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RESEARCH ARTICLE

IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOL LEAF EXTRACTS OF *RHINACANTHUS NASUTUS* (LINN.) KURZ. (ACANTHACEAE)

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ABSTRACT

In the present research work was to examine the possible antioxidant activities of the aqueous and ethanol leaf extract of *Rhinacanthus nasutus* (Linn.) Kurz. DPPH, Hydroxyl radical scavenging and reducing power assays were employed. The results showed that the DPPH activity of aqueous and ethanol leaf extract at the dose of 50µg/ml has exhibited in 63.81±0.013 and 79.36±0.028 inhibition with an IC_{50} value of 21.39 and 29.41µg/ml. The highest Hydroxyl radical scavenging activity showed aqueous and ethanol leaf extract at the dose of 50µg/ml has exhibited in 96.18±0.029 and 121.23±0.081 inhibition with an IC_{50} values of 30.19and 41.39µg/ml, the reducing power assay aqueous and ethanol leaf extract showed the 0.59 and 0.71 absorption at 700 nm extract at the dose of 50µg/ml suggested that promising antioxidant activity of crude aqueous and ethanol extract could be used as a source of natural antioxidants of *R. nasutus* and needs further studies to bring new natural products into pharmaceutical industries.

Keywords: *Rhinacanthus nasutus,* antioxidant activities, DPPH, Hydroxyl radical scavenging activity, reducing power assay.

1. INTRODUCTION

The large number of medicinal, aromatic, modern medicines, spice and other plants contain the phytochemical constituents exhibiting antioxidant activities. In oxidative process is one of the most important ways for producing free radicals in drugs, foods and even in alive systems (1). Mostly effective path to abolish and diminish the action of free radicals which can be cause the oxidative stress is antioxidative defense mechanisms. In antioxidants substance can be possessing free radical chain reaction breaking the properties. Recently there has been increases of importance in the therapeutic prospective medicinal plants have antioxidants to re-antioxidants in reducing oxidative stress induced tissue injury (2). Several plant species are naturally occurring to the antioxidants; phenol, ascorbic acid, carotenoids and phytochemical compounds in effective (3).

Rhinacanthus nasutus (Linn.) Kurz belong to the family Acanthaceae it is an important medicinal plant, widely distributed in several part of sub-continent of India, China, Thailand and East-Asia (4). Plant also commonly known as snake jasmine, it is called as Nagamalli in Tamil. The Naga means snake in Sanskrit and this plants treating with snake bite. The freshly root and leaves, injured and mixed with lime juice are used remedy for skin affections and ringworm. The seeds are more than effective in ringworm, bark and root is a very good remedy for dhobie's itch. Some people are said to possess extraordinary aphrodisiacal powers of roots boiled in a milk presence much active by Hindu practitioners. Roots, believed in some parts of India to be an antidote in the bites of poisonous snakes.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The plant *R. nasutus* collected from the wild areas of Western Ghats, Valparai. Then the plant identified and authenticated by referring herbarium. The collected plants were washed in tap aqueous to remove the impurities, separate the leaves and dried in a room temperature. The dried leaf materials pulverized and stored in an air tight container for further usage.

2.2. Preparation of extracts

50g leaves extract with 250 ml of ethanol using for the soxhlet extractor in 9-12 hours. And another set of leaves powder extracted to be aqueous placed in an aqueous bath at 100 ^{0}C for 2 hurs. The extract was filtered through what man No.1 filter with paper to remove all undisclosed substance, including to the cellular materials and other phytochemical constituents that are insoluble in the extraction solvents. Final extract were used in antioxidant activities.

2.3. DPPH radical scavenging activity

The scavenging effect of extracts on DPPH radials activity was determined according to the

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method of Shimada *et al.* (5). The various concentrations of plant extract (4 ml) were mixed with 1ml of aqueous and ethanol solution containing DPPH radicals, resulted in the final concentration of DPPH being 0.2mM. Then mixture were well shaken well and left to stand in 30min and absorbance was measured at 517nm. The percentage of inhibition was calculated according to the formula by: (A0-A1)/A0] × 100, where A0 was the absorbance of the control and A1 was the absorbance of the sample.

2.4. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging activity of plant extracts were assay by the method of (6). The reaction mixture 3.0 ml contained 1.0 ml of 1.5 mM FeSO4, 0.7ml of 6mM hydrogen peroxide, 0.3 ml of 20 mM sodium salicylate and various concentrations of the extract. After incubation period of 1 hour at 37°C, the absorbance of the hydroxylated salicylate complex was measured at 562nm. % inhibition = $[(A0-A_1)/A_0]\times100$,where A_0 is absorbance of the control (without extract) and A_1 is the absorbance in the presence of the extract, A_2 is the absorbance without sodium salicylate.

2.5. Reducing power

The reducing power performed according to the method of Oyaizu (7). Different concentrations of aqueous and ethanol extracts (10, 20, 30, 40 and 50µg/ml) of the study sample was mixed with 1ml of 200 mM sodium phosphate buffer (pH 6.6) and 1ml of 1% potassium ferriccyanide followed by incubation at 500 C for 20 minutes. After that 1ml of 10% trichloroacetic acid, was added and centrifuged at 3000 rpm for 10 minutes. Then, the supernatant was mixed with 2 ml of distilled water and 0.5 ml of 1% ferric chloride. After incubation of 10 minutes, the absorbance was measured at 700 nm.

3. RESULTS AND DISCUSSION

The effects of aqueous and ethanol leaf extracts of *R. nasutus* was investigated for its antioxidant activity on various *in vitro* models like DPPH, Hydroxyl radical scavenging and reducing power assays different levels. In the present study some free radical scavenging activities of aqueous and ethanol leaf extracts of *R. nasutus* was investigated by DPPH scavenging assay. Aqueous and ethanol leaf extract of *R. nasutus* have got profound antioxidant activity. DPPH antioxidant assay was based on the ability of the DPPH, a free radical are stable, which can be gets decolorized in the presence of antioxidants (8,9). The ability to scavenge the stable free radical DPPH was measured by decrease in the absorbance at 517 nm. The aqueous and ethanol leaf extracts of *R. nasutus* exhibited a significant at a dose dependent inhibition of DPPH activity. A concentration dependent assay was carried out with these extracts and the results are presented in table 1. The IC_{50} value of this plant both extract found to be 21.39 and 29.41µg/ml respectively. Generally the presence of phenolic compounds in *R. nasutus* extracts were responsible for the antioxidant activity and it could be due to the presence of hydroxyl group in the compounds which showed antioxidant activity.

Table 1.DPPH radical scavenging activity of aqueous and ethanolic leaf extracts of *R. Nasutus.*

S. No	Aqueous extract			Ethanolic extract		
	Concentration (µg/mL)	% of inhibition		Concentration (µg/mL)	% of inhibition	
1	10	14.36±0.024		10	18.34±0.018	
2	20	19.41±0.019	IC_{50} 21.39	20	26.93±0.019	IC_{50} 29.41
3	30	28.18±0.053		30	42.18±0.025	
4	40	48.56±0.036		40	65.13±0.048	
5	50	63.81±0.013		50	79.36±0.028	

Table 2. Hydroxyl radical-scavenging activity at various concentrations of aqueous and ethanolic leaf extracts of *R. nasutus.*

S. No	Aqueous extract			Ethanol extract		
	Concentration (µg/mL)	% of inhibition		Concentration (µg/mL)	% of inhibition	
1	10	21.67±0.037		10	31.46±0.031	
2	20	35.81±0.022		20	39.08±0.067	
3	30	46.31±0.048	IC_{50} 30.19	30	71.19±0.027	IC_{50} 41.39
4	40	67.23±0.019		40	95.46±0.041	
5	50	96.18±0.029		50	121.23±0.081	

Table 3. Reducing power at various concentrations of aqueous and ethanolic leaf extracts of *R. nasutus.*

S. No	Aqueous extract		Ethanol extract	
	Concentration (µg/mL)	% of inhibition	Concentration (µg/mL)	% of inhibition
1	10	0.29	10	0.39
2	20	0.34	20	0.41
3	30	0.39	30	0.59
4	40	0.42	40	0.63
5	50	0.59	50	0.71

It is capable of neutralizing the deleterious effect of free radicals and their redox properties. Fruits and vegetables are natural sources of antioxidants and they provide protection against harmful free radicals (10,11). The highly hydoxyl radical scavenging effect at 50µg /ml concentration. The aqueous and ethanol leaves extracts of *R. nasutus* showed higher scavenging activity showed in (Table 2). The IC_{50} value of this plant extracts found to be 30.19and 41.39µg/ml respectively. This ability of the both plant extracts shows in the quenching ability of hydroxyl radicals, which seems to be a very good scavenger, of active oxygen plant species thus reducing the rate of chain reaction. The reducing power assay leaves of both extract showed the 0.59 and 0.71 absorption at 700 nm extract at the dose of 50µg/ml in (Table 3). The reducing power activity is often used to be an study ability of natural occurring antioxidant to donate electron (12,13).

4. CONCLUSION

Finally, concluded that present research work plant extracts possessed variable but interesting antioxidant properties. These properties were significantly correlated to their total phenolics content and they could be used as a source of natural antioxidants in food, cosmetic and pharmaceutical industries. It can be also necessary that complete structural identification of the active phytochemical components of antioxidant of plants is, therefore, required and their biological properties could be investigated.

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