The Journal of Phytopharmacology 2021; 10(5):316-318
Online at: www.phytopharmajournal.com


Research Article

ISSN 2320-480X
JPHYTO 2021; 10(5): 316-318
September- October
Received: 09-07-2021
Accepted: 19-08-2021

doi: 10.31254/phyto.2021.10506

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# Qualitative Phytochemical analysis of *Rhinacanthus nasutus*

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## ABSTRACT

*Rhinacanthus nasutus* (Nagamalli) is a small slender shrub widely distributed in India, South East Asia and China. This herb is being used for treating snake bite by irula tribes in the remote villages of Nilagiri hills. The plant possesses a wide spectrum of phytochemical substances. The current study is aimed at measuring the qualitative phytochemistry of *Rhinacanthus nasutus* cultivated at herbal garden of Veterinary College and Research Institute, Orathanadu. The results suggest that the leaves and root of *Rhinacanthus nasutus* are potential sources for healthy phytochemicals especially alkaloids and phenols.

**Keywords:** Phytochemistry, Nagamalli, Methanol extract, Petroleum ether extract.

## INTRODUCTION

The native healers used varieties of herbs to treat domestic animals. Herbal products were used as medicine and hence they are intensively studied. Thereby medicinal plants became potential sources of new compounds of therapeutic value. Many secondary metabolites of plants are commercially important and found to be used in a number of pharmaceutical compounds [4]. Recent developments in the field of phytomedicine research have emphasized more on the scientific evaluation of medicinal herbs against usual drugs owing to their immense beneficial properties. As medicinal plants produce several phytochemical compounds they can be suitably used as an alternative source of medicine to synthetic drugs. Phytochemicals play important roles in plant defence mechanisms and are mainly classified into carotenoids and polyphenols [6].

*Rhinacanthus nasutus*, popularly known as Nagamalli in Tamil, is a profusely branching herb in the family Acanthaceae. It is distributed in the Indian sub-continent, Southeast Asia and China. Since 2019, the plant was widely cultivated at Herbal Garden of Veterinary College and Research Institute, Orathanadu as medicinal plant.

*Rhinacanthus nasutus* is a perennial shrub found as wild bushes that love shade. It grows to 58-74 cm in height. The stems are slender [9]. The plant has long leaves, narrow at both ends. The plant has spreading, leafy inflorescence. They have triple lobed flowers which are white with brownish dots. The plant has four seeded fruits. The leaf powder of *R.nasutus* of about 15 gm was a good antidote for cobra bite among the Irula tribes of Tamil Nadu [2].

In the present study, the qualitative phytochemistry of *Rhinacanthus nasutus* was assessed to find out its potential as therapeutic agent.

## MATERIALS AND METHODS

Fresh leaves of *Rhinacanthus nasutus* were collected from Herbal Garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu. The leaves were cleaned, shade dried and finely powdered for further analysis.

### Preparation of Alcoholic Extracts

The powdered sample (25g) was extracted with 125ml of Methanol for 6 hours in a Soxhlet Apparatus [10]. A second sample (25g) was extracted with 125ml of Petroleum ether for 6 hours in a Soxhlet Apparatus. The extracts were kept in a desiccator allowing the solvent to evaporate completely. The materials extracted were kept in Rotary Vacuum Splash Evaporator to remove the solvent in the extracted material [10]. The evaporator was rotated at 50 rpm under 50 ºC of temperature for cooling the circulator for collecting condensed solvent in the flask. The evaporation was done fast and the extract was quickly analyzed to prevent oxidation of the phenolics. The preliminary analysis of leaf extract was

done according to standard protocols to find the presence of tannins, saponins, alkaloids, phenols, glycosides, flavanoids, amino acids, carbohydrates, steroids, and terpenoids. The Gallic acid equivalence (GAE) method in which Folin- Ciocalteu's reagent was used to find the total phenolic compounds in root, stem and leaves [7]. A standard curve for total phenols was established using Gallic acid [5].

A Standard Gallic acid stock solution was made by dissolving 100 mg of gallic acid in 10 ml of 80%methanol. Followed by the addition of distilled water to make the volume to 100ml. A Standard Gallic acid stock solution with concentration of 1mg/ml was obtained.

The Standard Gallic acid stock solution was diluted with distilled water to obtain calibrated standard solutions (25 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of gallic acid.

The sample solution was prepared by dissolving 100 mg of dry extract in 10ml of 80% methanol, followed by addition of 80% methanol to make the volume to 100 ml to obtain a sample solution with 1mg/ml concentration.

**The estimation Process for total phenols**

The methanolic sample solution (1ml) was added to 9 ml of distilled water in a 25ml volumetric flask. The Folin-Ciocalteu phenol reagent (1ml) was added to the mixture. Then mixed well using stirrer for five minutes. A 7% sodium carbonate solution (10ml) was added to the mixture. The obtained mixture was diluted to 25 ml with distilled water. Then the mixture was allowed to incubate for ninety minutes at room temperature. An absorbance was determined against prepared blank reagent at 765 nm under a Ultra Violet visible spectrophotometer. The total phenolic contents were expressed in milligram of Gallic acid equivalent per milliliter [1]. The experiments and analysis were done thrice to ensure the precision of the study.

## RESULTS AND DISCUSSION

The preliminary phytochemical screening of the leaves extracted in methanol are given in Table 1. The results of preliminary phytochemical analysis for methanolic extract of *Rhinacanthus nasutus* leaves indicated the presence of alkaloid, carbohydrates, phenolics, tannins, volatile oils, hydrolysable tannins, flavonoids and glycerides. A wide range of chemical compounds such as carbohydrates, anthraquinones, flavonoids, benzenoids, glycoside and napthoquinone were identified in plant extracts [9].

From the leaves and roots of *Rhinacanthus nasutus* plant was isolated important napthoquinones such as Rhinacanthin A, B, C, D, G, H, I, J, K, L, M, N, O, P, and Q [8]. From the roots of *Rhinacanthus nasutus* plant dehydro α-lapachone was isolated. The Benzenoids like vanillic acid, syringic acid, methyl valinate and syringaldehyde were isolated from leaves and roots of *Rhinacanthus nasutus* plant [11].

The extracts of the leaves of *Rhinacanthus nasutus* showed secondary metabolites [3] such as carbohydrates, flavonoids, alkaloids, anthraquinones, polyphenols, phytosterols and saponins.

The total phenolic compound in leaf and roots of *Rhinacanthus nasutus* was estimated and presented in Table 2. In *Rhinacanthus nasutus* the petroleum ether extract of leaves had 340 mg GAE/ml of total phenolic compounds, petroleum ether extract of root had 290 mg GAE/ml of total phenolic compounds, Methanol extract of leaves had 310 mg GAE/ml of total phenolic compounds and Methanol extract of root had 160 mg GAE/ml of total phenolic compounds while 388.33mg Gallic acid equivalent per ml of total phenols was found in *Withania somnifera* and 513.33mg Gallic acid equivalent per ml of total phenols was found in *Asparagus racemoses* [7].

The present study revealed that petroleum ether extract of leaves had 42% higher level of total phenols as compared to Gallic acid solution, petroleum ether extract of root had 21%higher level of total phenols as compared to Gallic acid solution, methanol extract of leaves had 29% higher level of total phenols as compared to Gallic acid solution and methanol extract of root had 33% lower level of total phenols as compared to Gallic acid solution.

**Table 1:** Preliminary Phytochemical Screening of Methanolic leaf extracts

| Phytochemical compound | Method | *Rhinacanthusnasutus* |
|---|---|---|
| Alkaloids | Dragendorff test | Positive |
| Glycosides | Keller Killani Test | Negative |
| Tannins | Ferric chloride test | Positive |
| Saponins | NaOH test | Negative |
| Phenolics | Ferric chloride test | Positive |
| Flavonoids | Shinoda Test | Positive |
| Terpenoids | Noller Test | Negative |
| Volatile oil | Fluorescence test | Positive |
| Carbohydrates | Fehlings test | Positive |
| Phylobatannin | Foam test | Negative |
| Aminoacids | Ninhydrin test | Negative |
| Hydrolysable tannin | Braymer's test | Positive |
| Vitamin C | Ascorbic acid test | Negative |
| Steroids | Salkowski's test | Positive |

**Table 2:** Estimation of Total Phenol Content in *Rhinacanthusnasutus*

| Type of extract | OD value | GAE/ml extract (in mg) |
|---|---|---|
| Petroleum ether extract of leaves | 0.3177 | 340 |
| Petroleum ether extract of root | 0.267 | 290 |
| Methanol extract of leaves | 0.2876 | 310 |
| Methanol extract of root | 0.1369 | 160 |

## CONCLUSION

*Rhinacanthus nasutus* is rich in healthy phytochemical compounds, essentially the alkaloids and phenols. The presence of alkaloids and phenols indicates the potential of *Rhinacanthus nasutus* as a therapeutic agent.

**Acknowledgement**

The authors are grateful to TANII Scheme, Government of Tamil Nadu and Tamil Nadu Veterinary and Animal Sciences University for the essential resources provided during the course of this research.

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**HOW TO CITE THIS ARTICLE**
Sivagnanam S, Ranganathan V, Paramasivan S. Qualitative Phytochemical analysis of *Rhinacanthus nasutus*. J Phytopharmacol 2021; 10(5):316-318. doi: 10.31254/phyto.2021.10506