



# Phytochemical Analysis, In-Vitro Antioxidant Activity and Proximate Analysis on *Rhinacanthus Nasutus*(L) Kurz Leaf

## KEYWORDS

Phytochemical, *R.nasutus*, antioxidant, DPPH and  $H_2O_2$ .**Suman Bukke**

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**ABSTRACT**

The present study provides an information on phytochemical, antioxidant and proximate analysis of different extracts of *Rhinacanthus nasutus* leaf. Different parts of *R nasutus* have been used in folk medicine for treating liver disorders, several skin diseases and other pharmacological effects. Different extracts of *R.nasutus* were prepared based on the polarity in solvents of hexane, ethyl acetate, methanol and water for phytochemical analysis from leaves of *R.nasutus*. The phytochemical analysis leaf extract has revealed the presence of flavonoids, triterpenoids, polyphenols, steroids, saponins, alkaloids, carbohydrates, anthraquinones and least of tannins.

These principles at concentration of 250µg of preparation showed potential antioxidant properties as tested by the methods of radical scavenging activity, and methanolic extract showed more of peroxy radical scavenging property at all concentrations compared to hexane and ethyl acetate extracts. The Dried leaf powder Proximate analysis has revealed the presence of 85% of dry matter, 13% of crude fiber, 11% of total ash, 1% of acid soluble ash and 4% crude protein. Hence the present exploration of phytochemical antioxidants and proximate studies of *R.nasutus*, will be useful in the synthesis and preparation of new drugs of pharmaceutical importance.

**Introduction**

The identification of plants is useful to human beings from natural strands commenced in prehistoric studies. Experiments and trails with the two main ways through which humans have learnt various uses of the plants (Haseena Bhanu et al., 2010 and Suman Bukke et al., 2011). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. More than 13,000 plants have been studied during the last 5 year period (Dahanukar et al., 2000). Over three-quarters of the world population relies mainly on plants and plant extracts for health care. From plants the isolated and purified compounds in contrast, may lose their biological activity or fail to behave in the same way as in the complex matrix that the original item of food represents (Rao et al, 1998; Raveendra et al, 2008). According to the Food and Agriculture Organization (FAO), more than 50,000 plant species are used in the traditional folk medicine throughout the world (Schippmann et al., 2002). The drugs are derived from the whole plant or from different parts like leaves, stem, bark, root, flower, tuber and seed etc. More than 30% of the entire plant species, at one time or other was used for medicinal purposes. It has been estimated that in developed countries such as United States, plant drugs constitute as much as 25% of the total drugs, while in fast developing country such as India, the contribution is as much as 80% (Joy et al., 2001). In 19<sup>th</sup> century, medicinal plants and herbs were the soul source of active principles capable of curing man's ailments. Medicinal plants and herbs continue to be the source of proven medicaments and of new and revolutionary drugs (Chatterjee 2000). In early 20<sup>th</sup> century new trend has emerged, as several Botanists started surveying and identifying medicinal plants on the basis to use in tribal and rural areas. Thus, the economic importance of medicinal plants is much more to countries such as India than to rest of the world (Suman et al 2011) .

The *Rhinacanthus nasutus* commonly known as "Rangchita and Nagamalli" belongs to family Acanthaceae. The genus *Rhinacanthus* comprises of about 25 species confined to the Old World tropics and subtropics it is placed in the

Justiciinae subtype (Scotland and Vollesen, 2000). *R nasutus* is widely distributed in some parts of sub-continent, in the region of Southeast Asia and China (Farnsworth and Bunyapraphatsar 1992). The plant is a small slender shrub. The *R nasutus* is cultivated particularly as a medicinal plant has been used in treatments and preventions of diverse diseases as folklore medicines. Different parts of the plant has used in traditional medicine for the treatment in diseases such as eczema, pulmonary tuberculosis, herpes, hepatitis, diabetes, hypertension and several skin diseases (Siripong et al., 2006). The experimental evidences shows, that it has potential effects for treatment of cancer, liver disorders, skin diseases, peptic ulcers, helminthiasis, scurvy, inflammation and obesity (Suja et al., 2003). The leaves of this plant are also used in the preparation of shampoos. *Rhinacanthine* from roots induce apoptosis in human cervical carcinoma cells and hepatocellular cancers (Wu et al.1998;Thirumurugan et al.,2000; Siripong et al., 2006 and 2009). Therefore considering the significant importance of *R.nasutus* in folk medicine experiments were conducted on analysis of plant products and their biological activity.

**Materials and Methods****Collection of plants and preparation of extracts:**

The *R.nasutus* were identified and authenticated by plant Taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh and voucher specimen no SVUBH/ 579. The fresh leaves of *R nasutus* were collected from Sesaschalam hills (Tirumala Hills and Tirupati) Chittoor district of Andhra Pradesh. Fresh leaves of *R.nasutus* (L) were shade dried and milled to fine powder using a mechanical grinder. The powdered plant material was macerated with hexane, Ethyl acetate, methanol and water separately. The extract was then filtered with filter paper (Whatman No. 1) under reduced pressure using rota evaporator at 40°C. The concentrate was to obtain a dark molten mass then layered on aluminum foil and freeze dried for further use.

**Phytochemical screening**

Phytochemical examinations were carried out to detect the secondary metabolites (Alkaloids, saponins, carbohydrates, flavonoids, cardiac glycosides etc.) in *R.nasutus* extracts by us-

ing standard procedures/methods (Trease and Evans 1983; and Harbourne 1988).

#### DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging assay

Evaluation of antioxidant activity was done by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method (Burits and Bucar 2000) with some modifications. Antioxidant reacts with DPPH and convert it to  $\alpha, \alpha$ -diphenyl- $\beta$ -picryl hydrazine. One ml of plant extract was added to 4ml of 0.004% methanol solution of DPPH. After 20-30 mins incubation period at room temperature, DPPH solution with 2 ml of methanol was used as sample (blank) and test samples were done at different concentrations. The absorbance was read at against blank at 517nm. Inhibition of free radical by DPPH in percent (%) was calculated by using the following equation. The degree of discolouration indicates the scavenging potentials of the antioxidant extract.

$$\% \text{DPPH radical/scavenging} = [(\Delta \text{ in Absorbance of control-test sample}) / (\text{Absorbance of control})] \times 100$$

#### Total antioxidant activity by Phosphomolybdenum Method

The total antioxidant capacity of *R.nasutus* of different solvent extracts was evaluated according to the method, Prieto et al., 1999. The absorbance of the samples were measured at 695 nm in UV spectrophotometer. The higher absorbance value indicates higher antioxidant activity. Ascorbic acid was used as standard for comparison.

#### Hydrogen Peroxide Method

The hydrogen peroxide assay was determined by the method Vijayabaskaran et al 2010, with some modifications. A solution of hydrogen peroxide (20mm) was prepared in phosphate buffer. To 2 ml of methanol and 2 ml of hydrogen peroxide solution was added, and used as control. Methanol was used as blank and Ascorbic acid used as standard for comparison. After incubation for 10 minutes in dark, absorbance was recorded at 230 nm using UV-visible spectrophotometer.

The H2O2 scavenging property was calculated using the formula:

$$\% \text{ Scavenging} = (\text{OD of Control} - \text{OD of Test} / \text{OD of Control}) \times 100$$

#### Determination of Total phenolic assay

The total phenolic content of plant extract was determined by using the Folin-Ciocalteu assay (Singleton et al, 1965). Gallic acid and double distilled water were used as standard and blank. The reaction mixture was incubated for 90 mins at room temperature, the absorbance against prepared reagent blank was determined at 750nm with in UV-Vis Spectrophotometer. The total phenolic content was expressed as mg of gallic acid equivalents (GAE)/100g fresh weight. All samples were analysed in triplicates.

#### Proximate analysis

The Dried *R.nasutus* leaf powder was prepared for Proximate analysis. It includes to prepare the Dry mater, total ash, Crude protein, Crude fibre, Ether extract and Acid soluble in Ash. The analysis was carried out using the AOAC methods 1990

#### Results and Discussion:

##### Phytochemical analysis

Preliminary phytochemical screening of the leaf extracts of *R.nasutus* showed positive results for the presence of secondary metabolites like steroids, saponins, triterpenoids, alkaloids, carbohydrates, flavanoids, polyphenols and glycosides. Tannins are absent in all extracts prepared. Bioactive active compounds like steroids, alkaloids, carbohydrates, glycosides, polyphenols were present in high amounts in methanol extract (Table-01) These phytochemical compounds are

known to support bioactive activities in medicinal plants and thus responsible for the antioxidant activities of this plant extract used in this study. The presence of flavonoids in the plants is likely to be responsible for the free radical scavenging effects observed. Flavonoids and plant phenolics are a major group of compounds that may act as primary antioxidants or free radical scavengers (Polterait et al., 1997)

**Table-01: Phytochemical screening of different extracts of *R.nasutus* leaf**

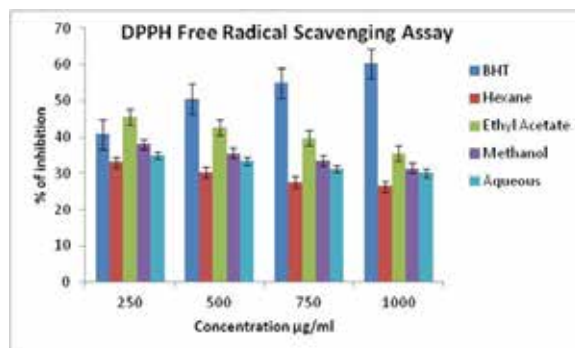
S. No	Secondary metabolites	Hexane Extract	Ethyl Acetate Extract	Methanol Extract	Aqueous Extract
1	Steroids	-	+	+	-
2	Triterpenes	-	+	+	-
3	Saponins	-	+	+	-
4	Alkaloids	-	+	+	-
5	Carbohydrates	-	-	+	+
6	Flavonoids	+	+	+	+
7	Cardiac glycosides	-	-	-	+
8	Anthroquinones	-	+	+	-
9	Polyphenols	-	+	+	+

Note: "+" = presence and "-" = absence

#### DPPH (2, 2-diphenyl-1-picryl hydrazyl) free radical scavenging assay

The hexane, ethyl acetate, methanol and water extracts of *R.nasutus* leaves were analysed for antioxidant property by using DPPH as recipient of radical. With increase in the range of 250-1000  $\mu\text{g/ml}$  of extract, the antioxidant activity was found to be decreased in all extracts of hexane, ethyl acetate, methanol and water when compared to BHT (Fig 01). The ethyl acetate and methanol extracts showed the relatively highest activity compared to other preparations. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Lee et al., 2003). Free radicals are involved in many disorders like skin diseases, cancer and pharmacology activity. DPPH is stable for free radical and is a sensitive way to determine the antioxidant activity of plant extracts (Koleva et al., 2002 and Suresh et al., 2008)

**Fig 01: Free radical (DPPH) scavenging activity of the *R.nasutus* leaf extracts**



Data are expressed as the mean of triplicate  $\pm$  SD

#### Total phenolic content

The selection of solvents used for the extraction of phenolic compounds depends on its solubility. It is well-known that phenolic compounds contribute to quality and nutritional val-

ue in terms of modifying colour, taste, aroma, and flavor also in providing health beneficial effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores (Vaya et al., 1997). Phenols are very important plant constituents because of their scavenging ability due to their hydroxyl groups and it involves directly to antioxdiative action (Hantano et al., 1989). There were no correlation between total phenolic content and antioxidant activity. Although some studies have demonstrated a correlation between phenolic content and antioxidant capacity (Yang et al., 2002), our results (Table 02) shows that methanol extracts contain highest antioxidant activity among all the solvents. Hexane extract shows the least phenolic activity compared with all solvents. Total phenolic content was measured in conc. of total phenolics mg/ GAE/g of extract.

**Table 02: Total Phenolic content and Phosphomolybdenum activity of the R.nasutus leaf extracts**

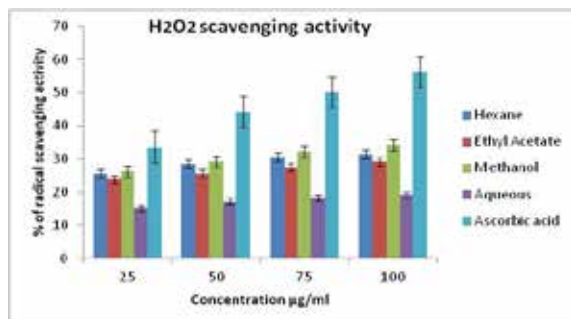
Solvents	Total Phenolic content (Conc. of total phenolics mg/GAEs/g of extract)	Phosphomolybdenum assay (mg AE/G of plant extract)
Hexane	19.6±1.9	57.06 ± 0.09
Ethyl Acetate	26.6±1.0	57.36 ± 0.16
Methanol	31.0±1.7	60.00 ± 0.02
Aqueous	24.5±1.4	59.56 ± 0.00

#### Phosphomolybdenum assay

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, tocopherol, and carotenoids (Prieto et al., 1999). Ascorbic acid, glutathione, cysteine, tocopherols, polyphenols, and aromatic amines have the ability to donate hydrogen and electrons. Generally, aqueous or alcohol is considered the best solvent for extracting phenolic compounds from plant materials (Negi et al., 2003). Our results among hexane, ethyl acetate, methanol and water, the methanol shows the best antioxidant activity and the hexane shows the lowest activity (Table 02). Gallic acid was used standard for this experiment. The plant extracts electron-donating capacity and thus they may act as radical chain terminators, transforming reactive free radical species into more stable non-reactive products. The antioxidants break the free radical chain by donating a hydrogen atom (Gordon 1990 and Dorman et al, 2003).

#### Hydrogen peroxide method

The hydroxyl radical is the most reactive free radical formed in biological systems and is considered to be one of the quick initiators of the lipid peroxidation process Hydrogen peroxide is weak oxidizing agent that inactivates a few enzymes directly, usually by oxidation of essential thiol (-SH) group. From the Fig 02 The H<sub>2</sub>O<sub>2</sub> was calculated in different concentrations in triplicates form that the increased in concentration decreases the values as the hexane values were decreased than methanol and aqueous this shows the stability of activity.



**Fig 02: Hydroxyl radical scavenging activity of R. nasutus leaf extracts**

Data are expressed as the mean of triplicate ±SD

#### Proximate analysis:

From our Proximate analysis of plant sample includes that the total ash is 11.4% in dried leaf, proteins shows the 4.46% and fibre content in 13.24% in plant samples (Table 03) In this study the analysis provided an insight into the composition of the tested medicinal plant in addition to their therapeutic potentials. It was concluded that the presence of these nutrients and phytochemically-active components in the plant sample might be responsible for their therapeutic activity

**Table 03: Proximate analysis of R.nasutus leaf powder**

S. No.	Proximate factors(Parameters)	Dried R.nasutus leaf powder (% w/w)
1	Dry Matter	84.6 %
2	Total Ash	11.4 %
3	Crude Protein	4.46 %
4	Crude Fibre	13.24 %
5	Ether extract	0.74 %
6	Acid soluble in ash	1.06 %

#### Conclusions

The main focus of this work was to extract the plant active principles R.nasutus with different solvents, characterization of the chemical constituents and evaluate the photochemicals and also antioxidant activities of extracts. Our studies provide a basis for various principles of R nasutus that are present and influence the free radical scavenging. Our results on these extracts showed that the plant products could serve as a good source for the therapeutic drugs for degenerative diseases and exhibit good antioxidant potency as reflected by the results of the analysis performed and the components responsible for its efficacy identified for its phytochemical nature. Among all the extracts prepared from R.nasutus leaves the bioactive compounds present are steroids, alkaloids, carbohydrates, glycosides, and polyphenols in high amounts. With regard to antioxidant activity all methanolic extractions have showed and confirmed by DPPH assay method. The hydrogen peroxide shows less inhibitory effect but in case of assay hexane, ethyl acetate extract showed less antioxidant activity compared to methanol extract. The results total phenolic and Phosphomolybdenum activity shows that methanol extracts shows highest among all the solvents hexane shows the less activity compared with all solvents. In this study the analysis provided an insight into the composition of the tested medicinal plant in addition to their therapeutic potentials. It was concluded that the presence of these nutrients and phytochemically-active components in the plant sample might be responsible for their therapeutic activity. The presence of the identified phytochemicals makes the leaves pharmacologically active. Their antioxidant activity may be responsible for their usefulness in the management and treatment of various diseases. The proximate analysis the leaf nutrients in plants that are useful for many pharmacological activity. We are currently studying other possible mechanisms of action of these leaves. Efforts to identify the constituent compounds responsible for this antioxidant activity are also in progress

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