections and dermatological infections, indicating its antimicrobial activity based on the traditional medicine (Rao, 1998). The well diffusion method was used to test the antimicrobial activity of different extracts on bacteria and fungi. Among the cold successive extracts and hot successive extracts, the cold water extracts have given better antibacterial activity against Gram-positive bacteria (*S. aureus*, *S. citreus* and *B. cereus*) than Gram-negative bacteria (*E. coli*). The cold water extracts were found to be more effective on Gram-positive bacteria than Gram-negative bacteria. This can be accounted by the presence of their thick murein

layer which prevents the entry of inhibitors in Gram-negative bacteria (Martin, 1995). This difference in the resistance may be attributed to the difference in the cell wall composition. The walls act as a diffusion barrier making Gram-negative bacteria less susceptible to the antimicrobial agents than Gram-positive bacteria (Nostro *et al*., 2000). Only hot acetone, ethanol and water extracts showed the zone of inhibition for *Salmonella typhi*, among them the water extract showed maximum inhibition. This is due to the presence of important secondary metabolites present in the extracts which is supported by the earlier findings of Chhajed *et al*. (2008) who reveled the presence of alkaloids, flavonoids and phenols in the extracts of *Flickingeria nodosa.*

In the case of antifungal activity, *A. flavus* and *C. parapsilosis* showed zone of inhibition with hot extracts. *C. albicans*, *T. rubrum*, *T. mentagrophytes* and *A. niger* showed good zone of inhibition with cold extracts. The cold extracts were more effective than hot extracts because the bioactive component present in the extracts might be thermolabile which might lose its activity when extracted under heat. As there are no previous studies, no comparison can be derived but can only be suggested that the extracts have good antimicrobial activity.

CONCLUSION

The plant can be a source material to herbal drug industry since it has some important antimicrobial components in the extracts that can be used for the development of therapeutic phytomedicine.

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