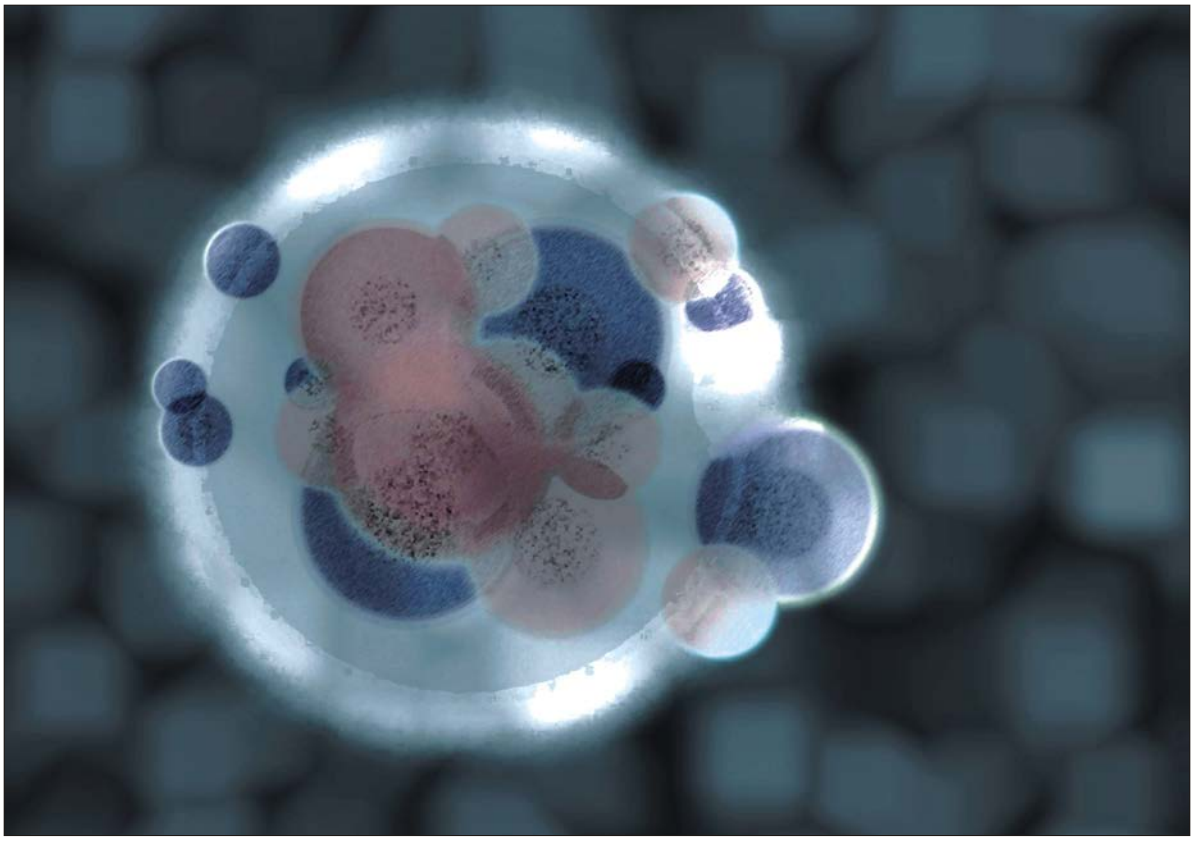

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Evaluation of Anti-fungal and Anti-bacterial Activity of a Local Plant *Rhinacanthus nasutus* (L.)

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Abstract: In traditional medicine of Southeast Asian region and other parts of the world *Rhinacanthus nasutus* (L.) is used in several skin complaints. It was found that aqueous ethanolic extract of *Rhinacanthus nasutus* (L.) exhibited a potent dose dependent anti-fungal activity against *Candida albicans* and *Trichophyton mentagorphytes* along with substantial anti-fungal activity against all the fungal strains tested. An anti-bacterial activity of the plant is also observed against gram-positive bacteria, however, was ineffective against gram-negative bacteria used in this study.

Key words: *Rhinacanthus nasutus* (L.), anti-microbial activity

INTRODUCTION

Rhinacantus nasutus (Acanthacae) is widely distributed in some part of the sub-continent India, in the region of Southeast Asia and China. The plant is a small slender shrub, 1-2 m in height. In traditional medicine preparations, leaves of this shrub are widely advocated for various skin problems. In India *Rhinacanthus nasutus* (L.) is used in poultice for the treatment of 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 Philippines it is traditionally used either as a sap or a decoction[1-3]. Numerous phytochemical studies have been carried out on this plant and as a result several compounds have been isolated and identified, notably 4-acetonyl-3, 5-dimethoxy-p-quinol, rhinacanthin-A, rhinacanthin-D, rhinacanthin-Q, rhinacanthone, dihydro-α-lapachone, p-hydroxybenzaldehyde, methyl vanillate, syrengaldehyde, lupeol, wogonin, oroxylin A, (+)-praeruptorin, allantoin, β-amyrin, stigmasterol, sitosterol stigmasterol-4-en-3-one, 2-methylanthraquinone, 2, 4-dimethoxybenzoquinone, 2-methoxy-4-propionylphenol, syringic acid, vanillic acid[4-8].

Some of these compounds have been tested for their biological activities such as cytotoxicity, anti-viral and anti-fungal[4, 6-8].

The present study intended to evaluate the folk medicinal use of a local species of this plant by studying the possible anti-fungal activity of 70% aqueous ethanolic extract of the leaves of *Rhinacanthus nasutus* (L.) using several pathogenic fungi. The extract was further tested for anti-bacterial activity using gram-positive and gram-negative bacteria known to be pathogenic in human.

MATERIALS AND METHODS

Plant material: *Rhinacanthus nasutus* (L.) Kurz. (Acanthaceae) were collected locally from a rural neighborhood of Penang, Malaysia during the period of May to June and authenticated by the Botanical Gardens Department, Penang, Malaysia. A voucher specimen is kept in our laboratory for future reference.

Leaves of *Rhinacanthus nasutus* (L.) were dried in a hot air oven and milled to fine powder using a mechanical grinder. The powdered plant material was macerated and shaken in 75:25 ethanol: water mixture at 60°C for 48 h using a bath shaker. The extract was then filtered with filter paper (Whatman Int. Ltd, Maidstone, UK) and concentrated to dryness under vacuum and reduced pressure using rotory evaporator at 40°C (Buchi, Switzerland). The concentrate was then layered on aluminium foil and freeze dried (Hetovac, Heto Laboratory Equipments, Denmerk). The yield was 26.9% in terms of dry leave weight. For anti-microbial study the extract was dissolved in deionised water and filter sterilised using

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Table 1: Anti-bacterial activity of *Rhinacanthus nasutus* (L.) extract [a]

Concentration of extract (mg mL^{-1})	2.5	5	10	50
Bacterial strains				
Gram-positive				
Bacillus cereus	7.9±0.5	8.0±0.4	9.0±0.4	11.9±1.8
Bacillus globigii	7.7±0.2	7.9±0.1	8.5±0.1	12.3±2.1
Bacillus subtilis	7.4±0.1	8.0±0.5	8.4±0.5	10.1±1.6
Staphylococcus aureus	--	--	7.4±0.2	9.3±0.5
Gram-negative				
Proteus morgani	--	--	--	--
Proteus mirabilis	--	--	--	--
Salmonella typhi	--	--	--	--
Pseudomonas aeruginosa	--	--	--	--
Escherichia coli	--	--	--	--

[a] Values presented are zone of inhibition (mm) in response to different dosages of extract and are Mean±SEM of three independent observations. --, No anti-bacterial activity observed.

Table 2: Anti-fungal activity of *Rhinacanthus nasutus* (L.) extract (Arbitrary scoring [a-d]).

Concentration of extract (mg mL^{-1})	0.0005	0.005	0.05	0.5	5.0
Fungal strains					
C. albicans	+	++	+++	+++	++++
T. meutagrophytes	+	++	+++	+++	++++
C. tropicals	++	++	++	+++	++++
C. parapsilosis	+++	+++	+++	++++	++++

+ - Weak effect; ++ - Good effect; +++ - Very good effect; ++++ - Complete inhibition

sterile filter papers (Whatman International Ltd, Maidstone, UK). A strict sterile condition is maintained in preparation and storage of the extract until it was used in the anti-microbial study.

Microorganisms used: Bacterial (Table 1) and fungal strains (*Trichophyton mentagrophytes* var *interdigitalae, Candida albicans, Candida tropicalis* and *Candida parapsilosis*) used in the present study were obtained from School of Pharmaceutical Sciences, Universiti Sains Malaysia, Penang, Malaysia.

Anti-bacterial activity: Conventional agar plate-diffusion method was used to evaluate the anti-bacterial activity of the *Rhinacanthus nasutus* (L.) extract. Overnight stock cultures of all the bacterial strains were prepared in nutrient broth (Oxiod, UK). A single volume, 0.5 mL of stock culture of each of the bacterial strains containing approximately 14x10^4 cells mL^{-1} were seeded and thoroughly mixed with nutrient agar (Oxoid, UK) at 45°C in individual petri dishes and allowed to solidify at room temperature. In these agar plate's, holes of 7 mm in diameter made using sterilized cork borer and filled in triplicate with a fixed volume, 0.2 mL of aqueous extract at four different concentrations (50, 10, 5 and 2.5 mg mL^{-1}). After a standing period of one hour the plates were incubated at 37°C for 18 h. The diameter of zone of inhibition was measured by Vernier calipers as index of putative anti-bacterial activity produced by the extract.

Anti-fungal activity: For anti-fungal activity a stock solution of the extract was serially diluted in glucose peptone agar (Oxoid, UK) media in desired concentrations. A volume of 0.5 mL of microorganism suspensions containing approximately 4×10^6 cells mL^{-1} were used to inoculate the surface of the solidified media and finally incubated at 28°C for eight consecutive days to observe the effect of the extract on fungal growth. We used an arbitrary scoring to describe the anti-fungal activity observed in the study.

RESULTS

Anti-bacterial activity: A dose dependent anti-bacterial activity was observed against all the gram-positive organisms (Table 1). Four different dosages (2.5, 5, 10 and 50 mg mL^{-1}) of the extract were used in this study and it was found that all dosages of the extract could inhibit the growth of *B. cereus*, *B. globigii* and *B. subtilis* as clear zones of inhibition were observed. However, only the higher dosages of the extract, 10 and 50 mg mL^{-1} could produce inhibitory effect on the growth of *Staphylococcus aereus*.

In case of gram-negative bacteria, none of the dosages of the extract could produce any inhibitory effect on their growth.

Anti-fungal activity: The *Rhinacanthus nasutus* (L.) extract showed moderate to potent (partial to complete inhibition of fungal growth) anti-fungal activity in relation to the dosages (0.0005, 0.005, 0.05, 0.5 and 5 mg mL^{-1}) applied. As presented in the Table 2, although only in case of *C. albicans* and *T. mentagrophytes* a clear dose dependent anti-fungal activity was observed, a spectrum of potent anti-fungal activity was observed in case of the highest dose (5 mg mL^{-1}) as it caused a complete inhibition of growth of all the fungal strains tested.

DISCUSSION

The 75% aqueous ethanolic extract of *Rhinacanthus nasutus* (L.) used was found to have potent anti-bacterial effect at the concentrations used against all the gram-positive bacteria tested in this study (Table 1). However, virtually no inhibitory effect of

the extract could observe on the growth of any of the gram-negative bacteria tested. Moreover, the anti-bacterial activity of the extract appeared to be dose dependent and was more active towards the *Bacilli* rather than *Cocci*.

The results of the anti-fungal activity (Table 2), revealed a complete inhibition of fungal growth in case of all the fungi tested at a dose of 5 mg mL^{-1} of extract. Moreover, a dose related inhibitory response was observed only in *C. albicans* and *T. mentagrophytes* var *interdigitalae* growth. Regarding the observed anti-microbial activity, in particularly the anti-fungal activity from previous reports by others, this study speculated that the presence of naphthoquinones and naphthopyran derivative of *Rhinacanthus nasutus* (L.) might be involved in the observed activity[6-8].

However, in the present study it was not within our scope to clarify how exactly these observed anti-microbial activities were brought about by the aqueous ethanolic extract of the leaves of this local *Rhinacanthus nasutus* (L.). Further study is underway in our laboratory to verify these activities and to isolate compound (s) that might be involved in such activities.

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