Assessment of Free Radical Scavenging Activity and Antioxidant Potential of the TLC separated Fractions of *Rhinacanthus nasutus* Root Extracts

Nirmaladevi R.,* Chithra P. and Padma P.R.
Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore, INDIA
*nimmysaro@yahoo.com

Abstract

The root powder was shade dried and powdered and subjected first to extraction by series of solvents on increasing polarity (Petroleum ether, Benzene, Chloroform, Ethyl acetate, Methanol and Water). Then the extracts were subjected to TLC and the spots obtained were eluted and used for the further antioxidant study. DPPH, ABTS, Hydroxyl radical, Hydrogen peroxide radical scavenging assays and determination of inhibition of in vitro nitric oxide and superoxide generation were determined to evaluate the free radical scavenging activity of the extract. The total antioxidant potential of the extract was studied by phosphomolybdenum assay and reduction potential assay. The results of our experiment showed that plant extract has highest radical scavenging activity.

Of the plant extracts tested, methanolic extract showed better radical scavenging activity compared to other extracts. The total antioxidant potential was found to be high in the methanolic extract of the roots of R. nasutus and it also shows good inhibition to superoxide and nitric oxide generation in vitro. The root extracts of Rhinacanthus nasutus 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.

Keywords: *Rhinacanthus nasutus*, TLC, ABTS, DPPH.

Introduction

Generally human beings live in a highly oxidative environment and many processes involved in metabolism may result in the production of more oxidants. It has been estimated that there are more than ten thousand oxidative hits to DNA per cell per day in humans. For protection against free radicals, organisms are endowed with endogenous and exogenous defense systems. However these systems are not sufficient in situations where the production of free radicals significantly increases. Plant based dietary components and additives are known to protect cells from deleterious effect of Reactive Oxygen Species (ROS)[1].

All over the world, several hundreds of plants are good sources of medicinal agents and used in traditional medicine. Traditional usage of plants in curing illness has deep roots in human history[2]. Screening of active components from plants has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases including cancer and Alzheimer's disease[3]. The medicinal properties of plants are due to their potent antioxidant properties[4].

Rhinacanthus nasutus Kurz is one such plant belonging to the family of Acanthaceae. It is a valuable plant which is widely distributed and cultivated in South China, Taiwan, India and also in Thailand. *Rhinacanthus nasutus* is well known as a source of flavonoids, steroids, triterpenoids, anthraquinones, lignans and especially naphthoquinone analogues[5].

Material and Methods

Preparation of the Extract: The roots of *Rhinacanthus nasutus* were collected from herbal garden, where the *Rhinacanthus nasutus* plant saplings purchased from Tamilnadu Agricultural University, Coimbatore are maintained. The roots were washed and shade dried, powdered and weighed (5g). The root powder was subjected to extraction based on polarity nature using different solvents namely petroleum ether, benzene, chloroform, ethyl acetate, methanol and water. After extraction, the solvent was evaporated at 60°C in a boiling water bath and the residue obtained was re-dissolved in DMSO to obtain a final concentration of 20mg/5µl. The respective extracts were first subjected to a phytochemical screening by Thin Layer Chromatography and the separated fractions were eluted and tested for antioxidant potential.

Thin Layer Chromatography: The dried, cleaned, TLC glass plates were coated with the slurry silica gel G-50 prepared using silica gel and water in the ratio 1:2, uniformly spreaded on the glass plate with the help of the spreader and activated at 105°C for 30 minutes. The solvent system used for the mobile phase was methanol and chloroform in the ratio 7:3. Then 10µl of the petroleum ether, benzene, chloroform, ethyl acetate, methanol and aqueous extracts of the candidate plant were spotted on the TLC plates and were kept in the TLC chamber saturated with mobile phase methanol and chloroform (7:3). Depending upon the partition coefficient, the bands were separated. The bands obtained were eluted with the respective solvents such as petroleum ether, benzene, chloroform, ethyl acetate, methanol and water and the eluted fractions were used for further analysis of the study.

Elution of the Bands: The separated bands of the extracts of petroleum ether, benzene, chloroform, ethyl acetate, methanol and water were dissolved in respective solvents and centrifuged at 3000 rpm for 10 minutes. The process was repeated for 3-4 times and the supernatant collected was evaporated at room temperature. The residue obtained contains fractions of interest and it was re-dissolved in DMSO. Then these eluted bands were taken for the analysis.

Free Radical Scavenging effect of *Rhinacanthus nasutus* root extracts- DPPH: Scavenging activity of *Rhinacanthus nasutus* root extracts was determined using the procedure adopted by Mensor et al.[6]

Determination of ABTS Scavenging Activity: ABTS scavenging activity was estimated according to Shirwaiker et al.[7] $ABTS^+$ radical cations were produced by reacting 7mM ABTS solution with 2.45 mM ammonium persulphate with 7mM ABTS solution and kept in dark for 12-16 hours at room temperature. The mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. 0.5 ml of root extract was added to 0.3 ml ABTS solution and the final volume was made upto 1ml with ethanol. Absorbance was read at 745 nm and the percentage inhibition of the radical formation was calculated using the formula:

$$\text{Percentage of ABTS Scavenged} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is absorbance of control and A_1 is absorbance in the presence of root extracts.

Determination of Hydroxyl Radical Scavenging Effect[8]: The reaction mixture contained 0.1 ml of deoxyribose, 0.1ml of EDTA, 0.1 ml of H_2O_2, 0.1 ml of ascorbate and 0.1ml of KH_2PO_4 – KOH buffer. 20µl of the root extracts were added such that the final volume was 1.0 ml. The reaction mixture was incubated for one hour at 37°C. Deoxyribose degradation was measured as TBARS (Thiobarbiturate reactive substances) formed by the addition of 0.5 ml TBA and 0.5ml HCl, boiled in a water bath for 20 minutes, cooled and the absorbance measured at 532 nm.

Percentage of inhibition of hydroxyl radical

$$= \frac{\text{Absorbance}}{H_2O_2\ \text{(Absorbance)}} \times 100$$

Determination of Hydrogen Peroxide Scavenging Activity[9]: A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer. Root extract at the concentration of 10mg/10µl was added to 0.6ml hydrogen peroxide solution (40 mM). The total volume was made upto 3.0 ml. The absorbance of the reaction mixture was recorded at 230 nm. The solution containing phosphate buffer without hydrogen peroxide acts as a blank. The percentage of hydrogen peroxide scavenged by the root extract was calculated by the formula:

$$\text{Percentage of hydrogen peroxide scavenged} = \frac{A_0 - A_1}{A_0} \times 100$$

where A_0 is absorbance of control and A_1 is absorbance in the presence of root extracts.

Determination of In Vitro Superoxide Generation[10]: 20 µl of the root extracts to the assay tubes containing 0.2 ml of EDTA (0.1 M containing 1.5 mg NaCN) was added to 0.1 ml of NBT, 0.05 ml riboflavin and 2.55 ml of phosphate buffer. The control tubes were also set up where DMSO was added instead of the root extract. All the tubes were vortexed and the initial optical density was measured at 560 nm. After that, these tubes were placed in an area where they received uniform illumination for 30 minutes. Again the optical density was measured at 560 nm. The difference in optical density before and after illumination is the generation of superoxide by the test sample which was calculated by comparison with the OD of the control:

Percentage inhibition of superoxide radical generation

$$= 100 - \frac{\text{Test X } 100}{\text{Control}}$$

Determination of In Vitro Niric Oxide Generation[11]: 20 µl of the root extract was added to the reaction mixture (3ml) containing sodium nitroprusside (10 mM in PBS) and it was incubated at 25 °C for 150 minutes. Control without test sample was kept in an identical manner. After incubation, 0.5 ml of reaction mixture was taken and 0.5 ml of Griess reagent was added to it. The absorbance was read at 560 nm and the percentage inhibition was calculated by the following formula:

Percentage inhibition of nitric oxide generation

$$= \frac{\text{Test OD}}{\text{Control OD}} \times 100$$

Determination of Total Antioxidant Status by Phosphomolybdenum Method[12]: The tubes containing 20 µl of the root extracts of *Rhinacanthus nasutus* and the reagent (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 minutes. The mixture was cooled to room temperature and the absorbance of each solution was measured at 695 nm against blank.

Reduction Potential[13]: 1.0 ml of the different concentration of various extracts of the sample was mixed with 2.5 ml potassium ferric cyanide and 2.5ml phosphate buffer (pH 6.6). The mixture was incubated at 50°C for 20 minutes. After the incubation, 2.5 ml of TCA (10%) was added to it and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of the supernatant was taken and 2.5 ml water and 0.5 ml of ferric chloride (0.1%) were added to it. The absorbance of the color was measured spectrophotometrically at 700 nm. In all the parameters analyzed, the samples were taken in triplicates.

Results and Discussion

The different root extracts were subjected to thin layer chromatography to identify the active compounds present in them. All the extracts analyzed showed single spot in the TLC plate, after the chromatogram was developed, except the aqueous extracts which showed no spots in its chromatogram and hence the aqueous extracts of *Rhinacanthus nasutus* roots were eliminated for further analysis of the study.

Determination of DPPH and ABTS Scavenging Activity of TLC Separated Fractions of *Rhinacanthus nasutus* Root Extracts: The DPPH has been largely used as a quick, reliable and reproducible parameter to find out the *in vitro* general antioxidant activity of pure compounds as well as plant extracts. The decrease in absorbance by DPPH radical with increase in concentration of the extract suggested that the extract has antioxidant activity due to its proton donating ability[14].

ABTS assay is commonly used to assess radical scavenging or antioxidant activity. The scavenging activity is measured at 745 nm and the absorbance decreases as the ABTS radical is scavenged[15]. The scavenging ability of *Rhinacanthus nasutus* root extracts to DPPH and ABTS was observed and depicted in figure 1.

The results showed that the methanolic extract has high DPPH and ABTS scavenging activity followed by chloroform, petroleum ether, benzene and ethyl acetate indicating that methanolic extract possesses components responsible for its higher free radical scavenging activity.

Effect of *rhinacanthus nasutus* root extracts on H_2O_2 induced damage to deoxyribose (hydroxyl radical scavenging activity): Hydroxyl radical is the most reactive among ROS; it has the shortest half life compared with others and is considered to be responsible for much of the biological damage in free radical pathology. The deoxyribose is the common method for determining the rate constant of hydroxyl radical reactions[1]. It is difficult to determine directly the hydroxyl radical. They are measured as ˙OH induced TBA-reactive substance (TBARS) formation. The rate of TBARS formation is dependent on the reaction of deoxyribose with hydroxyl radical[16].

The effect of *Rhinacanthus nasutus* root extracts on H_2O_2-induced damage to deoxyribose was quantified as the amount of TBARS formed and the results are presented as follows. The extent of DNA damage caused in H_2O_2 treated groups is fixed at 100 percentage and the damage caused to other groups was calculated relative to this value as depicted in figure 2.

From the figure it is clear that highest hydroxyl radical scavenging activity was found in the methanolic extracts of the roots of *Rhinacanthus nasutus* when compared to chloroform, petroleum ether, benzene and ethyl acetate. H_2O_2 exposure resulted in a steep increase in the extent of damage which was very effectively counteracted by the different extracts of *Rhinacanthus nasutus* roots. The effect of methanolic extract was more pronounced in scavenging hydroxyl radicals when compared to other extracts.

Therefore, from the results, it was found that the methanolic extract of the TLC separated fractions of *Rhinacanthus nasutus* root exhibits strong hydroxyl radical scavenging effect which reflects protective nature of the extract against oxidative damage to DNA molecules.

Determination of hydrogen peroxide scavenging activity of TLC separated fractions of *rhinacanthus nasutus* root extracts: Hydrogen peroxide is a weak oxidizing agent and can inactivate few enzymes directly, usually by oxidation. It can cross cell membranes rapidly and once inside the cell, it can probably react with Fe^{2+} and Cu^{2+} to form hydroxyl radicals and this may be due to their toxic effects. It is therefore advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[17]. The percentage scavenging of the root extracts of TLC separated fractions of *Rhinacanthus nasutus* to hydrogen peroxide are reported in figure 3.

The scavenging ability of TLC separated fractions of *Rhinacanthus nasutus* root extracts to hydrogen peroxide showed that the methanolic extract has the strongest hydrogen peroxide scavenging with 79.4% activity. Chloroform extract also showed significant scavenging effect. According to the results, it was made clear that the methanolic root extract can be a better antioxidant for removing hydrogen peroxide and these could protect the biological system from oxidative damage.

Determination of inhibition of *in vitro* super oxide and nitric oxide generation by TLC separated fractions of *rhinacanthus nasutus* root extracts: Superoxide anions are precursors to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage[18].Nitric oxide was generated from sodium nitroprusside under *in vitro* and measured by Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrate ions that can be estimated by the use of Griess reagent. Antioxidants compete with oxygen leading to decreased production of nitric oxide radicals.[19]

The extent of inhibition of *in vitro* super oxide and nitric oxide generation by TLC separated fractions of *Rhinacanthus nasutus* root extracts are produced in figure 4. From the results of study, it is indicated that the methanolic extract shows maximum inhibition for superoxide and nitric oxide radical generation when compared to other extracts. Thus it was confirmed that the TLC separated fractions of *Rhinacanthus nasutus* root

Results and Discussion

The different root extracts were subjected to thin layer chromatography to identify the active compounds present in them. All the extracts analyzed showed single spot in the TLC plate, after the chromatogram was developed, except the aqueous extracts which showed no spots in its chromatogram and hence the aqueous extracts of *Rhinacanthus nasutus* roots were eliminated for further analysis of the study.

Determination of DPPH and ABTS Scavenging Activity of TLC Separated Fractions of *Rhinacanthus nasutus* Root Extracts: The DPPH has been largely used as a quick, reliable and reproducible parameter to find out the *in vitro* general antioxidant activity of pure compounds as well as plant extracts. The decrease in absorbance by DPPH radical with increase in concentration of the extract suggested that the extract has antioxidant activity due to its proton donating ability[14].

ABTS assay is commonly used to assess radical scavenging or antioxidant activity. The scavenging activity is measured at 745 nm and the absorbance decreases as the ABTS radical is scavenged[15]. The scavenging ability of *Rhinacanthus nasutus* root extracts to DPPH and ABTS was observed and depicted in figure 1.

The results showed that the methanolic extract has high DPPH and ABTS scavenging activity followed by chloroform, petroleum ether, benzene and ethyl acetate indicating that methanolic extract possesses components responsible for its higher free radical scavenging activity.

Effect of *rhinacanthus nasutus* root extracts on H_2O_2 induced damage to deoxyribose (hydroxyl radical scavenging activity): Hydroxyl radical is the most reactive among ROS; it has the shortest half life compared with others and is considered to be responsible for much of the biological damage in free radical pathology. The deoxyribose is the common method for determining the rate constant of hydroxyl radical reactions[1]. It is difficult to determine directly the hydroxyl radical. They are measured as $\cdot$OH induced TBA-reactive substance (TBARS) formation. The rate of TBARS formation is dependent on the reaction of deoxyribose with hydroxyl radical[16].

The effect of *Rhinacanthus nasutus* root extracts on H_2O_2-induced damage to deoxyribose was quantified as the amount of TBARS formed and the results are presented as follows. The extent of DNA damage caused in H_2O_2 treated groups is fixed at 100 percentage and the damage caused to other groups was calculated relative to this value as depicted in figure 2.

From the figure it is clear that highest hydroxyl radical scavenging activity was found in the methanolic extracts of the roots of *Rhinacanthus nasutus* when compared to chloroform, petroleum ether, benzene and ethyl acetate. H_2O_2 exposure resulted in a steep increase in the extent of damage which was very effectively counteracted by the different extracts of *Rhinacanthus nasutus* roots. The effect of methanolic extract was more pronounced in scavenging hydroxyl radicals when compared to other extracts.

Therefore, from the results, it was found that the methanolic extract of the TLC separated fractions of *Rhinacanthus nasutus* root exhibits strong hydroxyl radical scavenging effect which reflects protective nature of the extract against oxidative damage to DNA molecules.

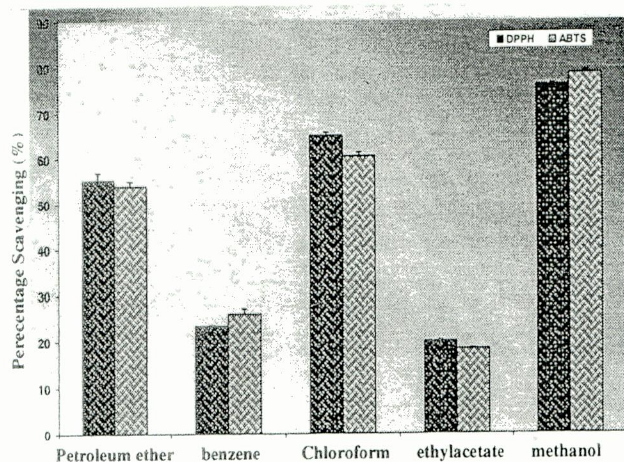
Determination of hydrogen peroxide scavenging activity of TLC separated fractions of *rhinacanthus nasutus* root extracts: Hydrogen peroxide is a weak oxidizing agent and can inactivate few enzymes directly, usually by oxidation. It can cross cell membranes rapidly and once inside the cell, it can probably react with Fe^{2+} and Cu^{2+} to form hydroxyl radicals and this may be due to their toxic effects. It is therefore advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate[17]. The percentage scavenging of the root extracts of TLC separated fractions of *Rhinacanthus nasutus* to hydrogen peroxide are reported in figure 3.

The scavenging ability of TLC separated fractions of *Rhinacanthus nasutus* root extracts to hydrogen peroxide showed that the methanolic extract has the strongest hydrogen peroxide scavenging with 79.4% activity. Chloroform extract also showed significant scavenging effect. According to the results, it was made clear that the methanolic root extract can be a better antioxidant for removing hydrogen peroxide and these could protect the biological system from oxidative damage.

Determination of inhibition of *in vitro* super oxide and nitric oxide generation by TLC separated fractions of *rhinacanthus nasutus* root extracts: Superoxide anions are precursors to active free radicals that have the potential of reacting with biological macromolecules and thereby inducing tissue damage[18].Nitric oxide was generated from sodium nitroprusside under *in vitro* and measured by Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrate ions that can be estimated by the use of Griess reagent. Antioxidants compete with oxygen leading to decreased production of nitric oxide radicals.[19]

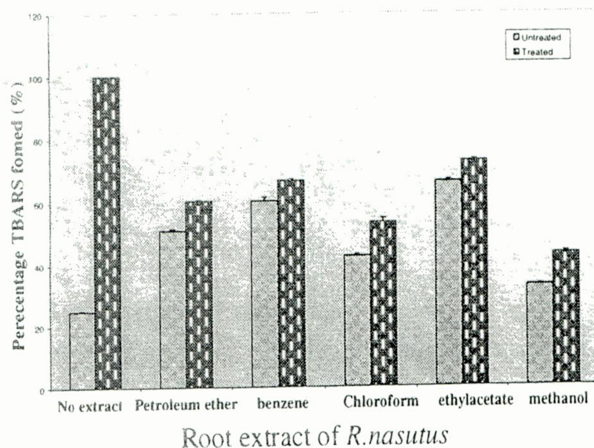
The extent of inhibition of *in vitro* super oxide and nitric oxide generation by TLC separated fractions of *Rhinacanthus nasutus* root extracts are produced in figure 4. From the results of study, it is indicated that the methanolic extract shows maximum inhibition for superoxide and nitric oxide radical generation when compared to other extracts. Thus it was confirmed that the TLC separated fractions of *Rhinacanthus nasutus* root

extract have shown good inhibition to super oxide and nitric oxide generation indicating the presence of phytochemical components which are responsible for its active antioxidant property.



Root extract of *R. nasutus*

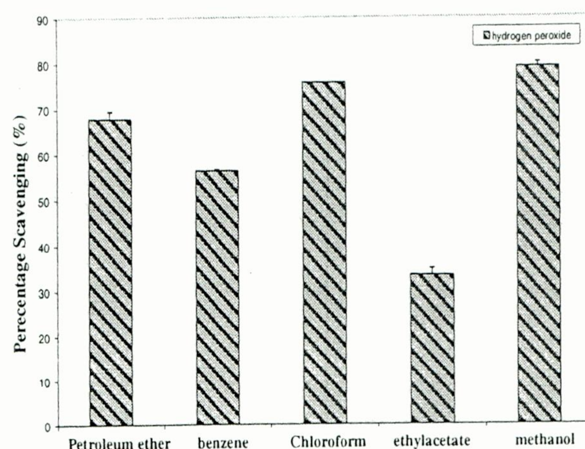
Figure 1: DPPH and ABTS scavenging activity of TLC separated fractions of *rhinacanthus nasutus* root extracts



Root extract of *R.nasutus*

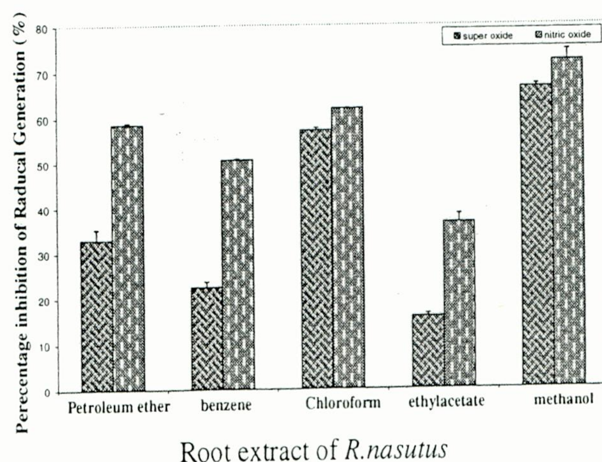
Figure 2: Hydroxyl radical scavenging activity of TLC separated fractions of *rhinacanthus nasutus* root extracts

Determination of total antioxidant potential of the TLC separated fractions of *rhinacanthus nasutus* root extracts by phosphomolybdenum assay and reduction potential method: The total antioxidant content of *Rhinacanthus nasutus* root extracts by phosphomolybdenum assay and reduction potential are presented in figure 5. According to results, the methanolic extract showed highest total antioxidant content compared to chloroform, petroleum ether, benzene and ethyl acetate root extracts. It was concluded from the above results that the methanolic root extracts of *R.nasutus* possess high total antioxidant content. This suggests that the root extracts possess considerable antioxidant substances which may be responsible for its free radical scavenging activity. In all the parameters analysed, methanolic extract of the TLC separated fractions of *Rhinacanthus nasutus* root showed a better radical scavenging effect and also prevented the generation of radicals to a maximum extent. The total antioxidant assay performed also revealed that the antioxidant potential was found to be higher in the methanolic extract. All the other extracts analyzed showed considerable radical quenching ability and protection against radical generation.



Root extract of *R.nasutus*

Figure 3: Hydrogen peroxide scavenging activity of TLC separated fractions of *rhinacanthus nasutus* root extracts



Root extract of *R.nasutus*

Figure 4: Effect of inhibition of *in vitro* super oxide and nitric oxide generation by TLC separated fractions of *rhinacanthus nasutus* root extracts

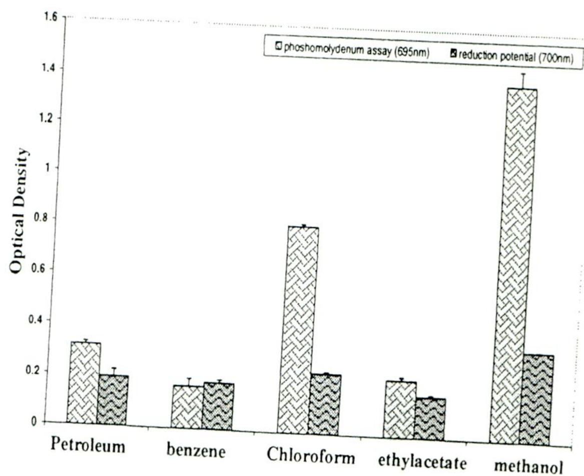
Conclusion

It can be concluded that the root extracts of the plant could serve as a good source for the therapeutic drugs for degenerative diseases. The root extracts of *Rhinacanthus nasutus* 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.

References

1. Jelili A. B., Oyeronke A.O., Efere M.O. and Oyeboade O.O., Phytochemicals and *in vitro* antioxidant potentials of defatted

methanolic extracts of *Holarrhena floribunda* leaves, *African Journal of Biotechnology*, **9**, 340-346 **(2010)**



Root extract of *R.nasutus*

Figure 5: Total antioxidant potential of the TLC separated fractions of *rhinacanthus nasutus* root extracts

2. Abimbola O.A., Ayanbamiji T.A., Yejide A.O., Ewegbenro and Ikeoluwa, Antimicrobial and phytochemical analysis of leaves and bark extracts from *Bridella ferruginea*, *African Journal of Biotechnology*, **9**, 1031-1036 **(2010)**

3. Roy S., Rao K., Bhuvaneshwari C., Giri A. and Mangamoori L.N., Phytochemicl analysis of *Andrographis paniculata* extract and its antimicrobial activity, *World J. Microbiol. Biotechnol.*, **28**, 85-91 **(2010)**

4. Patel V.R., Patel P.R. and Kajal S.S., Antioxidant activity of some selected medicinal plant in western region of India, *Advances in Biological Research*, **4**, 23-26 **(2010)**

5. Siripong P., Kanokmedakul K., Piyaviriyagul S., Yahuafai J., Chanpai R., Ruchirawat S. and Oku N., Antiproliferative naphthoquinone esters from *Rhinacanthus nasutus* Kurz roots on various cancer cells, *J. Trad. Med.*, **23**, 166-172 **(2006)**

6. Mensor L.L., Menezes F.S., Leitao G.G., Reis A.S., Santos T.C., Coube C.S. and Leitao S.G., Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytotherapy Research*, **15**, 127-130 **(2001)**

7. Shirwaiker A., Rajendran K. and Kumar C.D., *In vitro* antioxidant studies of *Annona squamosa* Linn leaves, *Ind. J. Exp Biol.*, **42**, 803-807 **(2006)**

8. Elizabeth K. and Rao M.W., Oxygen radical scavenging activity of Curcumin, *Int J Pharmaceu.*, **58**, 237-240 **(1990)**

9. Ruch R.J., Cheng S.J. and Klaunig J.E., Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea, *Carcinogenesis*, **10**, 1003-1008 **(1989)**

10. Winterbourn C., Hawkins R.E., Brain M. and Carell R.W., The estimation of red cell superoxide dismutase activity, *J. Lab. Clin. Med.*, **85**, 37-341 **(1975)**

11. Green L.C., Wagner D.A., Gloowski J., Skipper P.L., Wishnok J.S. and Tannenbaum S.R., Analysis of nitrate and nitrite (15N) nitrate in biological fluids, *Anal. Biochem.*, **126**, 131-136 **(1982)**

12. Prieto P., Pineda M. and Aguilar M., Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.*, **269**, 337-341 **(1999)**

13. Oyaizu M., Studies on products of browning reaction prepared from glucosamine, *Jpn. J. Nutr.*, **44**, 307-314 **(1986)**

14. Aliyu A.B., Ibrahim M.A., Musa A.M., Ibrahim H., Abdulkadir I.R. and Oyewale A.O., Evaluation of antioxidant activity of leaf extract of leaf extract of *Bauhinia rufescens* Lam (Caesalpiniaceae), *Journal of Medicinal Plants Research*, **3**, 563-567 **(2009)**

15. Osman H., Rahim A.A., Isa N.M. and Bakhir N.M., Antioxidant avtivity and phenolic content of *Paederia foetida* and *Syzygium aqueum*, *Molecules*, **14**, 970-978 **(2009)**

16. Mimura T., Yazaki K., Zawaki K., Ozawa T. and Kawaguchi M., Hydroxyl radical scavenging effect of guaiacol used in traditional dental pulb sedation, reaction kinetic study, *Biochemical Research*, **26**, 139 – 145 **(2005)**

17. Devi K.P., Suganthy N., Kesika P. and Pandian S.K., Bioprotective properties of Sea weeds: *In vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content, *BMC Complementary and Alternative Medicine*, **8**, 1-11 **(2008)**

18. Gulcin I., Huyut Z., Elmastas M. and Aboul-Enein H.Y., Radical scavenging and antioxidant activity of tannic acid, *Arabian Journal of Chemistry*, **3**, 43-53 **(2010)**

19. Ganapathy S., Chandrasekhar V.M., Chictme H.R. and Narsu M.L., Free radical scavenging activity of Gossypin and Nevadensin: An *in vitro* evaluation, *Indian J. Pharmacol.*, **39**, 281-283 **(2007)**.

(Received 24th August 2011, revised 25th December 2011, accepted 15th March 2012)

Authors will be pleased to know that our international peer reviewed quarterly journal

"Research Journal of BioTechnology"

being published from year 2006 is indexed and abstracted in

- **Chemical Abstracts**
- **BioTechnology Citation Index®**
- **Science Citation Index Expanded (SciSearch®)**
- **Journal Citation Reports/Science Edition**

SCI Impact Factor 0.284