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STUDIES ON ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANTS AGAINST HUMAN PATHOGENS

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Abstract

The Indian folk medicine practice is based on the use of plant extracts. Many phytochemicals like alkaloids, flavonoids, steroids, phenolics, terpenoids and saponins are isolated from the roots of medicinal plants like Ruellia tuberosa L. and Alangium salvifolium (L.F.) Wang. The plant and plant products are known for their therapeutic uses from thousands of years. In folk medicine it has been used as diuretic, Antipyretic, antidiabetic and analgesic agent. The plants are being used as medicinal plants as it is effective against many infectious diseases.

The Ruellia tuberosa L. of family Acanthaceae and Alangium salvifolium (L.F.) of family Alangiaceae were studied for its antibacterial properties against pathogenic bacteria. During the present study Aqueous and Methanolic extract of roots of Ruellia tuberosa L. and root extract of Alangium salvifolium (L.F.) are used. Root extracts were evaluated for their antibacterial activity. The antibacterial study was carried out by disc diffusion method against the pathogens Klebsiella pneumoniae, Staphylococcus auerus, Salmonella typhi, Proteus vulgaris and Pseudomonas aeruginosa, and Escherichia coli. To evaluate the antibacterial activity methanol and aqueous extracts were used.

The maximum zone of inhibition was observed in methanol extract as compared to aqueous extract for each bacterium was as Methanolic extract of roots of medicinal plants showed the zone of inhibition against the studied bacteria were S.typhi (12 mm), K. pneumoniae (15 mm), Proteus vulgaris (22 mm), Pseudomonas aeruginosa (18 mm) and Staphylococcus aureus(14 mm), and Escherichia coli.(12 mm). The present study demonstrates that the selected medicinal plants have antibacterial potential.



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Introduction

The medicinal plants are the herbal remedies in which one or more of its parts contains substances that can be used for therapeutic purposes. During the present studies the

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selected medicinal plants are Ruellia tuberosa L.of family Acanthaceae and Alangium salvifolium (L.F).

The root part of plants is used for the experiment.

The roots of *Ruellia tuberosa* are the tap roots. They are fasciculate tap roots. The roots are found to be contain many chemical compounds like alkaloids, tannins and coumarin. It is clear from the literature that these compounds have high pharmaceutical values. They serve as protective mechanism to the plants. They are found to be stored in the wood and bark of the roots. The compounds like tannins are found to be resistant to proteolytic enzymes. They are found to be used in the Indian system of medicine as astringent to intestinal tract. The roots are proved to be good astringent and can be used in diarrhea, dysentery, ulcers, piles and fissures. The roots of *Ruellia tuberosa* are not so popular like the vegetables in food and the nutraceutical. They contain starch and claims for food values. Considering these facts studies were undertaken for antibacterial properties of roots of *Ruellia tuberosa L*.

Alangium salvifolium (L.F) Wang is a deciduous shrub or tree belonging to the family Aangiaceae. Locally it is called Ankolam. This family consist one genus with twenty two species, out of which Alangium salvifolium (L.F) Wang is the only species used medicinally in India, China, and Phillipines. The different parts of this plant are used for a wide range of diseases. Root bark is an antidote for several poisons. The root is also useful for external application in acute case of rheumatism, leprosy and inflammation. Fruits are sweet, used to treat burning sensation, constipation, and haemorrhage. Medicinal plants represent a rich source of antimicrobial agents. Medicinal plants are believed to be important source of phytochemicals with therapeutic effects.

Material and Methods

- **a. Plant Source:** The plant roots of *Ruellia tuberosa* L.were collected from the campus of Yeshwant Mahavidyalaya Nanded and The plant roots of *Alangium salvifolium* (L.F) were collected from Shikarghat region of Nanded district Maharashtra. The collected roots were washed with water and shed dried. The dried roots were powdered in grinder and were stored in air tight containers for further use. The Preliminary phytochemical screening was performed according to Johansen (1940) and Harborne (1984).
- **b. Preparation of Plant extracts:** The roots of plant *Ruellia tuberosa* L. and (250 gms) was extracted with methanol solvents (450 ml) by using soxhlet apparatus for 8 hours at a

temperature not exceeding the boiling point of the respective solvent. The obtained extracts were filtered by using Whatman No.1 filter paper and then concentrated under vacuum at 40°c, dried in dessicator and packed in sterile plastic bottles.

Aqueous extract was made by cold maceration method. About 50 g of powdered material mixed with 500 ml of distilled water and kept for 7 days at 25 °c. The extract obtained from water was filtered through Whatmann filter paper No.1 and residue water content was evaporated (4°c) with heating mantle.

- **c. Preparation of test samples:** Test samples of the plant extract were prepared in DMSO (Dimethyl Sulfoxide).
- **d. Phytochemical Analysis:** The phytochemical screening of methanol extract was done to identify the phytoconstituents by their color reaction. (Evans, 1997).
- **e. Inoculum Preparation :** The microorganisms are pre-cultured in nutrient broth overnight in a rotary shaker at 37°c ,centrifuged at 10,000 rpm for 5 min. pellet was suspended in double distilled water and cell density was standardized Spectrophotometrically at 660 nm.
- **f. Antibacterial Activity:** The methanol root extract of *Ruellia tuberosa* L. and *Alangium salvifolium* (L.F) were tested by disc diffusion method (Anonymous, 1996). Different concentration of the extracts ($100 \mu g/ml$) was prepared by reconstituting with methanol. The test microorganisms were seeded in to respective medium by spread plate method. $10 \mu l$ with the 24 hrs culture of bacteria grown in nutrient broth. After solidification the filter paper discs (5 mm diameter) impregnated with the extract were placed on test organism-seeded plates. Streptomycin sulphate ($10 \mu g/ml$) used as negative control. The antibacterial assay plates were incubated at $37^{\circ}c$ for 24 hrs. The diameter of the inhibition zones were measured in millimeters.

Experimental Results

1. Phytochemical Analysis: The roots of *Ruellia tuberosa L*. and *Alangium salvifolium* (L.F) were collected shade dried and powdered. The powder is used to prepare extract in methanol and water (Aqueous extract) and screened for qualitative phytochemical analysis. The results are presented in table-1.

Table-1: Phytochemical Analysis

Sr.No.	Phytochemicals	Ruellia tuberosa L		Alangium salvifolium (L.F)		
		Methanol	Aqueous	Methanol	Aqueous	
1	Tannin	+	+	+	+	
2	Saponin	+	+	+	+	
3	Terpenoid	+	+	+	+	

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4	Phenolic compounds	+	+	+	+	
5	Flavonoids	+	+	+	+	
6	Alkaloids	+	+	+	+	

(+ Represents the presence of phytochemicals)

The results shows presence of tannins, saponins, tarpenoids, phenolic compounds, flavonoids and alkaloids.

2. Antimicrobial Activity

During the present studies the results obtained revealed that the tested plants have potential antibacterial activity against the human pathogenic bacteria as *Klebsiella pneumonia*, *Staphylococcus aureus*, *Salmonella typhi*, *Proteus vulgaris and Pseudomonas aeruginosa*, *Escherichia coli*. The methanolic extract showed significant antimicrobial activity compared to aqueous extract against all tested microorganisms when tested by disc diffusion method. The results are presented in table-2.

In the results it is found that the highest antibacterial activity recorded in *P. vulgaris* (22 mm),

P. aeruginosa (18 mm), *K.pneumoniae* (15 mm), *S.aureus* (14 mm), *S.typhi* (12 mm), and *E. coli*. (12 mm) for the plant *Ruellia tuberosa* root extracts.

The results obtained for the second plant *Alangium salvifolium* (L.f.) were also significant and showed antimicrobial property as *P. vulgaris* (20 mm), *P. aeruginosa* (18 mm), *K.pneumoniae* (16 mm), *S.aureus* (13 mm), *S.typhi* (12 mm), and *E. coli.* (12 mm) .The results obtained showed that significant antibacterial activity was observed in methanolic root extracts as compared to aqueous root extracts. The results are presented in table-2.

Table-2: Antibacterial activity of methanol and Aqueous extract (100 μg/ml) of *Ruellia tuberosa* L. and *Alangium salvifolium* (L.F) by disc diffusion method.

	Zone of Inhibition* (mm)						
Human pathogenic bacteria	Ruellia tuberosa L.		Alangium salvifolium (L.F)		Streptomycin sulphate (10 µg/ml)		
	Methanol	Aqueous	Methanol	Aqueous			
S.aureus	14	8	13	10	24		
S.typhi	12	6	12	8	21		
K.pneumoniae	15	9	16	7	18		
P. vulgaris	22	5	20	6	23		
P. aeruginosa	18	7	18	6	22		
Escherichia coli.	12	8	12	10	16		

Discussion

The plant material used as an alternative method to control pathogenic microorganisms (Aqil et. al.,2005) and many components of plant products have been shown to be specially targeted against resistant pathogenic bacteria (Nostro et.al.,2006).

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The present work explore the antimicrobial activity of medicinal plants *R.tuberosa and A. salvifolium*. These plants are reported to have antibacterial, antifungal, anthelmintic properties. (Mahesh and Satish, 2008). The methanol root extract of *R.tuberosa and A. salvifolium* showed the activity against all the six tested microorganisms but the activity was very significant against *P. vulgaris* and *P. aeruginosa*. The methanol and aqueous extract of medicinal plants were significantly active against Staphylococcus species (Selvamohan et. al., 2012).

The present study shows that the methanolic and aqueous extract of roots of *R.tuberosa and A. salvifolium* have all the phytochemical constituent studied with negligible variation. Thus, the significant activity against P. vulgaris and P. aeruginosa may be due to their phytochemical or secondary metabolites present in the medicinal plants.

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