Invitro Efficacy of antioxidant activity in ethanolic and aqueous leaf extracts of *Andrographis paniculata* Nees and *Rhinacanthus nasutus* Kurz

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**Article History:**
Received on: 12 Apr 2020
Revised on: 22 Jul 2020
Accepted on: 05 Jun 2020

**Keywords:**
Andrographis paniculata Nees,
Rhinacanthus nasutus Kurz,
In vitro,
Antioxidant,
DPPH,
ABTS,
FRAP,
SOD

**ABSTRACT**
Plants are abundantly and are very promising to be used as source of drugs in many diseases or infections and also it is a main agents of antioxidants which prevents the oxidative stress that are caused by the free radicals. There are numerous studies based on the pharmaceutical and classification of medicinal plants throughout the world. Leaves, fruits, roots are most frequently plant parts used in many research and studies. Here the present study was aimed to evaluate the *in vitro* antioxidant activity of aqueous and ethanol leaf extracts of *Andrographis paniculata* Nees and *Rhinacanthus nasutus* Kurz. Antioxidant is a substance which is used to prevent some types of cell damage in the body. Determination of their *in vitro* antioxidant activity were carried out by using methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl assay), ABTS (2,2′-azinobis (3-ethylbenzothiazolin 6-sulfonic acid assay), FRAP (ferric reducing antioxidant power assay) and SOD (super oxide anion scavenging) assay, H₂O₂ (hydrogen peroxide radical scavenging) assay. Moreover the ethanolic leaf extracts showed best antioxidant activity than the aqueous leaf extracts. Experimental results reveals that the leaves of *A. paniculata* have potent antioxidant and free radical scavenging activity than *R. nasutus*. Further investigation must be done for these two medicinal plants for the discovery of the bioactive compounds.

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**INTRODUCTION**
All living organisms involves many biological processes which produce harmful intermediates known as reactive oxygen species (ROS) or free radicals (Sen et al., 2010; Santhi et al., 2019). Radiation, bacteria, virus, toxins, smoking and alcohol are also reported to produce ROS. Over production of ROS implicated as a potential contributor to the pathogenesis and complications of some disease like diabetes, cancer, atherosclerosis, arthritis and ageing process (Khalaf et al., 2008). Oxidative stress is an imbalance between oxidants and antioxidants that cause damage in the biomolecules like nucleic acid,
protein, DNA and RNA (Droge, 2002).

Antioxidant acts as a defense mechanism that protects against deleterious effect of oxidative reaction produced by ROS (Jayachitra and Kirthiga, 2012). The antioxidant nutrient may be a major importance in disease prevention (Chanda and Dave, 2009). These days there has been a surge of interest for the helpful possibilities of therapeutic plants as cancer prevention agents is decreasing such free radical incited tissue injury (Pourmorad et al., 2006). Examinations of phenolic mixes in medicinal plants have picked up significance because of their high cancer prevention agent action. It has extraordinary incentive in forestalling the beginning or movement of numerous human ailments (Chang et al., 2007).

Medicinal plants which were studied in the recent investigation is Andrographis paniculata Nees and Rhinacanthus nasutus Kurz, belongs to the family Acanthaceae. Plenty of A. paniculata were distributed in India and China. It is an annual plant with 1–3 ft high. The A. paniculata leaves are used as antibacterial, antiviral, hepatoprotective, and hypoglycemic agent. The leaves of the plants which contains flavinoids, terpinoids, tannins etc (Hidalgo et al., 2013; Suja et al., 2017). R. nasutus are widely distributed in South China and India and this has been used for the treatment of diabetes, hepatitis, cancer, and hypertension etc (Siripong et al., 2006). The leaves of the plant has a rich source of triterpinoids, flavinoids, and steroids etc (Kupradinun et al., 2009). The present study was to evaluate the antioxidant activity of aqueous and ethanolic extract of A. paniculata and R. nasutus by in vitro model.

MATERIALS AND METHODS

Plant material and extraction

The fresh leaves of A. paniculata and R. nasutus were collected in and around Trichy and Coimbatore. The plants were identified and are authenticated with the specimen at St. Joseph’s College Herbarium, “THE RAPINAT”,Department of Botany, Trichy, Tamil Nadu. The plant parts were dried and then those dried plant parts are exposed to electrical blender to deliver a coarse powder. The plant was then extracted by utilizing water and ethanol. 5 g of plant powder was macerated with 100 ml of water and ethanol independently in a closed flask for 24 h. The substance was shaken as often as possible during the initial 6 h and permitted to stand for 18 h. At that point the filtrate was dried in a tarred flat-bottomed dish at 105 °C until consistent weight was acquired. The concentrates were preserved in the fridge for further examinations.

DPPH radical scavenging assay

The free radical scavenging capacity of the ethanolic concentrate of A. paniculata and R. nasutus was determined utilizing the DPPH strategy followed by Vijayakumar et al. (2015). DPPH (200 µM) solution was set up in 95% methanol. From the stock plant extract solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in four test tubes. 1 ml of newly prepared DPPH solution was incubated with test drug and after 15 min, the absorbance was taken as 517 nm utilizing a spectrophotometer. Standard ascorbic acid was utilized as reference.

Calculation

DPPH Scavenging activity (% inhibition =

\[
\frac{Control – Test}{Control} \times 100
\]

ABTS+ radical scavenging assay

ABTS+ assay was done by the technique for Gülçin et al. (2009). Changing the concentration (25, 50, 75, 100 µg/ml) of plant extracts and standard ascorbic acid solutions were taken into series of test tubes. 2 ml of ABTS solutions was included and the volume was made up to 1 ml with ethanol. To the control 2 ml of ABTS solution and 1 ml with ethanol was included. The solutions were perused promptly at 734 nm.

Calculation

ABTS+ Scavenging activity (%) =

\[
\frac{Control – Test}{Control} \times 100
\]

Ferric reducing power assay

FRAP assay was utilized by the technique of (Vijayalakshmi and Ruckmani, 2016). 1 ml of differing concentrations (25, 50, 75, 100 mg/ml) of plant extract was blended in with 3.5 ml phosphate buffer and 3.5 ml of potassium ferricyanide. The blend was incubated at 75 °C for 25 min. Aliquots of 3.5 ml of trichloroacetic acid were added to the blend, which was then centrifuged at 3000 rpm for 15 min. The upper layer of the solution (3.5 ml) was blended in with equivalent volume of distilled water; to this 1 ml of newly prepared ferric chloride solution was included and the absorbance was estimated at 700 nm. The expanded absorbance of the reaction blend demonstrates increase in reducing power.

\[
\text{(% Inhibition in reducing power} = \frac{A_{\text{Test}}}{A_{\text{Blank}}} \times 100
\]

Superoxide radical scavenging activity
Table 1: DPPH radical scavenging activity of *A. paniculata* and *R. nasutus* leaf extracts

| Concentration (µg/ml) | % inhibition of *A. paniculata* | % inhibition of *R. nasutus* | % inhibition of standard |
|-----------------------|---------------------------------|-------------------------------|--------------------------|
|                       | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous |
| DPPH activity         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 25                    | 36.86   | 33.5    | 36.05   | 30.76   | 34.82   |
| 50                    | 47.67   | 35.91   | 37.3    | 32.62   | 41.33   |
| 75                    | 52.58   | 36.26   | 40.04   | 34.11   | 49.02   |
| 100                   | 66.44   | 38.32   | 42.58   | 36.39   | 58.55   |
| IC<sub>50</sub>       | 60 µg/ml | -       | -       | -       | 76 µg/ml |

Table 2: ABTS<sup>+</sup> radical scavenging activity of *A. paniculata* and *R. nasutus* leaf extracts

| Concentration (µg/ml) | % inhibition of *A. paniculata* | % inhibition of *R. nasutus* | % inhibition of standard |
|-----------------------|---------------------------------|-------------------------------|--------------------------|
|                       | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous |
| ABTS<sup>+</sup> activity |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 25                    | 31.11   | 21.95   | 31.04   | 21.4    | 32.21   |
| 50                    | 52.72   | 39.69   | 49.67   | 33.43   | 53.22   |
| 75                    | 75.37   | 56.84   | 68.25   | 49.83   | 73.37   |
| 100                   | 99.55   | 76.26   | 90.4    | 62.95   | 93.01   |
| IC<sub>50</sub>       | 46 µg/ml | 64 µg/ml | 50 µg/ml | 77 µg/ml | 47 µg/ml |

Table 3: FRAP activity of *A. paniculata* and *R. nasutus* leaf extracts

| Concentration (µg/ml) | % inhibition of *A. paniculata* | % inhibition of *R. nasutus* | % inhibition of standard |
|-----------------------|---------------------------------|-------------------------------|--------------------------|
|                       | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous |
| FRAP activity         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 25                    | 28.61   | 21.75   | 26.37   | 18.22   | 28.16   |
| 50                    | 44.62   | 36.19   | 38.03   | 28.45   | 44.15   |
| 75                    | 60.56   | 50.84   | 57.7    | 38.25   | 58.91   |
| 100                   | 86.51   | 66.11   | 75.59   | 48.96   | 83.37   |
| IC<sub>50</sub>       | 56 µg/ml | 73 µg/ml | 63 µg/ml | -       | 58 µg/ml |

Table 4: Superoxide dismutase anion scavenging activity of *A. paniculata* and *R. nasutus* leaf extracts

| Concentration (µg/ml) | % inhibition of *A. paniculata* | % inhibition of *R. nasutus* | % inhibition of standard |
|-----------------------|---------------------------------|-------------------------------|--------------------------|
|                       | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous | Ethanol | Aqueous |
| SOD activity          |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |         |
| 25                    | 29.17   | 20.22   | 30.01   | 16.34   | 33.96   |
| 50                    | 49.56   | 36.81   | 46.41   | 24.33   | 52.81   |
| 75                    | 70.85   | 51.35   | 59.28   | 31.88   | 73.36   |
| 100                   | 93.35   | 70.25   | 70.67   | 42.67   | 93.14   |
| IC<sub>50</sub>       | 50 µg/ml | 71 µg/ml | 60 µg/ml | -       | 46 µg/ml |
The PMS-NADH system (phenazine methosulfate, nictotinamides adenine dinucleotide system) of (Fontana et al., 2001). It was utilized for the age of superoxide anion. 0.2–1 ml of ethanol and plant extract was blended in with 0.5 ml NBT and 0.5 ml NADH was blended. The reaction blend incubated at 50 °C for 5 min. After 5 min absorbance of the blend was estimated at 560 nm against the blank. The level of hindrance was dictated by looking at the consequences of control and test.

\[
\text{Super oxide radical scavenging activity (\%) = } \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100
\]

RESULTS AND DISCUSSION

The stable DPPH radical model is broadly utilized, generally quick and exact strategy for the assessment of free radical scavenging activity. DPPH is a steady free extreme it turns into a stable diamagnetic particle when it acknowledges an electron or hydrogen radical. Antioxidant on association with DPPH both exchange electron or hydrogen atom into to DPPH and hence killing its free radical character and convert it to 1,1-diphenyl-2-picolyl hydrazine and the level of staining shows the scavenging action of the drug (Vasanth and Bupesh, 2019). The reduced limit of DPPH radical is controlled by the abatement in its absorbance at 517 nm prompted by cancer prevention agents (Saran et al., 2019). The decrease in absorbance of DPPH radical achieved by malignant growth prevention agents because of the reaction between cancer prevention agent particles and radical progression which realizes the looking of the radical by hydrogen donation. It is visually noticeable as an adjustment in shading from purple to yellow. Henceforth, DPPH is commonly used as a substance to evaluate the antioxidant activity (Braca et al., 2001; Usmani, 2013).

Table 1 shows the inhibition action of ethanolic extracts of A. paniculata and R. nasutus shows most elevated hindrance of 66.44% in 100 µg/ml focuses and least inhibition of 36.86% in 25 µg/ml and the IC_{50} esteem is 60 µg/ml and the ethanolic concentrate of R. nasutus shows 42.58% most noteworthy and 36.05% lower inhibition in 100 µg/ml and 25 µg/ml individually and the outcomes were contrasted and standard ascorbic acid which utilized as a source of perspective and the IC_{50} value is 76 µg/ml.

In this examine, ABTS is changed over to its radical cation by the expansion of potassium persulfate. This ABTS radical cation is blue in shading and absorbs light at 734 nm. The ABTS radical cation is responsive towards most antioxidants, including thiols, phenolics, and ascorbic acid. During this response, the blue ABTS radical cation is converted to its colorless, neutral form (Ragavendra et al., 2013). Table 2 display the ethanolic leaf concentrates of A. paniculata and R. nasutus results the remarkable inhibition of 99.5% and 90.4% in 100 µg/ml separately, and 25 µg/ml shows 31.11% and 31.04% of hindrance and their IC_{50} esteems are 46 µg/ml and 50 µg/ml, and the standard ascorbic acid has IC_{50} estimation of 47 µg/ml.

The FRAP test used to measure the decreasing capability of a cell reinforcement responding with a ferric tripyridyltriazine (Fe3+-TPTZ) complex and delivering a colored ferrous tripyridyltriazine (Fe2+-TPTZ). The free extreme chain breaking happens through giving a hydrogen molecule. At low pH of about 3.6, decrease of Fe3+-TPTZ complex with blue shaded Fe2+-TPTZ happens, which has an absorbance at 593 nm. The outcomes acquired are highly reproducible and related directly to the molar convergence of the cancer prevention agents present. This is according to the results point by point by Benzie et al. (1999); Jeong et al. (2004).

Table 3 results noticeable electron move limit of ethanolic leaf concentrates of A. paniculata and R. nasutus reveals the most extreme hindrance of 100 µg/ml in 86.51% and 75.59% and the lower limitation of 25 µg/ml is 28.61% and 26.37% and the IC_{50} regards are 56 µg/ml and 63 µg/ml independently and the IC_{50} regard 58 µg/ml of standard ascorbic acid was differentiated and the result.

Superoxide dismutase (SOD) is a significant protein in a antioxidant resistance framework (Curtis et al., 1972). SOD converts over the superoxide anion into hydrogen peroxide and in this way decreases the harmful impact. The level of restraint of superoxide by SOD may decrease the cell harms. The current investigation demonstrates that the expanding grouping of the concentrate has a most extreme inhibitory action of SOD. Table 4 shows the potential action of 93.35% and 70.67% in 100 µg and minimal activity of 25 µg in 29.17% and 30.01% of ethanolic leaf concentrates of A. paniculata and R. nasutus and their IC_{50} esteems are 50 µg/ml and 60 µg/ml. The standard ascorbic acid shows 93.14% of most extreme restraint in 100 µg and the IC_{50} esteem is 46 µg/ml.

CONCLUSIONS

The current study demonstrates that the in vitro antioxidant activity of the Medicinal plants A. paniculata and R. nasutus were scientifically validated and its traditional utility. Further the efficacy of antioxidant activity was more in the ethano-
lic extract of A. canaliculata than R. nasutus.

ACKNOWLEDGEMENT

This is to thank Department of Human Genetics and Molecular Biology, Bharathiyar University for their Laboratory support to completing this work successfully.

Funding Support

The authors declare that they have no funding support for this study.

Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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