

IN VITRO ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOL EXTRACTS OF *RHINACANTHUS NASUTUS*- A MEDICINAL PLANT

R. Nanthakumar*, MR. Udhayasankar, V. Ashadevi, K. Arumugasamy and A. Shalimol

PG and Research Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamil Nadu, India.

ABSTRACT

Absolutely the plant kingdom holds many species of plants containing substances of medicinal value that have yet to be discovered. Therefore in recent years, considerable attention has been directed towards the identification of plants with antimicrobial activity. For this reason, *Rhinacanthus nasutus* a medicinal plant belonging to family Acanthaceae, the under shrub was selected for the present study. The study was formulated with the objective to assess the antibacterial activity of *R. nasutus*. The antibacterial and antifungal activities were assessed by the presence or absence of inhibition zones from aqueous and ethanolic leaf extracts of *R. nasutus*. The results showed that most effective activity against bacterial strains *Bacillus subtilis* and *Salmonella paratyphi*. The same extracts had exhibit highest inhibitory activity against the fungal strains *Candida albicans* and *Aspergillus flavus*. The present findings suggest the usefulness of leaf aqueous extracts of *Rhinacanthus nasutus* against pathogenic bacteria and fungal strains.

Keywords: *Rhinacanthus nasutus*, aqueous, ethanol, bacteria and fungus.

INTRODUCTION

Medicinal plants can provide a wealth of antimicrobial agents, and hundreds have been investigated for biological activities. Local people collect raw materials in small quantities and use them to treat diseases. Raw materials are also collected in huge amounts and traded in the marketplace to supply herbal industries (Uniyal et al., 2006).

Rhinacanthus nasutus Linn. (Acanthaceae) is available in India, Taiwan, Thailand, South china, Ceylon and Madagascar. It is locally known as Nagamulla and Nagamalligai. It is an under shrub with white flowers, and is extensively used in traditional medicine to treat skin diseases, antitumor, hepatoprotective, liver diseases, peptic ulcer, helminthiasis, scurvy, inflammation and obesity (Warrier et al., 1995). In indigenous system of medicine, the root and leaves of *R. nasutus* are used to treat herpes and other viral infections (Kernan et al., 1997).

MATERIALS AND METHODS

Collection of plant material

The plant *R. nasutus* collected from the wild areas of Western Ghats, The Nilgiris. Then the plant identified by a plant taxonomist. The collected plants were washed in tap aqueous to remove the impurities, separate the leaves and dried in a room temperature. The dried leaf materials pulverized and stored in an air tight container for further usage.

Preparation of extracts

50g of leaf powder extracted with 250ml of ethanol using the soxhlet extractor for 9-10 hours. And another set of leaf powder extracted with aqueous placed in the aqueous bath at 100 C for 2 hours. The extract was filtered through what man No.1 filter paper to remove all undisclosed matter, including cellular materials and other constituents that are insoluble in the extraction solvents. Final extract was used in antibacterial and antifungal screening activities.

Test microorganisms

Authentic pure cultures of human pathogenic bacteria like *Bacillus subtilis* (Gram positive), *Streptococcus faecalis* (Gram positive), *Salmonella paratyphi* (Gram negative), *Salmonella paratyphi* (A) (Gram negative), *Aspergillus flavus*, *Candida albicans*, *Trichoderma viride* and *Penicillium sp* were obtained from the Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore.

Antimicrobial Screening

Disc diffusion method

The antimicrobial activity of leaves extracts of *R. nasutus* was evaluated by disc diffusion method the culture media were prepared and autoclaved at 121°C at 15 psi for 20 minutes and stored in refrigerator. The media were melted before the process of inoculation. The clean dry sterile petri dishes were poured with nutrient agar medium (for bacteria) and potato dextrose agar medium (for fungus). Ten number of 10 ml broths were prepared separately for nutrient agar medium and potato dextrose agar medium in test tubes and plugged with cotton and autoclaved. The test tubes were labeled according to the microbes to be inoculated. The bacterial strains were inoculated into the nutrient broth and fungi were inoculated onto potato dextrose broth under aseptic conditions and incubated at 37±0.5°C for 18 hours. After incubation, the bacteria and fungi were smeared on the nutrient agar and potato dextrose agar plate respectively using a sterile cotton swab. A sterile disc of 6 mm diameter was loaded with known quantity of 10mg of dried crude extracts of both ethanol and aqueous extracts which was dissolved in 10ml of DMSO. These discs were placed on the surface of the media. The positive control antibiotics viz., tetracycline for bacteria (30µg) and ampicillin for fungi (10µg) were maintained. Then the Petri dishes were incubated at 37±0.5°C for 12 to 14 hours. The diameter of inhibition zone was measured. Triplicates were maintained for all tests (Bauer et al., 1966).

RESULTS AND DISCUSSION

The anti-bacterial activity of leaf extracts of the studied species was assayed *in vitro* by disc diffusion method against four bacterial such as *Bacillus subtilis*, *Streptococcus faecalis*, *Salmonella paratyphi*, and *Salmonella paratyphi* (A) (Table-1). The antimicrobial activity of the

aqueous leaf extract against *Bacillus subtilis* showed maximum activity in 100mg/ml solution (21mm diameter inhibitory zone) and ethanolic extract showed 18mm inhibition zone. Followed by *Salmonella paratyphi* showed 17mm diameter inhibitory zone in aqueous extract and 12mm in ethanol extract where the 100/mg/ml solution same extracts showed minimum activity against *Salmonella paratyphi*(A) (13 mm diameter inhibitory zone in aqueous and 10mm in ethanol extract)). On the other hand no activity was observed in *Streptococcus faecalis*. The aqueous extract showed maximum activity than the ethanolic extract.

The anti-fungal activity of leaf extract of the studied species was assayed *in vitro* by disc diffusion method against four fungal strains such as *Aspergillus flavus*, *Candida albicans*, *Trichoderma viride* and *Penicillium sp.*(Table-2). The antifungal activity of the aqueous leaf extract against *Candida albicans* showed maximum activity in 100mg/ml solution (20mm diameter inhibitory zone) and ethanolic extract showed 14mm inhibition zone. Followed by *Aspergillus flavus* 16mm diameter inhibitory zone in aqueous extract and 14mm in ethanol extract where the 100mg/ml both aqueous and ethanol extracts showed minimum activity against *Trichoderma viride* (13 mm and 12mm diameters inhibitory zone). On the other hand no activity was observed in *Penicillium sps* in both extracts. The inhibitory activity was higher for the extracts of aqueous than the ethanol.

It may be attributed to polarity of the solvents which naturally have high degree of extracting capacity (Aiyelaagbe et al., 2007). And Zakaria et al. (2010) pointed out that more than one compound in the crude extract might result in improved efficiency due to their synergistic effect. The overall study on antimicrobial activity reports that the studied plant species contains many active compounds of medicinal importance, particularly to cure infectious diseases. These heterogeneous compounds functioned synergistically to combat the pathogens. Balandrin et al., 1985 & Eseawi and Srour, 2000 explained that the increasing of heterogeneity of phytochemical generally increased the antimicrobial activities. Alam, 2009 also highlights the importance of secondary metabolites in plants for antimicrobial activities.

Table 1: Antibacterial activity of aqueous and ethanol leaf extracts of *Rhinacanthus nasutus*

S. No	Bacterial strains	Zone of inhibition(mm)					
		Aqueous leaf extracts			Ethanol leaf extracts		
		Concentration(mg/ml)					
		Control	50	100	Control	50	100
1	<i>Bacillus subtilis</i>	12	16	21	10	14	18
2	<i>Salmonella paratyphi</i>	19	-	17	15	-	12
3	<i>Salmonella paratyphi (A)</i>	17	-	13	13	-	10
4	<i>Streptococcus faecalis</i>	10	15	-	9	12	-

Table 2: Antifungal activity of aqueous and ethanolic extracts of *Rhinacanthus nasutus*.

S. No	Fungal strains	Zone of inhibition(mm)					
		Aqueous leaf extracts			Ethanol leaf extracts		
		Concentration(mg/ml)					
		Control	50	100	Control	50	100
1	<i>Candida albicans</i>	9	14	20	8	10	14
2	<i>Penicillium sps</i>	7	17	-	8	12	-
3	<i>Trichoderma viride</i>	13	12	13	11	10	12
4	<i>Aspergillus flavus</i>	15	-	16	12	-	14

CONCLUSION

Based on the results, it can be concluded that the *Rhinacanthus nasutus* both aqueous and ethanolic leaf extracts have great potential as antimicrobial components against microorganisms and they can be used in the treatment of infectious diseases caused by resistant microorganisms. Future work is needed to isolate the pure compounds from the leaf extract studied in order to test specific activity.

REFERENCES

- Uniyal SK, Singh KN, Jamwal P and Lal B. Traditional use of medicinal plants among the tribal communities of chhota bhagal, Western Himalaya. Journal of ethnobiology and ethnomedicine. 2006; 2:14-16.
- Warrier PK, Nambiar VPK and Raman kutty C. Indian medicinal plants Compendium of 500 species. 4. Orient Longmans India. 1995:417.
- Kernan MR, Sendi A, Chen JL, Jolad S D, Blanc P, Murphy JT, Stoddent CA, Nanakor W and Rozhon EJ. Two new lignin's with activity against influenza virus from the medicinal plant, *Rhinacanthus nasuta*. J Nat Prod. 1997;60:635-637.
- Alam S. Antimicrobial activity of natural products from medicinal plants, Comal Journal of Medicinal Science. 2009;7(1): 72-78.
- Aiyelaagbe OO, Adeniyi BA, Fatunsin OF and Arimah BD. In vitro antimicrobial activity and photochemical analysis of *Jatropha curcas* roots. Intern. J. Pharmacol. 2007;3(1):106-110.
- Balandrin MFJ, Kjocke A and wurtele E. Natural plant chemicals: sources of industrial and mechanical materials. Science. 1985;228:1154-1160.
- Eseawi T and Srour M. Screening some palestinian medicinal plants for antibacterial activity. J of Ethanopharmacol. 2000;70:343-349.
- Zakaria ZA, Desa AM, Ramasamy K, Ahmat N, Mohamad AS, Israf DA and Sulaiman MR. Lack of antimicrobial Activities of *Dicranopteris linearis* extracts and fractions. African Journal of Microbiology Research. 2010; 4(1):071-075.
- Bauer AW, Kirby WMM, Sherris JC and Turck M. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. American Journal of Clinical Pathology. 1966;45:493-496.