



EFFICACY OF ANTIMICROBIAL ACTIVITY OF RHINACANTHUS NASUTUS (LINN) LEAVES IN DIFFERENT EXTRACTIONS

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ABSTRACT

The in vitro antimicrobial activity of *Rhinacanthus nasutus* leaves was investigated against various strains of bacteria and fungi viz. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli* and *Aspergillus niger* by disc diffusion method. The Methanolic and ethyl acetate extract in a dose of 20 mg/ml showed significant activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* when compared with the standard antibiotic ciprofloxacin (10 µg /disc) and ampicillin (10 µg/disc) respectively. Chloroform extraction showed great significant result against *A.niger*.

KEY WORDS

Rhinacanthus nasutus (Linn) Leaves, antibacterial, anti fungal activity.

INTRODUCTION

Rhinacanthus nasutus (Linn) belongs to Acanthaceae family is a shrub and is well known for its medicinal uses, commonly called as Nagamali, Kaligai, anichi in Tamil, Nagamalli in Telugu, doddapatika in kannada, jupani in Hindi, yuthikaparni in Sanskrit and Gajakarni in Marathi¹⁻⁴. *Rhinacanthus nasutus* is widely distributed in some parts of the sub-continent India and in the region of Southeast Asia and china. The plant is small slender shrub 1-2 meter height. It has been used as Thai traditional medicine for the treatment of various cancers, e.g. cervical and liver cancers⁵. Various parts of this plant have been also used for the treatment in various other diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, and various skin

diseases, and the active components of this plant have been widely investigated⁵⁻¹⁵. In India *Rhinacanthus nasutus* is used in poultice treatment for eczema and ringworm. In Thailand a tincture either with alcohol or vinegar is prepared to cure certain forms of ringworm. In Malay Peninsula the leaves are pounded with benzoin and sulphur and the paste is applied to the area infected by ringworm. In Phillipines it is traditionally used either as a sap or a decoction¹⁶⁻¹⁸. In Thailand, the roots and leaves of *R.nasutus* are used for the treatment of cancer¹⁹. It is also used in the treatment of obesity, leprosy, eczema, scurvy and dhobi's itch. Leaves, roots and seeds act as an antidote for snake bites. In Madagascar, the juice of leaves and the root bark were used in the treatment of Herpes Circiinat²⁰⁻²². Since the plant was used as antiseptic, anti parasitic and in ringworm infestation it



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was thought worthwhile to investigate the antibacterial and antifungal activity.

MATERIALS AND METHODS

(i) Collection of the plant material

Fresh leaves of *Rhinacanthus nasutus* Linn were collected in the period of August - October 2008 from Seshachala Hills, Tirumala, Andhra Pradesh and authenticated at Botany department, Sri Venkateswara University, Tirupati, Andhra Pradesh. A voucher specimen has been preserved in our herbarium.

(ii) Preparation of extracts

Five hundred grams of coarse powder of shade dried leaves of *Rhinacanthus nasutus* was extracted successively with chloroform, ethyl acetate and methanol in soxhlet extractor for 48 h. Dark green residues were obtained after concentrating the extract under reduced pressure (yield 6.2%, 5.8% and 6.5% respectively). The aqueous extract was obtained by macerating 500 g of coarse powder of *Rhinacanthus nasutus* leaves with 5 L of distilled water for 72 h. The extract was filtered and concentrated under reduced pressure to obtain green colored residue (yield 4.8%). The plant extracts were diluted with respective solvents to the final concentration of 20 mg/ml. Microorganisms like *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* were used for testing.

ANTIMICROBIAL ASSAY

The antimicrobial activity of the extracts of each sample was evaluated by using disc diffusion method²³⁻²⁴. Petri plates containing 15 ml of respective media were seeded with selected microbial strains. Five milliliters of nutrient broth was inoculated with a loop (6 mm) of bacteria/yeast and incubated at 35°C for 6 h. Standard antibiotic discs viz., Ampicillin and Ciprofloxacin obtained from Hi-

Media, Mumbai, were used as controls. Ampicillin and Ciprofloxacin at conc. of 10µg/disc was used as a standard. The filter paper impregnated with extracts (separately in each extracts at a concentration of 20 mg/ml) and ampicillin, ciprofloxacin discs were placed aseptically on the seeded agar medium (Hi-Media Pvt. LTD, Mumbai) which was already swabbed with the test organisms and incubated at 37°C for 24 h. The assessment of antimicrobial activity was based on measurement of inhibition zones formed around the discs. Three independent trials were conducted for each concentration. The zone of inhibition in mm and MIC values were measured and recorded in Table 1 and 2.

ANTIFUNGAL EVALUATION

The antifungal activity of the crude extracts was determined against *Aspergillus niger* by disc diffusion method. The filter paper disc impregnated with various extracts (20 mg/ml) individually and ketoconazole disc were placed aseptically on the seeded sabouraud dextrose agar medium (Hi-Media Pvt Ltd., Mumbai) which was already swabbed with the test organism and incubated at 37°C for 48 h. The zone of inhibition was measured in mm by taking the average result of triplicate experiment.

RESULTS AND DISCUSSION

Six crude extracts of *Rhinacanthus nasutus* were investigated for their potential antibacterial activities. Standard antibiotics ciprofloxacin (10µg/disc) and ampicillin (10 µg/disc) showed good inhibitory action on the microorganisms tested. Chloroform and ethyl acetate extracts showed antibacterial activities against all the organisms tested except *Pseudomonas aeruginosa*. Methanolic extract showed significant activity only against *Staphylococcus aureus*. Chloroform extract showed best significant activity on the fungi *A.niger*. Aqueous extract was ineffective for the organisms tested. The



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antibacterial and antifungal activity may be due to the presence of phytosterols and terpenoids in chloroform and ethyl acetate extracts. However, in the present study it was proved that the leaf extract of *R. nasutus* is found to be active and can provide resistance capacity to the users. I conclude that the plant extract showed an excellent result against all the microorganisms which are causative of many diseases.

CONCLUSION

Herbs are an integral part of nature. Plants contain natural substances which promote and stabilize the health conditions of humans. Antimicrobial activity of Leaf extract of *Rhinacanthus nasutus* found to be very effective against many number of bacteria and fungi. So in future these plant products can be used as an advanced medicine for the diseases caused by the above mentioned bacteria and fungi.

Table 1.

Antimicrobial activity of Rhinacanthus nasutus (Linn) in different extracts.

S.No	Microorganism	Zone of inhibition in (mm)						
		Std	CE	ME	EE	AE	Ampicillin	Ciprofloxacin
1	<i>S. aureus</i>	31	15	16	20	06	23	20
2	<i>P. aeruginosa</i>	20	--	03	04	--	21	23
3	<i>K. pneumonia</i>	25	07	13	21	02	22	26
4	<i>E coli</i>	21	10	04	11	02	25	22
5	<i>A.niger</i>	22	20	03	07	--	21	24

-- = no activity

CE – Chloroform extract

EE - Ethyl acetate extract

ME - Methanol extract

AE - Aqueous extract

Table 2.

Minimum Inhibitory Concentration (MIC) mg/mL of Methanolic extracts of Rhinacanthus nasutus leaf.

S.NO	Microorganisms	Minimum Inhibitory Concentration (MIC) mg/mL
1	<i>S. aureus</i>	0.50
2	<i>P. aeruginosa</i>	1.25
3	<i>K. pneumonia</i>	0.75
4	<i>E coli</i>	0.50
5	<i>A.niger</i>	0.50



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