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IN VITRO ANTIBACTERIAL AND ANTIFUNGAL EVALUATION OF *VALLARIS SOLANACEA (ROTH) KUNTZE*

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ABSTRACT

The aim of the study is to assess the antimicrobial activity and MIC in the extracts of *Vallaris Solanacea* on selected bacterial and fungal strains. The antibacterial property in the extracts has been evaluated against two gram positive- *Staphylococcus aureus*, *Bacillus subtilis* and two gram negative - *Salmonella typhi* and *Escherichia coli* clinically isolated bacterial pathogens and antifungal activity was tested on *Candida albicans* and *Aspergillus niger*. The pet-ether, chloroform and ethanol extracts were used for the study of antimicrobial activity by agar well diffusion method. Zone of inhibition was measured around the wells to check the antibacterial activity of extracts. Results presented in the tables indicated that all the extracts were effective against tested organisms in comparison with standards. Pet-ether extract exhibited relatively higher zone of inhibition against *S.typhi*, *E.coli*, *A. niger* and *C.albicans*. Chloroform and ethyl alcohol extracts showed satisfactory zone of inhibition against all the pathogens as compared to standards.

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Key Words

Vallaris Solanacea, Soxhlet extraction, antimicrobial activity, Zone of inhibition, MIC.

INTRODUCTION

In the recent times, the rapid development of multiresistant bacterial and fungal strains of clinically important pathogens fetches the interest of scientist to develop newer broad spectrum antimicrobial agents¹. The less availability, high cost and greater side effects of new generation antibiotics necessitates looking for the substances from alternative medicines which claimed antimicrobial activity. A number of herbs with significant antimicrobial activity have been reported in literatures²⁻⁴. Now it is aimed to explore scientifically the antimicrobial potential of traditional plant *Vallisneria spiralis* and substantiate the folklore claims.

Vallisneria spiralis (Roth)Kuntze is a shrub climbing, often twisting to 10m, bark dirty whitish grey, flowering branchlets and distributed in China, India, Pakistan, Sri Lanka, Cambodia, Myanmar, Thailand, Vietnam and Indonesia. It belongs to Apocynaceae family found in Malnad region of Karnataka, India. It has very good medicinal values used in the treatment of skin diseases, wound healing etc. Pet-ether extract possesses pronounced inhibitory activity for gram positive bacteria, *S.aureus* followed by *B. subtilis*. Gram negative bacteria, shown equipotent inhibitory effect compared to standard. Relatively higher Minimum Inhibitory Concentration (MIC) was obtained for both gram positive bacteria *S. aureus*, *B. subtilis* and gram negative bacteria *E. coli* and *S.typhi*, with all the extracts. Antimicrobial activity of crude extracts of *Vallisneria spiralis* was carried out to validate the use of traditional herbal medicine and the results of this study tend to give credence to the common use of *Vallisneria spiralis* plant.

MATERIALS AND METHODS

Stem bark of *Vallisneria spiralis* was collected from Vodnala village of Sagar taluk, Shimogga

district, Karnataka state. The plant was identified by the department of Applied botany, Kuvempu University where the herbarium (KU/ST/09-DPN05) was deposited⁵. The plant product was shade dried, powdered and subjected to Soxhlet extraction using Pet-ether, Chloroform and ethyl alcohol respectively. The resulting extracts were evaporated in vacuum to dryness and stored in desiccator for future use.

MICROBIAL STRAINS

In total of six microbial strains used for investigation were collected as pure culture from National College of Pharmacy, Shimogga. Both gram positive, gram negative bacteria and fungi were taken for the test. The micro organisms were maintained on nutrient agar medium.

ANTIMICROBIAL ACTIVITY

Antimicrobial activity of crude extracts was carried against six microbial strains by the agar well diffusion method⁶⁻⁸. The in vitro antimicrobial activity was carried out against 24 h culture of four bacterial strains viz., Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative, *Salmonella typhi* and *Escherichia coli*. Two fungal strains used were *Aspergillus Niger* and *Candida Albicans*. The extracts were tested at 40 µg/ml concentration against both bacterial and fungal strains. DMSO was used as a vehicle. Ciprofloxacin (40 µg in 100µl) and Fluconazole (40 µg in 100µl) were used as standard drugs for the comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with standard drug after 24 h of incubation at 37°C for antibacterial activity and 72 h at 25°C for antifungal activity. The results are recorded in Table 1.

TABLE 1:- ANTIMICROBIAL ACTIVITY**ZONE OF INHIBITION (mm)**

Extract	Antibacterial strains				Antifungal strains	
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.typhi</i>	<i>E.coli</i>	<i>A.niger</i>	<i>C.albicans</i>
Pet-ether	22.9 ±0.06	20.23±0.15	23.9±0.06	24.13±0.19	23.0±0.21	21.7± 0.15
Chloroform	23.0±0.12	21.13±0.19	24.3±0.17	23.97±0.15	22.93±0.18	20.93 ±0.23
Ethyl alcohol	23.93±0.12	22.13±0.09	23.17±0.17	23.03±0.09	23.0±0.06	22.13±0.09
Ciprofloxacin	24.0±0.06	21.1±0.06	24.13±0.09	24.1±0.06	-	-
Fluconazole	-	-	-	-	24.0±0.06	22.1±0.06

Values are mean ± S.D of the triplicate *P<0.05; **P<0.

Standard drug : Bacteria-Ciprofloxacin (40 µg in100µl) Fungal-Fluconazole (40 µg in100µl)

Extract : (40 µg in 100µl)

Control : DMSO (dimethyl sulphoxide)

Sample used : Crude extract

MINIMUM INHIBITORY CONCENTRATIONS (MIC)

Minimum Inhibitory Concentration is defined as the lowest concentration of test samples that result in a complete inhibition of visible growth. MIC of all extracts was determined by a micro dilution method⁹⁻¹¹. The respective clinical strain was spread separately on the

medium. The wells were created using a stainless steel sterilized cork borer under aseptic conditions. The extracts at different concentrations viz. 10, 20, 30, 40 and 50 µg was dissolved respectively in 25, 50, 75, 100 and 125 µL of DMSO and later loaded into corresponding wells. The standard drug Ciprofloxacin (40 µg in100µl) and Fluconazole (40 µg in100µl) were used as standard drugs for the comparison of antibacterial and antifungal activities respectively. The zone of inhibition was compared with the standard drug after 24 h of incubation at 37⁰C for antibacterial activity and 72 h at 25⁰C for antifungal activity. The results are recorded in Table 2.

TABLE 2:- MINIMUM INHIBITORY CONCENTRATIONS (MIC)

MIC ($\mu\text{g}/\mu\text{L}$)						
Extract	Antibacterial strains				Antifungal strains	
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>S.typhi</i>	<i>E.coli</i>	<i>A.niger</i>	<i>C.albicans</i>
Pet-ether	30.1 \pm 0.06	30.0 \pm 0.06	30.1 \pm 0.06	40.03 \pm 0.03	40.03 \pm 0.09	29.97 \pm 0.03
Chloroform	30.1 \pm 0.06	40.03 \pm 0.03	30 \pm 0.06	29.97 \pm 0.03	30.03 \pm 0.09	29.97 \pm 0.09
Ethyl alcohol	30.03 \pm 0.09	30.1 \pm 0.06	29.97 \pm 0.03	40.03 \pm 0.03	30.1 \pm 0.06	29.97 \pm 0.09

Values are mean \pm S.D of the triplicate *P<0.05; **P<0.01

Standard drug : Bacteria-Ciprofloxacin (40 μg in100 μl)
 Fungal -Fluconazole (40 μg in100 μl)
 Control : DMSO (dimethyl sulphoxide)
 Sample used : Crude extract

RESULTS

ANTIMICROBIAL ASSAY

The extracts of *Vallis Solanacea* showed pronounced in vitro potential of antimicrobial activity against all four bacteria and two yeast species. The extracts were tested at 40 $\mu\text{g}/\text{mL}$ concentration against both bacterial and fungal strains. The data obtained from the disc diffusion method (Table 1) indicated that the pet-ether extract displayed high degree of antimicrobial activity on *S.typhi*, *E.coli*, *A.niger* followed by *S. aureus*, *B.subtilis*, *C.albicans*. The Chloroform extract showed maximum effect against *S.typhi*, *E.coli* with the strongest inhibition zones (24mm, 24mm). The ethanol extract exhibited high antimicrobial activity against *S.aureus* with inhibition zone of 24 mm in comparison with standard drug.

The result of minimum inhibitory concentration (MIC) presented in table-2 indicated that pet-ether extract showed high sensitivity against *S.aureus*, *B.subtilis*, *S.typhi*, *C.albicans* and satisfactory sensitivity to *E.coli* and *A.niger* While Chloroform extract showed great sensitivity against *S.aureus*, *B.subtilis*, *S.typhi*, *E.coli*, *C.albicans* and *A niger*. Ethanol extract was more potent to *B.subtilis*, followed by *S.aureus* *S.typhi*, *E.coli*, *A.niger* and *C.albicans* compared to standard drug.

DISCUSSION

The investigation suggested that all three extracts have almost similar effect against the tested organisms. Thus, in search of novel broad spectrum antimicrobial agent, the formulation comprising different proportions of these extracts may be proven good. This study has shown the scientific basis for the therapeutic uses of traditional plant, *Vallis Solanacea* and confirmed its ethno medicinal claims.

The results mentioned revealed that *Vallis solanacea* possess considerable antimicrobial activity against selected bacterial and fungal strains. The properties of these extracts encourage developing a

novel broad spectrum antimicrobial formulation in future. The isolation of bioactive components of these extracts responsible for the activity is in progress. The extracts of *Vallisneria spiralis* may be useful as an alternative antimicrobial agent as natural medicine for the treatment of diseases caused by microbes.

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