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In Vitro Antimicrobial Activity of *Alangium salviifoliium* L. Roots.

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A B S T R A C T

The antimicrobial activity of the roots of *Alangium salviifoliium* L. studied against bacteria, *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Streptomyces pneumoniae* and fungi *Aspergillus niger, Candida albicans* and *Saccharomyces cerevisiae* by the agar well diffusion method. Antimicrobial activity was recorded in the order of hexane, chloroform, methanol, ethanol and water extracts. Three concentrations (50, 75 and 100 mg/ml) of each extracts were studied in the antimicrobial activity which involved the determination of inhibition zone values. As the extract dosage level increases the inhibitory effect also increased. All the extracts have showed antimicrobial activity against all tested bacteria and fungi. Ethanol extract has exhibited the highest antimicrobial activity against *Proteus vulgaris* followed by *Candida albicans* and *Saccharomyces cerevisiae*, while methanol extract exhibited the highest activity against *Streptomycis pneumoniae*. The antimicrobial activity of the extracts was compared to the standard antibiotics, Tetracycline and Clotrimazole. The minimum inhibitory concentration of the chloroform, methanol, ethanol and water extracts were determined by the agar dilution method ranged between 10-1000 µg/ml and 10 µg/ml with that of *Proteus vulgaris* being the least phytochemical screening of the plant revealed the presence of tannins, alkaloids, flavonoids and saponins. The results of this study support the traditional use of *Alangium salviifoliium* L. root as an antimicrobial agent.

Introduction:

Plant based antimicrobials represent a vast untapped source for medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plant origin have enormous therapeutic potential (1). Human infections particularly those involving microorganisms i.e. bacteria, fungi, viruses. They cause serious infections in tropical and subtropical countries of the world. In recent years, multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of such diseases (2,3)

Alangium salviifoliium L. (Akoul) is a small deciduous tree or shrub. It belongs to Alangiaceae family, which groves in the wild throughout the hotter parts of India (4). Locally it called as oodugu. The major phytochemical constitutes of the plant are alangine A & B, alangicine, markindine, lamarckinine and emetine (5). Roots are used in diarrhoea, paralysis, piles & vomiting (6). The Indian system of medicine roots as an acrid, diuretic, astringent and antidote for several poisons. Root is useful for external application in acute case of rheumatism, leprosy and inflammation and internal application in cases of bites of rabbit and dogs (7). It has been reported that it used to cure skin diseases (8,9) and sexual diseases and as contraceptives for pigs and cattle rearing by the tribes in Malayalies (10). The fruits

(mucosa) of the plant are useful in treating burning sensation and haemorrhages (11). However few scientific reports (12,13,14,15) are available regarding its antimicrobial activity. An investigation of *Alangium salviifoliium* as an antimicrobial agent is the objective of our present study.

Materials and methods

Plant material

The roots of *Alangium salviifoliium* were collected from the Sudikonda forest, East Godavari District, Andhra Pradesh, India, and were authenticated by the curator of Botany Department, Andhra University, Visakhapatnam, where a voucher specimen was deposited.

Preparations of extracts

The roots were collected, cleaned and shade dried for two to three weeks. The dried roots were pulverized by a mechanical grinder and passed through a 20-mesh sieve. A powdered root 200 g was extracted successively with hexane, chloroform, methanol and ethanol using a soxhlet apparatus and water extracted by hot maceration. The extraction was carried out for 24 hr at room temperature with mild shaking (16). The extracts were filtered and concentrated at 45^0C, and were placed in a refrigerator and stored in darkness at 4^0C for the duration of the assays.

Screening of antimicrobial activity

The antimicrobial activity was evaluated by employing 24 hrs cultures of *Bacillus subtilis* (MTCC 2763), *Escherichia coli* (MTCC 2960), *Klebsiella pneumoniae* (MTCC 4032), *Pseudomonas aeruginosa* (MTCC 6642), *Proteus vulgaris* MTCC 1771), *Staphylococcus aureus* (MTCC 7443), *Streptomyces pneumoniae* (MTCC 1935), *Aspergillus niger* (MTCC 4360), *Candida albicans* (MTCC 4748), and *Saccharomyces cerevisiae* (MTCC 4742). The strains employed in the study were obtained from microbial type culture collection (MTCC), IMTECH, Chandigarh, India.

Nutrient broth was applied for growing and diluting the microorganism suspensions. Bacterial strains were grown to exponential phase in nutrient broth at 37^0C for 18 hrs, where as fungal strains grown at 28^0C for 48 hrs. For susceptibility testing the agar well diffusion assay was performed. Sterile nutrient agar/Sabouraud dextrose agar plates were prepared by pouring the inoculated media in sterile petriplates under aseptic conditions. Wells were made at the size of 6 mm diameter in the agar plates using the sterile borer. About 50 µl of each extract was filled in well by adjustable digital finn pipette. A standard disc with antibiotic Tetracycline (5 µg/ml) was used as positive antibacterial control whereas Clotrimazole (5 µg/ml) was used as positive antifungal control. DMSO used as negative control. The plates were maintained at 4^0C for 1hr to allow the diffusion solution into the medium. All the plates containing bacteria were inoculated at 37^0C for 24 hr and that of fungi at 28^0C for 48 hr (17).

Each experiment was carried out in triplicates and diameter of the zone of inhibition surrounding each well was recorded. The extracts that showed antimicrobial activity were subjected to minimum inhibitory concentration (MIC) assay by using serial two-fold dilution method (18).

Results and Discussion

The result shows that the chloroform, methanol, ethanol as well as water extracts of root of *Alangium salviifoliium* L. possessed antimicrobial activities (Table 1). Ethanol extracts were relatively more active than the methanol and water extracts, the later being were equally effective.

Table: 1. Antimicrobial activity of extracts of *Alangium solviifoliium* L.

Extract/ Antibiotic	BS	EC	KP	PA	PV	SA	SP	AN	CA	SC
	A B C	A B C	A B C	A B C	A B C	A B C	A B C	A B C	A B C	A B C
Hexane	- - -	- - 10	- - 10	10 12 14	10 12 14	- 10 12	- - 10	- - -	- - 10	- 10 12
Chloroform	10 12 14	12 14 16	11 13 15	11 13 15	10 12 14	10 12 14	10 12 14	- 10 12	- 10 12	12 14 16
Methanol	12 14 16	16 18 20	12 14 16	13 15 17	13 15 17	11 13 15	18 20 22	10 12 14	12 14 16	16 18 20
Ethanol	18 20 24	20 22 24	19 21 23	18 20 24	24 26 28	19 21 23	20 22 24	12 13 15	21 23 25	21 23 25
Water	10 12 14	10 12 14	16 18 20	12 14 16	12 14 16	18 19 21	14 16 18	12 15 17	16 18 20	15 17 19
Tetracycline	24	21	19	24	20	26	24	-	-	--
Clotrimazole	-	-	-	-	--	-	-	18	26	24

BS: *Bacillus subtilis* AN: *Aspergillus niger* PV: *Proteus vulgaris*
EC: *Echerichia coli* CA: *Candida albicans* SA: *Staphylococcus aureus*
KP*: Klebsiella pneoumoniae* SC: *Saccharomyces cerevisiae* SP: *Streptomyces pneumoniae*
PA: *Pseudomonas aeruginosa* A, B, C: 50, 75 and 100 mg/ml, respectively

From the zone of inhibition produced by the extracts it was observed that ethanol extract showed the prominent antimicrobial activity against all microorganisms compared to other extracts. Methanol and water extracts were equally effective. Ethanol extract showed maximum zone of inhibition against *Proteus vulgaris* followed by *Candida albicans* and *Saccharomyces cerevisiae*. Methanol extract showed maximum inhibition against *Streptomycis pneumoniae* followed by Escherichia coli, and *Saccharomyces cerevisiae*. Water extract showed maximum inhibition zone of inhibition against *Staphylococcus aureus* followed by *Klebsiella pneumoniae* and *Candida albicans*. Surprisingly, no inhibitory effect has been noted for non polar hexane, this could be attributed to the extraction of active components of *Alangium salviifoliium* in other solvents rather than hexane. The antimicrobial activities of ethanol extract of *Alangium salviifoliium* root was higher than those of tested Tetracycline and Clotrimazole, so that it proved as the most effective solvents for extracting broad spectrum of antimicrobial compounds.

From the MIC values (Table 2), it was observed that ethanol extract showed significant antimicrobial activity followed by methanol and water. The MIC values of ethanol were 10 µg/ml for *Proteus vulgaris* and 25 µg/ml for *Candida albicans* and *Saccharomycis cerevisiae*. The MIC values of methanol extracts for *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Streptomycis pneumoniae*, *Saccharomycis cerevisiae* was 500 µg/ml. Water extract exhibited MIC values as 125 µg/ml for *Proteus vulgaris* and 250 µg/ml for *Klebsiella pneumoniae*, *Candida albicans*, *Saccharomycis cerevisiae*. The chloroform extract showed the best activity (1000 µg/ml) against *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.

Table: 2. MIC of the root extract of *Alangium solviifoliium* L.

Microorganism	Chloroform extract	Methanol extract	Ethanol extract	Water extract
Bacillus subtilis	>1000	1000	50	>1000
Echerichia coli	1000	250	50	>1000
Klebsiella pneoumoniae	1000	1000	50	250
Pseudomonas aeruginosa	1000	500	50	1000
Proteus vulgaris	>1000	500	10	1000
Staphylococcus aureus	>1000	1000	50	125
Streptomyces pneumoniae	>1000	125	50	500
Aspergillus niger	>1000	>1000	1000	500
Candida albicans	>1000	1000	25	250
Saccharomyces cerevisiae	1000	250	25	250

In the present study in broth dilution technique, the ethanol and methanol extracts of *Alangium salviifoliium* successfully control the *Proteus vulgaris, Candida albicans, Saccharomyces cerevisiae* and *Staphylococcus aureus*. MIC value also revealed that almost all tested bacterial strains were sensitive to the ethanol and methanol extracts of our study plant. From the earlier studies it is also revealed that the organic solvent extract is better than aqueous extracts. *Proteus vulgaris* is an inhibition that either the plant extracts are less effective on some Gram-positive bacteria or that the organism has the potential of developing antibiotic resistance, while low MIC values for other bacteria is an indication of the efficiently of the plant extraction. Now the present study revealed that the ethanol and methanol extracts were effective control the bacterial growth than the aqueous extract. This probably indicates that there are bioactive ingredients that are able to inhibit the growth of these common pathogens.

According to this study, plant based antibacterial drug have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobial agents. The present results revealed that the extract of *Alangium salviifoliium* was effective against both Gram-positive and Gram-negative bacteria. Presence of chemical compounds viz. alkaloids, tannins, flavanoids and saponins of *Alangium salviifoliium* may inhibit the bacterial growth. Traditionally, *Alangium salviifoliium* was employed using/mixing with aqueous for treating the antibacterial and other infections (19). Naturally, the biological active compounds whose activity can be enhanced in the presence of ethanol and methanol could have been produced number of active compound responsible for antibacterial activity. The present study provided the scientific information about the plant extract of *Alangium salviifoliium* and supports the usage of this for curing many bacterial diseases by traditional healers. Further, phytochemical separation and immunological studies of this plant is in under progress.

Conclusion

The result of this study have shown that the root extraction of *Alangium salviifoliium* have great potential as antimicrobial agents in the treatment of infectious organisms. Further, detailed investigation of the active compounds of the plant for the exact mechanism of action will contribute greatly to the development new pharmaceuticals.

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