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Anti-oxidant, pediculicidal and pesticidal activities of leaves of *Rhinacanthus nasutus* (Linn) grown in Sri Lanka

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Abstract: *Rhinacanthus nasutus* is an ethnomedical plant used in the traditional system of medicine for treating skin diseases. As no adequate studies available on *R. nasutus* grown in Sri Lanka, this study aimed on exploring anti-oxidant, anti-parasitic and pesticidal activity of leaves of the plant. Antioxidant capacity of the crude extract of leaves was evaluated using DPPH free radical scavenging assay and FRAP assay using ascorbic acid and FeSO₄.7H₂O as the standards respectively. Pediculicidal efficacy was carried out against head lice using 0.0010% (w/v) methanolic, ethyl acetate and aqueous extracts of leaves by following modified filter paper diffusion bioassay. Pesticidal activity of leaves was evaluated using modified filter paper contact bioassay against mealybugs, *P. marginatus*, resides on papaw and guava trees. The IC₅₀ value of DPPH assay was found to be 514.3 µg mL⁻¹ and FRAP value was found to be 594.33±2.93 µmol Fe²⁺/g in anti-oxidant assay. The LT₅₀ (median Lethal Time) values for 0.0010% (w/v) concentration of methanolic, ethyl acetate and aqueous extracts were 263, 347 and 676 min respectively in pediculicidal assay. LC₅₀ (50% mortality) value was found to be 0.0011% (w/v) after 240 minutes whereas LT₅₀ values of 0.0020% (w/v) of methanol, ethyl acetate and aqueous fractions of *R. nasutus* for adult were found as 31, 124 and 189 hours and for nymph were 14, 18 and 29 hours respectively in pesticidal assay. Accordingly, methanolic extract was effective in controlling nymph stage of mealybug life cycle than adults with the LC₅₀ of 0.0015% (w/v) within 14 hours. This study confirmed promising pediculicidal and pesticidal activity and significant anti-oxidant capacity of leaves of *R. nasutus*.

Keywords: *Rhinacanthus nasutus*; pediculicidal activity; pesticidal activity; DPPH assay; FRAP assay

1. Introduction

Plants and plant based products have been used for medicinal and health enhancement purpose since human civilizations and indeed since the evolutionary origin of humans. With the development of science, focus on plant research has increased all over the world and the research on plants that have been used in traditionally for various healthcare purposes have been intensified with the purpose of finding novel bioactive material and potential applications as greener value-added products, pesticides and insecticides (Cordell and Colvard, 2012; Anand *et al.*, 2019; Hamid *et al.*, 2010; Bhattacharya, 2015; Ayoub and Mishra, 2107). Infestation by head lice (*Pediculus humanus capitis*) is one of the most common parasitic diseases in childhood worldwide, and there is statistics that infestations have increased during the last decade (Falagas, 2008).

The plant, *Rhinacanthus nasutus* (Acanthaceae), commonly known as “Heen aniththa is an ethnomedical plant which is Aniththa,” native to Sri Lanka and widely distributed in North Central and Southern provinces of the country. It is a perennial woody shrub which flowering period is November to December. The history indicated the use of this plant in Sri Lankan traditional system of medicine to treat various ailments including eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension, several skin diseases, cancers, curing ring-worms related diseases and parasitic skin diseases, healing wounds, reducing dermatitis, (Devanarayana *et al.*, 2015; Bukke *et al.*, 2011). Some bioactive compounds and some pharmacological activities have been discovered from leaves of *R. nasutus* by researchers around the globe (Thongrakard and Tencomnao, 2010;

Brimson and Tencomnao, 2011; Punturee, 2004; Zubaid, 2009). The main bioactive constituents identified in the leaves were naphthoquinones and rhinacanthones which are known to have anti-proliferative activity against cancer cells (Siripong, 2006).

In Sri Lanka context, no adequate reports are available on scientific investigation of this multi-functional native plant in Sri Lanka, and so far no studies has been done to explore its pediculicidal and pesticidal activities. Therefore, this study was aimed on investigation of anti-oxidant capacity, pediculicidal and pesticidal activities of leaves of *R. nasutus* grown in Sri Lanka.

2. Materials and Methods

2.1. Chemicals used in the study

All the chemicals and reagents including methanol, hexane, ethyl acetate, chloroform, L-ascorbic acid, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) used in this study were of analytical grade and purchased from Sigma-Aldrich.

2.2. Plant material

Leaves of *R. nasutus* were collected from a domestic cultivation of Puttalam district, Sri Lanka and authenticated. Healthy leaves were washed and dried under breeze at room temperature for one week. The dried leaves were grounded into fine powder using a blender and were stored in sealed containers at 4 °C until usage.

2.3. Extraction of plant constituents

The methanolic extract of leaves was prepared by macerating in methanol for five days with frequent agitation. The macerated solution was filtered and concentrated under vacuum, and suspended in distilled water. The water suspended material was subjected to sequentially extraction with n-hexane, chloroform, ethyl acetate and aqueous as the remain fraction. All the extracts were concentrated under vacuum and stored at 4 °C until tests were performed (Bulugahapitiya, 2013).

2.4. Determination of antioxidant capacity

Anti-oxidant capacity was determined using 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay (DPPH assay) and ferric reducing antioxidant power assay (FRAP assay), and ascorbic acid and $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ were used as the standards respectively (Gohari *et al.*, 2011; Amaeze *et al.*, 2011; Soonwera, 2016).

2.4.1. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The concentrations of plant extracts were prepared as 25, 50, 75, 100, 200, 400, 600, 800 and 1000 $\mu\text{g mL}^{-1}$. Ascorbic acid was used as the standard, and the similar concentrations were prepared with the plant extract. The test solutions were prepared by mixing 1 mL of the extract and standard with 3 mL of DPPH solution. The solutions were kept in dark for 30 minutes at room temperature. The absorbance was measured at 517 nm using the UV-Vis spectrophotometer (UH5300 -HITACHI). The free radical scavenging activity was expressed as IC_{50} using % inhibition vs. concentration plot. The % inhibition was calculated from $[(\text{AB}-\text{AA})/\text{AB}] \times 100$, Where AB is the absorption of the DPPH without extract and AA is the absorption of the DPPH solution containing plant extract or ascorbic acid.

2.4.2. Ferric Reducing Antioxidant Power (FRAP) assay

Freshly prepared FRAP reagent (3.00 mL) was mixed with 0.4 mL of extract and absorbance was recorded at 593 nm after 30 min incubation at 37 °C. An aqueous solution of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (20-150 $\mu\text{g mL}^{-1}$) was used as the standard for calibration.

2.5. Pediculicidal assay

Adult head lice were collected from infested children 7-15 years old with the approval of their parents. The lice were collected by visual search of the hair and combing the scalp using a fine-toothed anti-lice comb. Viable head lice were obtained and pooled by removing them from the comb into clean glass boxes and kept cool and shaded (Kosalge and Fursule, 2009). Modified filter paper diffusion *in-vitro* bio assay was used to evaluate pediculicidal activity of plant extracts within an hour after collection (Capinera, 2008). Filter paper discs and strips coinciding with internal surfaces of petri dish were placed and lice were kept on filter papers and exposed to fixed concentration 0.0010% (w/v) crude extracts dissolved in water using sonication. Then the petri dishes were covered with the cellophanes with small holes allowing ventilation. Distilled water was used as negative control and commercially available head lice lotion containing 0.5% (w/v) permethrin was used as the positive

control. Lice mortalities were monitored using magnifying lens in specific time intervals at 0.5, 1, 2, 4 and 8 hours after treatment. The criteria used to evaluate survival of lice were defined as the complete absence of any vital signs. If any signs of life such as leg movements were observed, the lice were considered as alive. The lice were judged as dead if there were no vital signs at all. The mortality was calculated using the following formula (Finney, 1952).

$$\text{Mortality} = \frac{\text{Total number of dead lice}}{\text{Total sample size}} \times 100$$

The time which kill a 50% of head lice (LT₅₀) were determined varying the concentrations (Majeed *et al.*, 2018).

2.6. Pesticidal assay

Mealybugs were used to investigate pesticidal activity of the plant. They are common pests of houseplants. These species remove plants sap from above ground plant parts, especially stem tips, leaf junctures and new growth. Their feeding weakens and stunts plants, and causes foliar yellowing, defoliation, wilting and general plant decline. Mealybugs were collected from infested plants of papaw and guava cultivation which is located around University of Ruhuna, Sri Lanka. They were taxonomically identified as *P. marginatus* with the help of Department of Zoology, University of Ruhuna, Sri Lanka. Then adult mealybug individuals and nymphs were separated in to glass box using a tiny brush (Karamaouna *et al.*, 2012).

Filter paper contact bioassay with slight modifications was conducted to evaluate the pesticidal activity (Maheshu *et al.*, 2010). The setup of the experiment was same as the above pediculicidal assay. Two life stages of mealybugs such as nymphs and adult females were tested in this assay. Distilled water was used as negative control. Pest mortality was recorded in specific time intervals at 2, 4, 6, 12 and 24 hours after application. The mealybugs were judged as dead if there were no body movements at all. The median lethal time (LT₅₀) and lethal concentration (LC₅₀) was calculated as above using probit analysis.

2.7. Statistical analysis

Mortality data of head lice and mealybugs at all treatments were subjected to probit analysis to determine the LT₅₀ and LC₅₀ values of each solvent extracts of *R. nasutus*. R statistical software was used for all these statistical analyses.

3. Results and Discussion

3.1. Anti-oxidant activity

The results of percentage inhibitions of standard ascorbic acid and methanolic extract with respect to different concentrations are given in the Figure 1.

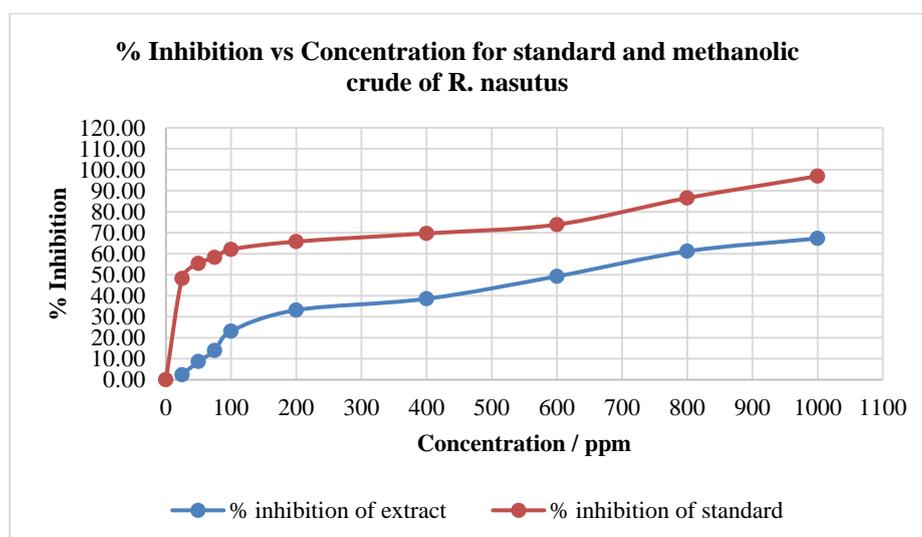


Figure 1. Percentage inhibition vs concentration for the standard series and the methanolic extract of *R.nasutus* in DPPH assay.

According to the percentage inhibition of DPPH, the calculated IC₅₀ values were 40.7µg/mL⁻¹ and 514.3 µg/mL⁻¹ for ascorbic acid and methanolic extract of *R. nasutus* respectively. DPPH is a stable free radical which is reduced in the presence of hydrogen donating antioxidants. Polyphenols present in plants are main responsible

for anti-oxidants activity exerted. Bukke *et al.* (2013) has reported the IC_{50} value of $230\mu\text{g mL}^{-1}$ for dry powder of the plant. They have also reported the antioxidant power of hexane, ethyl acetate, methanol and water extracts of *R. nasutus* leaves using DPPH assay, and IC_{50} values were lower than values obtained in this study (Bagavan *et al.*, 2009).

In FRAP assay, the antioxidant capacity was determined using the reducing power of the plant extract. The mean FRAP equivalent value was found to be $594.33\pm 2.93 \mu\text{mol Fe}^{2+} / \text{g}$ for crude methanolic extract. The FRAP assay treats the antioxidant in the sample as reductants in redox linked colorimetric reaction and the value reflect the reducing power of the antioxidants. In this assay, the antioxidants react with Fe^{3+} -TPTZ to produce a coloured Fe^{2+} -TPTZ complex which is measured at 593 nm. FRAP value of this study showed the highest FRAP value indicating highest total antioxidant capacity. Notably, there are no available records from Sri Lankan studies to compare the antioxidant capacity of *R. nasutus* grown in Sri Lanka.

3.2. Pediculicidal activity

The average percentage mortality of head lice in methanol, ethyl acetate and aqueous extracts of *R. nasutus* is shown in Table 1.

Table 1. Average percentage mortalities of head lice at different crude solvent extracts of *R. nasutus*.

Time /min	Average % mortality				
	Methanolic crude	Ethyl acetate crude	Aqueous crude	Negative control	Positive control
30	0	0	0	0	90
60	0	0	0	0	100
120	10	10	0	0	100
240	50	30	10	0	100
480	90	60	40	0	100

The LT_{50} values obtained from probit analysis for mortality values of 0.0010% (w/v) of methanolic, ethyl acetate and aqueous extract were found to be 263, 347 and 676 min. The methanolic fraction were highly active against head lice.

The whole bioassay procedure was repeated for active methanolic extract of *R. nasutus* with varying concentrations as 0.0005%, 0.0010%, 0.0015%, 0.0020% and 0.0025% in w/v basis. The LC_{50} (50% mortality) value was found to be 0.0011% (w/v) after 240 min which was calculated using the graph of probit mortality versus log concentration as indicated in the Figure 2.

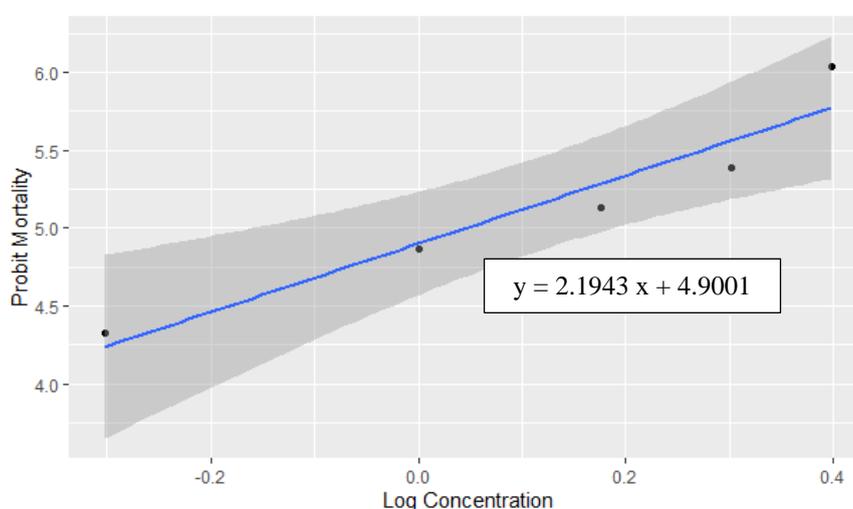


Figure 2. The graph of Probit mortality vs. Log concentration in Pediculicidal Assay.

The data in this bioassay highlights that methanolic extract of *R. nasutus* is highly toxic to head lice with 90% mortality at 480 min. Further, the 0.0010% (w/v) of methanolic extract has lowest LT_{50} value. Therefore, methanol extract showed high levels of mortality on adult lice in short time than the other extracts. Based on the above results, it is evident that *R. nasutus* has high potential to kill lice and hence, therefore there is an extensive scope for development into products of pediculicidal activity. It is noteworthy that there is no recorded literature

available for this plant species in terms of pediculicidal studies and this work is recognized as the first study related to this

3.3. Pesticidal assay

The average percentage mortality values for adults and nymph stages of mealybug are given in the Table 2.

Table 2. Average percentage mortalities of adult mealybugs and nymphs at different crude extracts of *R. nasutus* with respect to different exposure times.

Time hours	Average percentage mortality / %						
	Adult mealybugs			Nymphs of mealybugs			
	Methanolic crude	Ethyl acetate crude	Aqueous crude	Methanolic crude	Ethyl acetate crude	Aqueous crude	Aqueous crude
2	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0
6	0	0	0	15	5	0	0
12	10	0	0	60	35	10	10
24	35	20	10	85	60	45	45

Based on the results different extracts of *R. nasutus* it shows that nymphal mortality is significant compare to the adult mealybug mortality. The LT_{50} values of each 0.0020% (w/v) of methanolic, ethyl acetate and aqueous fractions were 31, 124 and 189 hours for the adult mortality and 14, 18 and 29 hours for the nymph mortality respectively. Methanolic fraction was highly active against both adult and nymph stage of the mealybug and the LC_{50} (50% mortality) value recorded as 0.0015% (w/v) after 14 hours which was calculated by using the graph of probit mortality versus log concentration as indicated in the Figure 3.

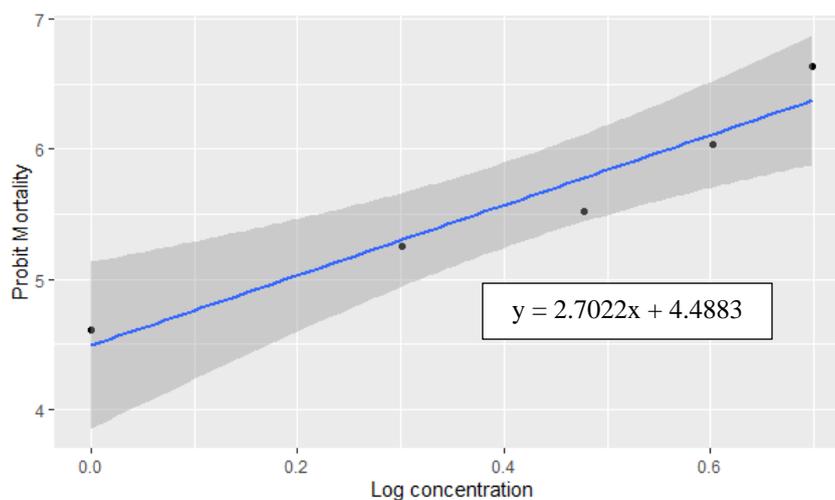


Figure 3. The graph of Probit mortality vs. Log concentration in Pesticidal assay.

The data in this pesticidal assay brought to notice that nymph stage of mealybug life cycle is the most susceptible stage to the plant extracts in term of pesticidal activity, and methanol extract showed 50% of mortality on nymph stage of mealybugs in 14 hours, with LC_{50} value of 0.0015% (w/v). It is noteworthy that there is no recorded literature available for this plant species in terms of pesticidal activity against mealybugs. However, *R. nasutus* has been studied against few pest insects by other researchers (Fadli *et al*, 2017; Peschiutta *et al*, 2018). However, contact pesticides are less effective against mealybugs because of their cryptic habitats in plants and the water proof waxy layer on the body. Therefore, the field trials should be necessary in order to check the reliability of plant extracts in control of mealybugs. Also, these results give an insight into the potential use of the *R. nasutus* as botanical pesticide especially against mealybug species on cultivated fruit crops in Sri Lanka.

4. Conclusions

This study confirmed that the leaves of *R. nasutus* exert promising pediculicidal activity and pesticidal activity against mealybugs. The methanolic extract of *R. nasutus* encompasses the highest activity in all performed tests.

The high antioxidant activity of leaves signifies the multifunctional therapeutic potential of *R. nasutus*. Further clinical trials and field trials will be needed to assess the accuracy of the laboratory results of *in-vitro* pediculicidal and pesticidal assays in order to develop it into value added products.

Conflict of interest

None to declare.

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