



ISSN 2320-3862
JMPS 2015; 3(6): 51-55
© 2015 JMPS
Received: 22-09-2015
Accepted: 26-10-2015

V Baskar Ananda Raj
Department of Pharmacognosy,
Padmavathi College of
Pharmacy and Research Center,
Krishnagiri Main Road,
Periyanaahalli, Dharmapuri
district, 635205, Tamil Nadu,
India.

S Suresh Kumar
Department of Pharmacognosy,
JKK Nataraja College of
Pharmacy, Komarapalayam,
Namakkal district, 638183,
Tamil Nadu, India.

KL Senthil Kumar
Department of Pharmaceutics,
Padmavathi College of Pharmacy
and Research Center, Krishnagiri
Main Road, Periyanaahalli,
Dharmapuri district, 635205,
Tamil Nadu, India.

Correspondence

V Baskar ananda Raj
Research Scholar, Department of
Pharmacognosy, Padmavathi
College of Pharmacy, Krishnagiri
main road, Periyanaahalli –
635205, Dharmapuri district,
Tamilnadu, India.

Journal of Medicinal Plants Studies

www.PlantsJournal.com

HPTLC Standardization and Quantification of *Rhinacanthus nasutus*

V Baskar ananda Raj, S Suresh Kumar, KL Senthil Kumar

Abstract

Background: Ethnobiological status of the plant *R.nasutus* (*Rhinacanthus nasutus*) has been analyzed by HPTLC standardizing and quantifying of flavonoids metabolites.

Methods: HPTLC method using 10 μ l of ethanol extract of *R.nasutus* at a concentration of 10mg/ml along with 10 μ l of each 1mg/ml standard concentration of Rutin and Quercetin were applied to the pre-coated silica gel F₂₅₄ HPTLC plates, and the plate was developed in a twin trough TLC chamber saturated with Ethyl acetate: Formic acid: Glacial acetic acid: water (11:1.1:1.1:2.6). Evaluation by using a scanning TLC scanner was carried out in the absorbance mode at 254 and 366 nm and the result was visualized using CAMAG Visualizer.

Result: The extract *R.nasutus* were estimated for the presence of standards Quercetin and Rutin by HPTLC method done by comparing the R_F values and peak matching with HPTLC chromatogram and quantified to be Quercetin - 11.6% and Rutin - 5.4% respectively.

Conclusion: Standardized criteria for studying the secondary metabolites such as flavonoids with reliable standard methods are often lacking. Efforts are underway to establish a pharmacoepidemiological evidence base regarding safety and practice of Ayurvedic medicines.

Keywords: *Rhinacanthus nasutus*, traditional, Ayurveda, HPTLC, chromatogram, Quercetin, Rutin, Flavonoids and phytomedicine.

Introduction

In traditional medicine research, clinical experiences, observations or available data are starting points, which are issues related to their quality assurance remain vitally important. In many cases, the exact chemical constituents and percentage of active constituents present in the plant needed to give a definite and specific biological effect have yet to be verified. These constituents need to be limited to a biological effect that in turn is relevant to a therapeutic target. This approach may be obtained by determining the natural compounds present in the selected botanical, followed by biological testing, to identify biological fingerprints of the plant extracts and their effective concentration [1].

Although several studies have been well performed, there have been conflicting clinical data, suggesting a lack of specific activity or inappropriate preparation. Owing to these numbers of new chemical/molecular entities that are approved are declining. This situation calls for critical assessment of the current reduction strategies, where only new chemical entities are valued as potential new drugs. Currently, many pharmaceutical companies and research organizations' have demonstrated renewed interest in investigating higher plants as sources for novel lead structures and also for the development of standardized phytomedicines with reasonable efficacy, safety and quality [2].

Botanical extracts can only be declared pharmacologically active if a reliable correlation can be made between a measurable active principle and its declared medicinal effect. Therefore, there is a requirement to look for secondary metabolites from plant sources. Consequently, this has led to the discovery of potentially useful preliminary active ingredients that can serve as a source and template for the synthesis of new drugs from the plant *R.nasutus*, thus by proving through quantification and standardization by using HPTLC method.

Plant Source

Rhinacanthus nasutus commonly known in English as "Snake Jasmine" due to the shape of its flowers [3] and as "Nagamalli" in Tamil belonging to family Acanthaceae. The plant is a small, slender shrub widely distributed in some parts of the sub-continent, in the region of Southeast

Asia and China. The genus *Rhinacanthus* comprises of about 25 species confined to the Old World tropics and subtropics it is placed in the Justiciae subtype. Traditionally roots are used against snake venoms. Rhinacanthine present in roots induce apoptosis in human cervical carcinoma cells and hepatocellular cancers. The leaves, root and whole plant are used for the treatment of ringworm, skin diseases, *Tinea versicolor*, inflammation, abscess, haemorrhoid, fungal infection, cancer, rheumatism, pulmonary tuberculosis, poisoning, septic ulcer, eczema, leprosy, dhobie's itch, herpes, and scurvy, proved to be antidiabetic in rats^[4]. Kurumba tribes in India apply the leaf paste externally to treat wounds. Many secondary metabolites are isolated such as flavonoids, triterpenoids, steroids, benzenoids, lignans, glycosides, naphthoquinones,^[5, 6, 7] coumarins, anthraquinone, quinone, glycosides, carbohydrates, sterols, dehydroalanine, *p*-hydroxybenzaldehyde, methyl vanillate, syringaldehyde, lupeol, wogonin, oroxylin A, (+)-praeruptorin, allantoin³, rhinacanthone, rhinacanthin^[8], rhinacanthin-C, rhinacanthin-D, rhinacanthin-N, rhinacanthin-Q, rhinacanthin (A-D,G-Q) and rhinacanthone have been reported to possess antiproliferative activity against a panel of cancer cells. Rhinacanthone (3, 4-dihydro-3,3-dimethyl-2H-naphthol [1,2-b] pyran-5,6-dione) was first isolated from the shrub which is essential for the tumor activity^[9].

Materials and Methods

Procurement of Plant materials

The leaves of *R.nasutus* were collected during morning hours/flowering season, from Palakkad district, Kerala, India, authenticated and Voucher specimens (RN/148/14) was submitted in Department of Pharmacognosy, Padmavathi College of Pharmacy, Dharmapuri district, Tamilnadu, India.

Extraction of Herb

Herbs for extraction were powdered, softer herbs (*R.nasutus* - leaves) powdered to moderately coarse. The leaves of *R.nasutus* were dried at 35-40 °C for several days and crushed into small pieces. The 100g of powdered leaves were extracted using ethanol as solvent by hot continuous Soxhlet apparatus for 6 hours. The extract thus obtained was cooled at room temperature and evaporated to dryness under reduced pressure in a rotatory evaporator^[10].

Hptlc Standardization and Quantification

Reagents and Standard

Analytical grade N-butanol, Glacial acetic acid were obtained from S.D. Fine chemicals, Mumbai, India. Standard amino acids were purchased from Sigma- Aldrich, USA.

Standard Preparation

5 mg of Rutin and Quercetin were dissolved in 5ml of ethanol (1mg/ml concentration)

Sample preparation

Crude extract preparation: 100 mg of ethanol extract was dissolved in 10 ml of ethanol and slightly warmed on a water bath for 5-10 min and after cooling the liquid was filtered through 42 sizes Whatman filter paper. The filter process was repeated for three more times and the same solution is employed for HPTLC analysis. (10mg/ml concentration).

Chromatographic conditions for determination of Rutin & Quercetin

Stationary phase : Precoated Silica gel F₂₅₄ Plates (MERCK KGaA)

Mobile phase: Ethyl acetate: Formic acid: Glacial acetic Acid: water (11:1.1:1.1:2.6)

Saturation: 60 mins

Development chamber: CAMAG twin trough development chamber

Applicator: CAMAG "Linomat V_ 171118" S/N 171118 (1.00.12)

Scanner: CAMAG TLC "Scanner_171019" S/N (2.01.02), Switzerland

Illumination instrument: CAMAG Visualizer 171217

Digital Camera type: snr& lens: DXA252:490823510. Computar, 12mm, f4.0

Mode of scanning: Absorption (deuterium)

Detection wavelength: 254 nm, 366 nm

Solvent front position: 80.0 mm

Volume: 10.0 ml

Volume applied (standards): 10 µl of the above prepared standard solution was applied.

Volume applied (sample): 10µl of the above prepared crude extract was applied.

HPTLC Plates

The precoated and preactivated TLC plates (E. Merk No.OB575863) of silica gel F₂₅₄ with the support of aluminum sheets having a thickness of 0.25 mm and size 20 × 20 cm were cut into smaller size according to required dimensions (10×10 cm).

Selection of solvent

Solvent system (10 ml), Ethyl acetate: Formic acid: Glacial acetic acid: water (11:1.1:1.1:2.6) (v/v/v) was poured into CAMAG twin trough chamber and allowed for chamber saturation to 15 min. After chamber saturation the TLC plates were developed in the solvent system for 20 minutes up to 9 cm length, by ascending technique. The plates were removed from the chamber after the development and dried.

Application of standard and sample

Ethanol extracts *R.nasutus* was dissolved in ethanol to get a concentration of 10mg/ml, were applied to the pre-coated silica gel F₂₅₄ HPTLC plates (10×10 cm, 6 mm band thickness, E. Merck, Germany) along with 10µl of each standard concentration of Rutin and Quercetin by means of CAMAG LINOMAT V an automatic sample application device. The quantity of test extract and standards applied was 10µl. After 10 min of drying at room temperature, the plate was developed in a twin trough TLC chamber (20×10 cm, CAMAG, Switzerland) saturated with ethyl acetate: Formic acid: Glacial acetic acid: water.

Evaluation of both standards and sample were performed. Again, the plate was dried at room temperature for 30min and then scanning was performed in TLC scanner (CAMAG, Switzerland) in the absorbance mode at 254 and 366 nm, the result was visualized using CAMAG Visualizer 171217 WINCATS software. A graph was plotted of the sample peak area against concentration of standards Rutin, Quercetin and linear regression was applied without forcing the intercept at x = 0, while plotting graph^[11, 12].

Post Chromatographic treatment of Plates:

Chromatography is a separation technique employed in the separation of a mixture of compounds into its individual compounds by distributing between the two immiscible phases a mobile and a stationary phase.

The detection of spots in the TLC plates was carried out by spraying 0.1% Ninhydrin reagent and viewing at 254, 366 nm.

The TLC plate is heated at 100°C for 5-10 minutes and observed in visible light [11, 12].

After development of chromatogram the silica gel plates were scanned by using a scanner and the marker compounds present in the extract are quantified by using the formula:

Quantification of marker compounds in the *R.nasutus*

$$\frac{\text{Sample peak area}}{\text{Standard peak area}} \times \frac{\text{Std. concentration}}{\text{Vol. dilution}} \times \frac{\text{Vol. dilution}}{\text{Sample. Con}} \times \frac{\text{Standard applied}}{\text{Sample applied}} \times 100$$

Result and Discussions

Qualitative and quantitative analysis was carried out by HPTLC method

The ethanol extract of *R.nasutus* were estimated for the presence of standards Quercetin and Rutin by HPTLC method done by comparing the R_F values and peak matching with HPTLC chromatogram and quantified to be Quercetin 11.6% and Rutin - 5.4%. The result is shown in Fig no 1-3.

***R.nasutus* extract (Rutin)**

Totally 11 peaks observed in *R.nasutus* extract, in this 7th peak with R_F(0.50) (Fig no 3) is matching to standard Rutin with R_F

value of (0.51) (Fig no 2) at the 3rd peak by peak area comparison, the presence of rutin in the extract was confirmed and quantified.

***R.nasutus* extract (Quercetin)**

Totally 11 peaks observed in *R.nasutus* extract, in this 11th peak with R_F (0.98) (Fig no 3) is matching to standard Quercetin with R_F value of (0.98) at the 6th peak by peak area comparison, the presence of Quercetin in the extract was confirmed and quantified.

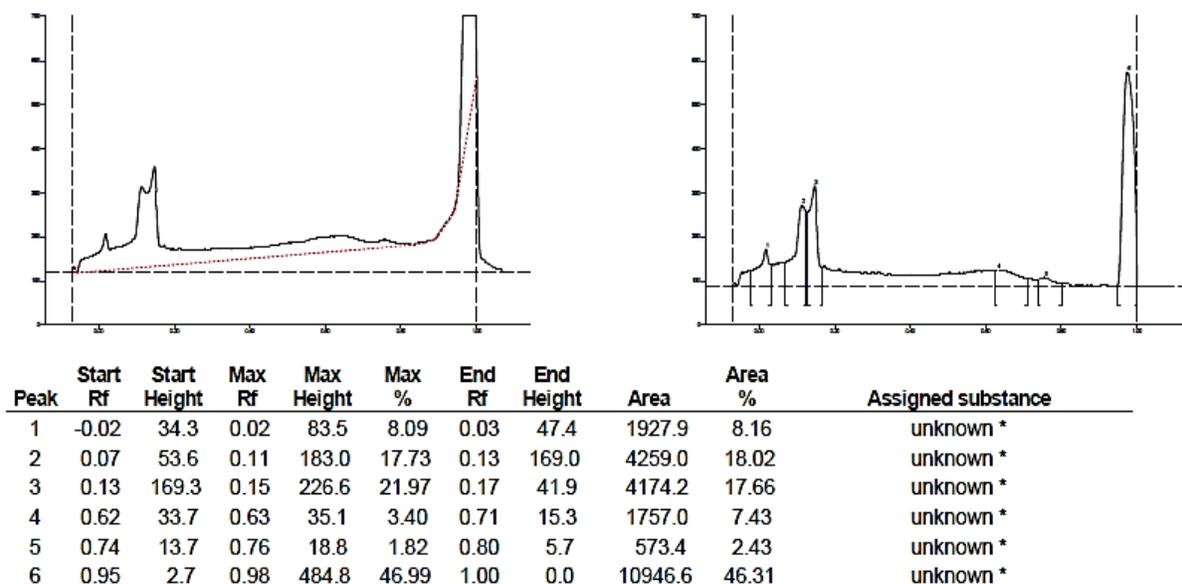


Fig 1: HPTLC of Standard Quercetin

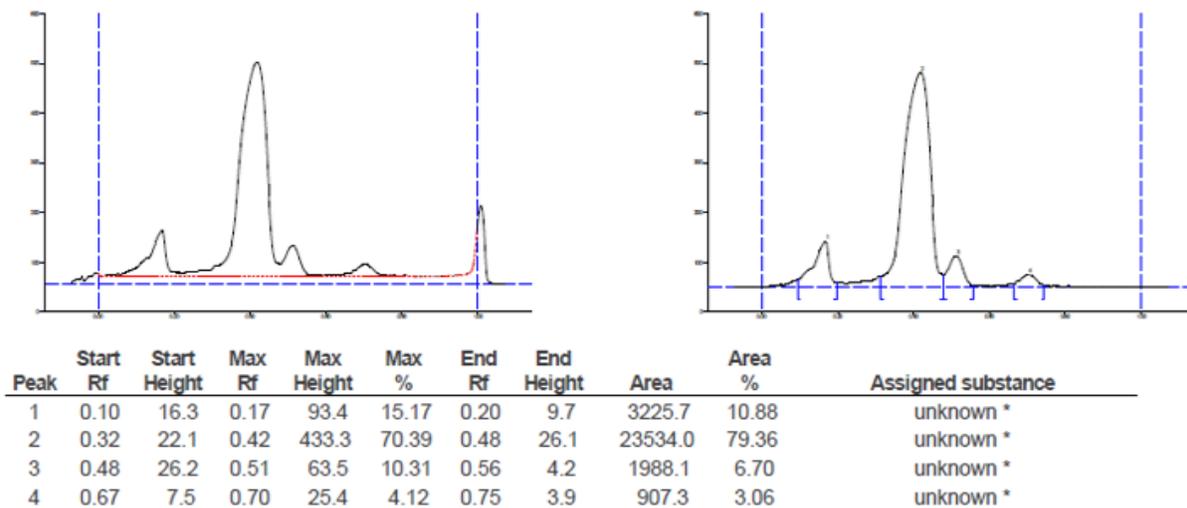


Fig no 2: HPTLC of Standard Rutin

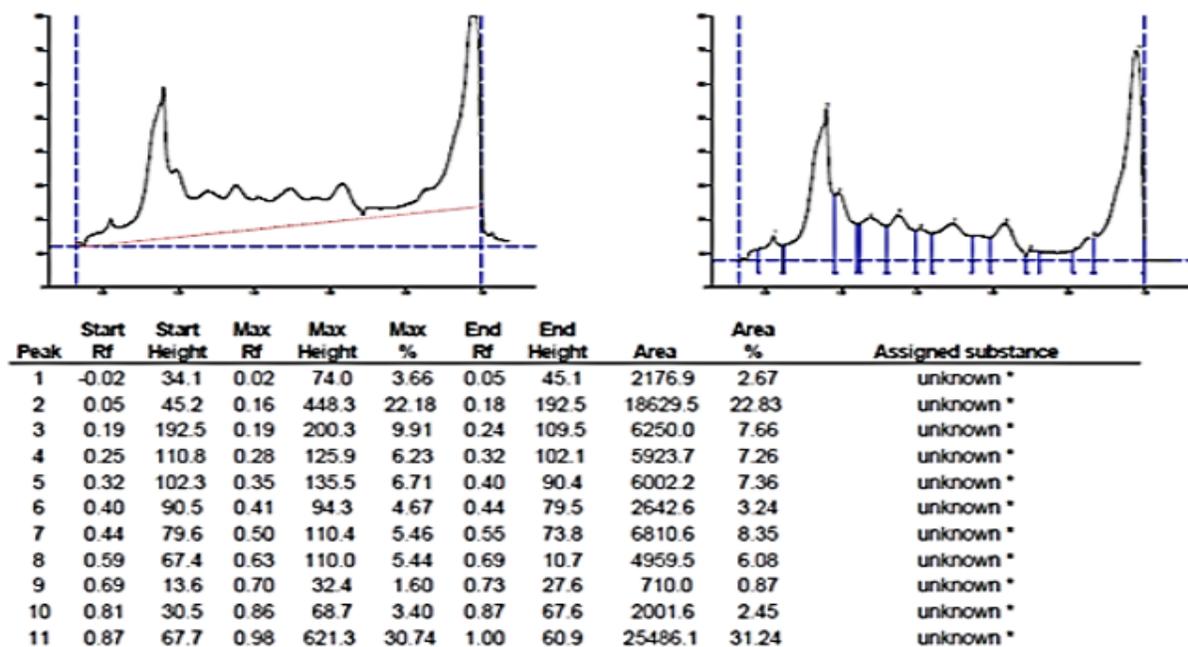


Fig no 3: HPTLC of extract *Rhinacanthus nasutus*

Discussions

Nature is the paradise of medicinal principles offers to the humanity through plants which act as the richest source of phytochemicals since time immemorial. India has been known to be a rich repository of medicinal plants. Ayurveda is gaining momentum and prominence as the natural system of health care all over the world. It's therefore essential to search for the efficacious plants of medicinal value for better manifestations [13].

According to Prashant Tiwari *et al*, [14] the purpose of standardized extraction procedures for crude drugs is to attain the therapeutically desired portions and to eliminate unwanted material by treatment with a selective solvent. Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent employed in the extraction procedure. Properties of a good solvent for plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate.

The choice of solvent depends on what is intended with the extract. Higher concentrations of more bioactive flavonoids compounds were detected with ethanol 70% due to its higher polarity than pure ethanol. By adding water to the pure ethanol up to 30% for preparing ethanol 70% the polarity of solvent, was increased. Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against micro-organisms are aromatic or saturated organic compounds, they are most often obtained through initial ethanol or methanol extraction. Methanol is more polar than ethanol, but due to its cytotoxic nature, it is unsuitable for extraction in certain kind of studies as it may result in incorrect results.

Soxhlet extraction is only required where the desired compounds have a limited solubility in a solvent, and the impurity that is insoluble in the solvent. The advantage of this system is possible that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled.

Conclusion

Efforts are underway to establish a pharmacoepidemiological evidence base regarding safety and practice of ayurvedic medicines to evaluate their therapeutic potential and are represented herein. In this way, by remembering of the past research, the study was led by utilizing ethanol as a dissolvable for more valuable extraction of flavonoid compounds which proved good percentage of Quercetin and Rutin by evaluation and standardization by using simple, precise, reliable and useful HPTLC method.

References

1. Awang D, Bejar E. Plant bioassays, the promise and the challenge. *Journal of Herbs Spices and Medicinal Plants*. 2001; 8:1-5.
2. Odunayo R, Akinsulire Ibukun E, Aibinu Tayo Adenipekun, Toyin Adelowontan, Tolu Odugbemi. In vitro antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata*. *African Journal of Traditional, Complementary and Alternative Medicine*. 2007; 4(3):338-344.
3. James Brimson M, Tewin Tencomnao. *Rhinacanthus nasutus* Protects Cultured Neuronal Cells against Hypoxia Induced Cell Death. *Molecules* 2011; 16:6322-6338.
4. Suman Bukke, Venkataswamy Mallepogu, Thyagaraju Kedam. *In Vitro* Antioxidant Activity and Proximate Analysis on *Rhinacanthus nasutus* (L) Kurz Leaf. *Phytochemical Analysis* 2013; 3(5):32-25.
5. Narathip Pradidphol, Ngampong Kongkathip, Pichamon Sittikul, Nonlawat Boonyalai, Boonsong Kongkathip. A First synthesis and anticancer activity of novel naphthoquinone amides. *European Journal of Medicinal Chemistry*. 2012; 49:253-270.
6. Supinya Tewtrakul, Pimpimon Tansakul, Pharkphoom Panichayupakaranant. Effects of rhinacanthins from *Rhinacanthus nasutus* on nitric oxide, prostaglandin E2 and tumor necrosis factor-alpha releases using RAW264.7 macrophage cells. *Phytomedicine* 2009; 16:581-585.
7. Meena K, Cheruvathura Sivub AR, Pradeep NS, Dennis Thomas T. Shoot organogenesis from leaf callus and ISSR

- assessment for their identification of clonal fidelity in *Rhinacanthus nasutus* (L.) Kurz. A potent anticancerous ethnomedicinal plant. *Industrial Crops and Products* 2012; 40:122-128.
8. Rajasekaran A, Sundaranadavalli S, Murugesan S. Antibacterial and Antifungal evaluation of the leaves of *Rhinacanthus nasutus* linn. *Int J Chem Tech Res.* 2009; 1(3):574-576.
 9. Upendra Rao M, Munna Sreenivasulu, Chengaiah B. *Rhinacanthus nasutus* (Linn.) Kurz: A comprehensive review. *International Journal of Pharmaceutical Research and Development.* 2010; 2(7):1-8.
 10. Baskar Ananda Raj V, Senthil Kumar KL, Suresh Kumar S. Traditional Indian Medicinal Plants as a potential Anti-inflammatory phytomedicine for Psoriasis control. *Journal of Pharmacognosy and Phytochemistry.* 2015; 4(3):118-122.
 11. Palanisamy Hariprasasad, Natesan Ramakrishnan. Chromatographic finger print analysis of *Rumex vesicarius*. L by HPTLC technique. *Asian Pacific Journal of Tropical Biomedicine.* 2012, S57-S63.
 12. Subramanian Sampath Kumar, Ramakrishnan N. Chromatographic finger print analysis of *Naringi crenulata* by HPTLC Technique. *Asian Pacific Journal of Tropical Biomedicine.* 2011, S195-S198.
 13. Debashisha Panda, Santhosh Kumar Dash, Gouri Kumar Dash. Phytochemical examination and antimicrobial activity of various solvent extracts and the selected isolated compounds from roots of *Tragia involucrata* linn. *International Journal of Pharmaceutical Science and Drug Research.* 2012; 4(1):44-48.
 14. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur, Harleen Kaur. Phytochemical screening and extraction: A Review. *International Pharmaceutica Scientia* 2011; 1(1):98-106.