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ANTIOXIDANT PROPERTIES OF THE LEAVES AND STEMS OF *ARISTOLOCHIA INDICA*

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Abstract

Aristolochia indica is a traditionally used medicinal plant claimed to possess antioxidant, antidiabetic and anti-inflammatory activity. The present study was conducted with the purpose to evaluate the *in vitro* antioxidant activity in leaves and stem of *Aristolochia indica*. The enzymic antioxidants such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione-s-transferase (GSH) and non-enzymic antioxidants (ascorbic acid, α-tocopherol, reduced glutathione, total carotenoids and flavonoids) were found to be present. The free radical scavenging activity (inhibition of nitric oxide and super oxide generation, *in vitro* lipid peroxidation and DPPH free radical scavenging and hydrogen peroxide radical scavenging activity) of different extracts of *Aristolochia indica* was also observed. The results obtained in the present study indicated that the *Aristolochia indica* might be a good source of natural antioxidant.

Key words : *Aristolochia indica*, Garudakodi, antioxidants, free radical scavenging activity, lipid peroxidation.

Introduction

Reactive oxygen species (ROS) including superoxide radical, hydroxyl radical, singlet oxygen and hydrogen peroxide are often generated as byproducts of biological reactions or from exogenous factors and molecules are responsible for cellular injury and aging processes (Wu *et al.*, 2008). Lifestyle related diseases of stroke, cancer, heart diseases, diabetes, kidney diseases and hypertension have been considered to be associated with reactive oxygen species including free radicals (Hiramatsu *et al.*, 2008). Antioxidant activity of plant is known for protection against ROS generation, general health maintenance, anti-aging and chemoprevention to be mainly provided by phenolic acids and flavonoids (Pyo *et al.*, 2004).

Aristolochia indica (Garudakodi) is one of the plant species with medicinal properties belong to the family *Aristolochiaceae*. The *Aristolochiaceae* family contains about 400 species in 7 genera of cosmopolitan distribution, many of them are of economic importance due to aristolochic acids and terpenoids. The phenolic such as terpenoids are the important components present in *Aristolochia indica* (Thirugnanasampandan *et al.*, 2008). The root of the plant is used in indigenous system of medicine as an antidote for the snake bites, gastric stimulant and bitter tonic. Among the tribal inhabitants, the roots are ground with black pepper seeds and made into pills administered to treat rheumatism and diabetes (Goverdhan *et al.*, 2008).

The present study was designed to investigate the "Antioxidant properties of the leaves and stems of *Aristolochia indica*" with the following objectives (i) to assess the levels of antioxidants present in *Aristolochia indica* (ii) to analyze the extent of inhibition of lipid peroxidation in the aerial parts of *Aristolochia indica* (iii) to determine the free radical scavenging effects of *Aristolochia indica*.

Materials and Methods

Collection of the sample

The root and the leaves of *Aristolochia indica* were collected from the local market, Coimbatore and duly authenticated. They were shade dried, powdered to coarse size and stored at room temperature until use.

Assessment of the activities of enzymic and non-enzymic antioxidants

The leaf and stem samples of *Aristolochia indica*

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was analysed for the enzymic and non-enzymic antioxidants such as catalase (Luck, 1974), superoxide dismutase (Misra and Fridovich, 1972), glutathione reductase (David and Richard, 1983), glutathione peroxidase (Rotruk *et al.*, 1973), glutathione S-transferase (Habig *et al.*,1974), ascorbic acid (Roe and Kuether, 1953), α – tocopherol (Rosenberg, 1992), reduced glutathione (Moron *et al.*, 1979), total carotenoids (Zakaria *et al.*, 1979) and flavonoids (Cameron *et al.*, 1943).

Free radical scavenging activity

The leaf and stem of *Aristolochia indica* powder were subjected to extraction with solvent like petroleum ether, benzene, chloroform, acetone, methanol and water in soxhlet apparatus for 18-20 h. The solvents were evaporated and resulting solid residue was dissolved in dimethyl sulfoxide (DMSO) and used for further studies. Each extracts were used for the determination of free radical scavenging activity such as DPPH (Mensor *et al.*, 2001), inhibition of *in vitro* lipid peroxidation (Okhwa *et al.*, 1979), inhibition of superoxide (Mc Cord and Fridovich, 1968), nitric oxide generation (Green and Hill, 1984) and hydrogen peroxide scavenging (Ruch *et al.*, 1989) activity.

Statistical analysis

Statistical analysis was performed according to student t-test procedure. The values ($P < 0.05$) were considered to be significant.

Results and Discussion

Enzymic and non-enzymic antioxidants

Activities of various enzymic antioxidants like catalase, superoxide dismutase, glutathione reductase, glutathione-S- transferase and glutathione peroxidase and non enzymic antioxidants such as ascorbic acid, α-tocopherol, total carotenoids and reduced glutathione in the leaves and stems of *Aristolochia indica* were tested and the results obtained are depicted in table 1.

From the table, it is clear that the activity of the enzymic antioxidants was found to higher in both the leaf and stem. The leaves exhibited the highest activity of glutathione reductase (11.3±2.5U/g) and the stems exhibited the highest activity of superoxide dismutase (11.5±0.4U/g). The above table reveals that *Aristolochia indica* contains moderate levels of ascorbic acid, α-tocopherol and reduced glutathione. A significant difference in the levels of α-tocopherol, carotenoids, flavonoids and reduced glutathione was seen between the leaves and stems of *Aristolochia indica.*

Since, *Aristolochia indica* contains significant activities of all the enzymic antioxidants analysed, it may have the ability to reduce the risk of serious diseases caused by reactive oxygen species like malignancy, cardiovascular diseases and severe neural diseases.

***in vitro* free radical scavenging activity**

Free radical often exist in the form of peroxyl (ROO□), alkoxyl (RO), hydroxyl (OH˙) and nitric oxide (NO), all of which retain surplus non-paired electrons being highly reactive to macromolecular compounds including proteins and nucleic acids (Chi *et al.*, 2007). The ability of *Aristolochia indica* to scavenge free radicals is shown in table 2.

All the extracts of *Aristolochia indica* were capable of scavenging 1, 1-Diphenyl-2- picryl hydrazyl (DPPH) radicals. The aqueous extract of the leaves of *Aristolochia indica* was found to have a strong inhibition (76 per cent) against DPPH radical, whereas the chloroform fraction showed only 23 per cent of inhibition when compared with other extracts. The aqueous extract of stem showed a good radical scavenging activity of about 52 per cent, whereas the benzene and acetone fractions were found to have 20 per cent of inhibition against DPPH radicals when compared to other fractions. The free radical scavenging properties of the extracts were determined by the DPPH assay, where the DPPH radical is reduced by the antioxidant compound to its hydrazine derivative (Rocha *et al.*, 2008).

The percentage inhibition of *in vitro* lipid peroxidation exerted by the leaves of *Aristolochia indica* in the extracts of petroleum ether, benzene, chloroform, acetone, methanol and aqueous was 17, 51, 26, 21, 55 and 8.4 per cent, respectively. Among the six extracts, the methanol extract was found to possess a strong inhibitory action on *in vitro* lipid peroxidation than that of the others. The benzene extract of the stem of *Aristolochia indica* showed the highest (36 per cent) antioxidant activity, whereas the petroleum ether extract showed a lower activity in comparison to other extracts.

The acetone (stems) extracts showed a strong inhibitory activity against superoxide radicals than the other solvent extracts. The aqueous extract of *Aristolochia indica* stem showed the lowest activity on superoxide radical inhibition. The scavenging effects of different extracts of leaves were in the order of: acetone > methanol > petroleum ether> benzene > chloroform> aqueous which were 79, 58, 57, 28, 10, and 6 respectively.

The aqueous extract of leaves shows more inhibitory (47 per cent) activity against nitric oxide radicals. The methanol extract of stem shows high inhibitory activity. The lowest inhibitory activity was found to be in leaf

Table 1 : Effect of enzymic and non-enzymic antioxidant in *Aristolochia indica.*

Enzymic antioxidants (U/g)	*Aristolochia indica*		
	Leaf	Stem	t value
Catalase[1]	235 ± 13.9	257 ± 27.0	1.02[ns]
Superoxide dismutase[2]	6.5 ± 2.1	11.5 ± 0.4	9.01*
Glutathione reductase[3]	11.3 ± 2.5	5.6 ± 1.04	2.98*
Glutathione peroxidase[4]	0.5 ± 0.04	0.4 ± 0.01	3.38*
Glutathione-S-transferase[5]	0.6 ± 0.2	0.3 ± 0.05	2.134*
Non enzymic antioxidants (mg/g)	***Aristolochia indica***		
	Leaf	**Stem**	**t value**
Ascorbic acid	1.01 ± 0.01	1.06 ± 0.04	1.14[ns]
α-tocopherol	0.62 ± 0.04	0.36 ± 0.01	5.78*
Carotenoids	2.13 ± 0.02	1.74 ± 0.04	33.97*
Flavonoids	3.30 ± 0.99	0.51 ± 0.25	3.10*
Reduced glutathione	0.03 ± 0.01	0.06 ± 0.01	2.21*

Values are mean ± SD of triplicates, * - Significant at 0.05 level, NS - not significant.

1- Amount of enzyme required to the optical density by 0.05

2- Amount that cause 50 per cent reduction in the extent of NBT oxidation.

3- μ moles of CDNB conjugated/minute 4- μ moles of NADPH utilized

5- μ moles of GSH utilized/minute

Table 2 : *in vitro* free radical scavenging activity *Aristolochia indica.*

% inhibition of free radicals / Extracts		DPPH	*in vitro* lipid peroxidation	Superoxide radicals	Nitric oxide radicals	Hydrogen peroxide radicals
Petroleum ether	L	55	17	61	21	47
	S	50	10	27	8	20
Benzene	L	61	51	28	33	66
	S	20	36	14	56	63
Chloroform	L	23	26	10	32	39
	S	28	17	39	21	43
Acetone	L	30	21	79	32	28
	S	20	30	63	34	6
Methanol	L	40	55	68	40	37
	S	35	26	48	67	22
Aqueous	L	76	2	6	47	62
	S	52	15	5	30	67

L : Leaf extract, **S :** Stem extract.

extracts of petroleum ether and stem extracts of chloroform. A strong inhibitory action was observed against the nitric oxide generation in the methanol extract, whereas a least inhibition was observed in petroleum ether extract of stem of *Aristolochia indica*. The results showed that both aqueous (52 per cent) and benzene (56 per cent) extracts of leaf sample produce strong hydrogen peroxide scavenging activity.

The hydrogen peroxide scavenging effect of the extracts of *Aristolochia indica* is shown in order aqueous

> benzene > chloroform > methanol> petroleum ether > acetone. Hydrogen peroxide can be formed *in vitro* by many oxidizing enzymes such as superoxide dismutase, cross membranes and slowly oxidize a number of compounds (Ozen and Kinalioglu, 2008).

Conclusion

Since, *Aristolochia indica* is exhibiting significant *in vitro* free radical scavenging activity, it may be effective in acting against cell or tissue damage which are caused by lipid peroxidation and therefore reduce the risk of aging, cardiovascular disease and cancer.

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