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Evaluation of Antibacterial Activity of *Rhinacanthus* Species in Sri Lanka

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ABSTRACT

Rhinacanthus nasutus (L.) Kurz is a valuable medicinal plant belonging to the family Acanthaceae that has many applications in the Ayurvedic system of medicine in Sri Lanka. *Rhinacanthus polonnaruwensis* Cramer is a more recently discovered species endemic to Sri Lanka, but its medicinal properties have not been recorded so far. The objective of the present study was to screen the antibacterial activity of leaf extracts of *R. nasutus* and *R. polonnaruwensis* against clinically isolated Gram-negative and Gram-positive bacteria. The study was carried out on six bacterial species, *Escherichia coli*, *Staphylococcus aureus, S. saprophyticus, Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexi.* Polar extracts of *R. nasutus* and *R. polonnaruwensis* were obtained by grinding the leaves with sterilized water and boiling the leaves in distilled water to obtain a decoction. A decoction of the concentration of 0.2 ml/ml of *R. nasutus* inhibited growth of all standard Gram-positive bacteria; *Staphylococcus aureus* and MRSA, whereas 0.2 ml/ml of decoction of *R. polonnaruwensis* inhibited growth of *Staphylococcus aureus*. Clinically isolated *Staphylococcus saprophyticus* was inhibited by both decoctions of *Rhinacanthus* species. None of the tested concentrations of the

two *Rhinacanthus* species inhibited the growth of any Gram-negative bacteria; *Pseudomonas aeruginosa*, *Escherichia coli*, and *Shigella flexi*. In this study, we demonstrated that the leaf extracts of both *R. nasutus* and *R. polonnaruwensis* were effective at inhibiting Gram-positive bacteria. However, the decoction of *R. nasutus* was found to be more effective against the tested Gram-positive bacteria than *R. polonnaruwensis*.

1. INTRODUCTION

The potential benefits of natural plant extracts have received attention in recent years, encouraging the development of natural products that effectively treat various diseases. *Rhinacanthus* belongs to the family Acanthaceae. There are two known species of *Rhinacanthus* used for medicinal purposes in Sri Lanka, *R. nasutus* (L.) Kurz and *R. polonnaruwensis* Cramer. *Rhinacanthus nasutus* is native to India, and widely distributed and cultivated in India, Madagascar, South China, South Africa, Sri Lanka, Taiwan, and Thailand (Siripong *et al*., 2006). *Rhinacanthus nasutus* is used as a traditional medicinal plant for thousands of years in the Ayurvedic system of medicine. Various parts of this plant have been used for the treatment of various diseases (Perry, 1980). It has been reported that rhinacanthin-C, rhinacanthin-D and rhinacanthin-N isolated from *R. nasutus* possessed antifungal, antibacterial, antiviral, anti-inflammatory, anti-allergic, hemorrhoid and various types of cancers (Puttarak, *et al*., 2010). *Rhinacanthus polonnaruwensis* Cramer was described more recently, and is native to Sri Lanka (Cramer, 1990), and found mostly in the Polonnaruwa district. Though it has been well documented about the antibacterial activity of *R. nasutus*, there are no such records about *R. polonnaruwensis*. Further, there are no studies on the investigation of the antibacterial properties of polar extracts of *Rhinacanthus* species found in Sri Lanka. Therefore, the objective of the present study was to evaluate the antibacterial properties of polar extracts of *R. nasutus* and *R. polonnaruwensis* grown in Sri Lanka and investigate the possibility of their application for medicinal purposes.

2. MATERIALS AND METHODS

Leaf extracts of *R. nasutus* and *R. polonnaruwensis* were prepared by two methods. The aqueous preparations were obtained by grinding 12g of fresh leaves in 7 ml of sterilized water. The decoctions were prepared by boiling of 12g of fresh leaves in 200 ml of distilled water and concentrating to 25 ml.

Four bacterial strains; *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* NCTC 6571, Methicillin-resistant Staphylococcus aureus (MRSA) 106 and Methicillin-resistant *Staphylococcus aureus* (MRSA) 112, were used to assess the antibacterial properties of the aqueous extracts of *R. nasutus* and *R. polonnaruwensis. Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* NCTC 6571 were used as the controls. These two strains are commonly used as a control strain for susceptibility testing to antibiotics, and they are sensitive to a variety of antibiotics, including methicillin (Treangen *et al*., 2014).

The decoctions and aqueous extracts of of *R. nasutus* and *R. polonnaruwensis* were tested for antibacterial properties using the two control bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* NCTC 6571), four standard bacteria (Methicillin-resistant Staphylococcus aureus (MRSA) 106 and Methicillin-resistant Staphylococcus aureus (MRSA) 112), *Pseudomonas aeruginosa* NCTC 10662, *Escherichia coli)*, and five clinically isolated bacteria from wounds of patients from the National Hospital, Peradeniya (coagulase-negative *Staphylococcus aureus, Staphylococcus saprophyticus, Shigella flexi,*

Salmonella typhi, *Pseudomonas aeruginosa* ESBL 111 and *Pseudomonas aeruginosa* ESBL 114).

The antibacterial activity of *R. nasutus* and *R. polonnaruwensis* was determined by the agar dilution method, using two different concentrations of aqueous extracts (0.05 ml/ml and 0.1 ml/ml) and three different concentrations of decoctions (0.05 ml/ml, 0.1 ml/ml and 0.2 ml/ml). For the preparation of 0.05 ml/ml and 0.1 ml/ml of aqueous extracts, 1 ml and 2ml of Muller-Hinton agar (Muller and Hinton, 1941) were removed respectively from a total volume of 25 ml, and equal volumes of the aqueous extracts were added. For the preparation of 0.05 ml/ml, 0.1 ml/ml and 0.2 ml/ml of the decoction concentrations, 1 ml, 2ml and 4 ml of Muller-Hinton agar (Muller and Hinton, 1941) were removed respectively from a total volume of 25 ml, and equal volumes of the decoctions were added. The agar media were poured into petri dishes and kept few minutes to settle. The inocula were prepared by adding 0.5 ml of direct colony suspension equivalent to 0.5 McFarland turbidity standards (McFarland, 1907) and 4.5 ml of distilled water to yield 1:10 dilution. Each type of bacteria was spotted on labeled petri dishes, and the petri dishes were incubated overnight at 37^0C. Each assay was replicated six times and repeated twice.

3. RESULTS AND DISCUSSION

The antibacterial activity of the aqueous extracts of *R. nasutus* and *R. polonnaruwensis* is shown in Table 1. The aqueous extracts did not show any significant activity against the tested bacteria. Therefore, we suggest that the aqueous extracts of 0.05 ml/ml and 0.1 ml/ml of *R. nasutus* and *R. polonnaruwensis* were ineffective against *Staphylococcus aureus* NCTC 6571, *Staphylococcus aureus* ATCC 25923, MRSA 106 and MRSA 112 bacteria.

Table 1. Antibacterial activity of aqueous extracts of *R. nasutus* and *R. polonnaruwensis*

Bacterial strain	*R. nasutus*		*R.polon-naruwensis*		Control
	0.05 ml/ml	0.1 ml/ml	0.05 ml/ml	0.1 ml/ml	
Staphylococcus aureus NCTC 6571	+	+	+	+	+
Staphylococcus aureus ATCC 25923	+	+	+	+	+
MRSA 106	+	+	+	+	+
MRSA 112	+	+	+	+	+

+ denotes presence of colonies

- denotes absence of colonies.

Previous studies have shown that *n*-hexane and chloroform extracts of roots and *n*-hexane extract of leaves showed potent antibacterial activity against Gram-positive bacteria (Munavaar *et al*, 2004, Siripong *et al*, 2006), whereas the aqueous extracts of all parts, methanolic extracts of stems and leaves as well as 85% ethanolic extract of stems were inactive. None of the extracts showed activity against Gram-negative bacteria (Siripong *et al*, 2006). It can be suggested that the extraction of bioactive compounds in *R. nasutus* requires non-polar solutions instead of polar solutions such as methanol, water, ethyl acetate or ethanol.

To investigate the antibacterial activity of the decoctions, we included an additional higher concentration (0.2 ml/ml) as the aqueous extractions at 0.05 ml/ml and 0.1 ml/ml concentrations of the two species did not inhibit the growth of any of the tested Gram-positive bacteria except *Staphylococcus saprophyticus* and *Staphylococcus aureus* NCTC at the concentration of 0.1 ml/ml (Table 2).

Table 2. The antibacterial activity of the decoctions of *R. nasutus* and *R*. *polonnaruwensis* against standard and clinically isolated bacterial strains.

Bacterial strain	*R. nasutus*			*R. polonnaruwensis*			Control
	0.05 ml/ml	0.1 ml/ml	0.2 ml/ml	0.05 ml/ml	0.1 ml/ml	0.2 ml/ml	
A- Coagulant-negative *Staphylococcus aureus* (clinically isolated, Gram-positive)	+	+	+	+	+	+	+
B- *Staphylococcus saprophyticus* (clinically isolated, Gram-positive)	+	-	-	+	+	-	+
C- *Shigella flexi* (clinically isolated, Gram-negative)	+	+	+	+	+	+	+
D- *Pseudomonas aeruginosa* (clinically isolated, Gram-negative)	+	+	+	+	+	+	+
E- *Salmonella typhi* (clinically isolated, Gram-negative)	+	+	+	+	+	+	+
F- *Pseudomonas aeruginosa* ESBL 111 (clinically isolated, Gram-negative)	+	+	+	+	+	+	+
G- *Pseudomonas aeruginosa* NCTC 10662 (standard Gram-negative)	+	+	+	+	+	+	+
H- *Escherichia coli* (standard, Gram-negative)	+	+	+	+	+	+	+
I- *Pseudomonas aeruginosa* ESBL 114 (clinically isolated, Gram- negative)	+	+	+	+	+	+	+
J- *Staphylococcus aureus* NCTC 6571 (standard, Gram-positive)	+	-	-	+	+	-	+
K- *Staphylococcus aureus* ATCC 25923 (standard Gram-positive)	+	+	-	+	+	+	+
L- MRSA 106 (standard Gram-positive)	+	+	-	+	+	+	+
M- MRSA 112 (standard Gram-positive)	+	+	-	+	+	+	+

+ denotes presence of colonies
- denotes absence of colonies.

The results presented in the Table 2 demonstrate that the decoctions obtained from the leaves of both *R. nasutus* and *R. polonnaruwensis* showed potent antibacterial activity towards all standard Gram-positive bacteria at the concentration of 0.2 ml/ml. *Rhinacanthus polonnaruwensis* did not show such remarkably potent antibacterial activity. It inhibited the growth of standard Gram-positive *Staphylococcus aureus* NCTC 6571 and clinically isolated Gram-positive *Staphylococcus saprophyticus* at 0.2 ml/ml. The growth of the same two bacterial strains were inhibited by the decoction of *R. nasutus* at a lower concentration (0.1 ml/ml). Neither *R. nasutus* nor R. *polonnaruwensis* was active against any Gram-negative bacteria tested. It could be hypothesized that this difference of the susceptibility of Gram positive and Gram-negative bacteria towards *Rhinacanthus* extracts may be due to the differences of their cell wall structure. Further studies will be required to test this hypothesis.

4. CONCLUSIONS

Both *R. nasutus* and *R. polonnaruwensis* leaf extracts possess antibacterial properties and effective against the Gram-positive bacteria, Staphylococcus aureus NCTC 6571, *Staphylococcus aureus* ATCC 25923, MRSA 106 and MRSA 112. However, none of the tested concentrations inhibited the growth of any tested Gram-negative bacteria, *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi* and *Shigella flexi*. Out of the two *Rhinacanthus* species, *R. nasutus* has the highest antibacterial activity. The leaf extracts taken by boiling the leaves of *R. nasutus* and *R. polonnaruwensis* in water (decoctions) were effective against *Staphylococcus saprophyticus* isolates taken from the wounds of patients having skin diseases. These two medicinal plant species can be potentially used in the preparation of

herbal medicines for skin wounds caused by *Staphylococcus saprophyticus.*

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REFERENCES

Cramer, L.H. (1990) Flora of the Polonnaruwa District. *Journal of National Science. Council, Sri Lanka, 18*(2), 168-170

McFarland J. (1907). Nephelometer: an instrument for media used for estimating the number of bacteria in suspensions used for calculating the opsonic index and for vaccines. *Journal of American Medical Assoc*iation, *14*, 1176-1178.

Mueller, J. H., Hinton, J. (1941). A protein-free medium for primary isolation of the *Gonococcus* and *Meningococcus*. *Experimental Biology and Medicine*. *48* (1), 330–333.

Munavaar, A.S., Abdullah, N.A., Abdul, H.K. and Noor, A.M. (2004). Antifungal and antibacterial activity of local plant, *Rhinacanthus nasutus* L. *Journal of Biological Sciences, 4*, 498-500.

Perry, L.M. (1980). *Medicinal plants of East and Southeast Asia: Attributed properties and uses*. Cambridge, Massachusetts and London: The MIT Press, p:5.

Puttarak, P., Charoonratana, T., and Panichayupakaranant, P. (2010). Antimicrobial activity and stability of rhinacanthins-rich *Rhinacanthus nasutus* extract. *Phytomedicine, 17*(5), 323-327.

Siripong, P., Wongseri V., Piyaviriyakul, S., Yahaufai, J., Chanpai, R. and Kanokmedakul, K. (2006). Antibacterial potential of *Rhinacanthus nasutus* against clinically isolated bacteria from Thai cancer patients. *Mahidol University Journal of Pharmaceutical Sciences*, *33*: 15-22.

Treangen, T. J., Maybank, R. A., Enke, S., Friss, M. B., Diviak, L. F., R. Karaolis, D. K., Koren, S., Ondov, B., Phillippy, A. M., Bergman, N. H., & Rosovitz, M. J. (2014). Complete genome sequence of the quality control strain *Staphylococcus aureus* subsp. *aureus* ATCC 25923. *Genome Announcements*, *2*(6).