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Full Length Research Paper

Antioxidant properties of some medicinal Aristolochiaceae species

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Antioxidant properties of five Aristolochiaceae species namely *Aristolochia brasiliensis* Mart. and Zucc., *Aristolochia bracteolata* Retz., *Aristolochia indica* Linn., *Apama siliquosa* Lamk. and *Aristolochia tagala* Cham. were investigated. Antioxidant and 2,2-diphenyl picrylhydrazyl (DPPH) radical scavenging activities, reducing powers, and the amount of total phenolic compounds of the extracts were studied. The highest antioxidant activity was shown by *A. tagala* and the lowest one was *A. brasiliensis*. The highest DPPH radical scavenging activity was shown by *A. tagala* and the least one was shown by A. *brasiliensis*. The highest reducing power and amount of total phenolic compounds was shown by *A. tagala* and lowest one was A. *brasiliensis*.

Key words: Antioxidant activity, Aristolochiaceae, phenolic compounds, radical scavenging, reducing power.

INTRODUCTION

Plants are sources of natural antioxidants, and some of the compounds have significant antioxidative properties and health benefits (Exarchou et al., 2002). Antioxidants are compounds that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu et al., 1998). The potential of the antioxidant constituents of plant materials for the maintenance of health and protection from coronary heart disease and cancer is also raising interest among scientists and food manufacturers as consumers move toward functional foods with specific health effects (Lo liger, 1991). The antioxidative effect is mainly due to phenolic components, such as flavonoids (Pietta, 1998), phenolic acids, and phenolic diterpenes (Shahidi et al., 1992). Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Also many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Koleva et al., 2002; Mantle et al., 2000; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. The Aristolochiaceae family contains about 400 species in 7 genera of cosmopolitan distribution, many of them of economic importance due to aristolochic acids and terpenoids. The phenolics such as terpenoids are the important components present in Aristolochiaceae plants namely *Aristolochia brasiliensis* Mart. and Zucc., *Aristolochia bracteolata* Retz., *Aristolochia indica* Linn., *Apama siliquosa* Lamk. and *Aristolochia tagala* Cham.

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MATERIALS AND METHODS

Plant material collection

Five plants belonging to the family Aristolochiaceae such as *A. brasiliensis* Mart. and Zucc., *Aristolochia bracteolata* Retz., *A. indica* Linn., *A. siliquosa* Lamk. and *A. tagala* Cham. were collected from Coimbatore, Tamil Nadu, India. Plants, parts used, ailment treated and administration are presented in Table 1.

Preparation of plant extracts

Fresh leaves were dried under shadow at room temperature and ground into fine powder. The leaf powder (50 g) was extracted separately with 100 mL of petroleum ether, chloroform, ethyl acetate using Soxhlet apparatus until the solvent became colorless. The extracts were filtered and evaporated to dryness by a rotary evaporator at 30ºC. The extracts were dissolved in dimethyl sulfoxide at a concentration 50 mg/5 ml. From the above extracts 500 µL was taken and the following assays were performed. Place, date

Table 1. Botanical names, parts used, ailments treated, and preparations of Aristolochiaceae species.

Botanical name	Parts used	Ailment treated	Administration
Aristolochia brasiliensis Mart. and Zucc.	Leaves	Dry cough	Decoction
Aristolochia bracteolata Retz.	Leaves	Anti inflammatory	Decoction
Aristolochia indica, Linn.	Leaves/Roots	Cardiotonic, diuretic	Decoction
Apama siliquosa, Lamk.	Leaves/Roots	Healing ulcers, burns	Decoction
Aristolochia tagala, Cham.	Whole plant	Anti-implantation	Decoction

Table 2. Plants, place, date, altitude, parts used and extraction solvents of the Aristolochiaceae species.

Species	Place	Collection date	Altitude (m)	Extraction solvent
Aristolochia brasiliensis Mart. and Zucc.	Coimbatore	12 Sep-2006	400	Petroleum ether, Chloroform, Ethyl acetate
Aristolochia bracteolata Retz.	Coimbatore	12 Sep-2006	400	Petroleum ether, Chloroform, Ethyl acetate
Aristolochia indica Linn.	Coimbatore	1 Aug-2006	400	Petroleum ether, Chloroform, Ethyl acetate
Apama siliquosa Lamk.	Coimbatore	1 Aug-2006	400	Petroleum ether, Chloroform, Ethyl acetate
Aristolochia tagala Cham.	Coimbatore	1 Aug-2006	400	Petroleum ether, Chloroform, Ethyl acetate

of collection, altitude, and extraction solvent are tabulated in Table 2.

Antioxidant activity

The antioxidant activity was determined by ammonium thiocyanate assay (Lee et al., 2002). 500 µL of the extract, 200 µL of diluted linoleic acid (25 mg/mL 99 ethanol) and 400 µL of 50 mM phosphate buffer (pH 7.4) was mixed and incubated at 40ºC for 15 min. Aliquot (100 µL) from the reaction mixture was mixed with reaction solution containing 3 mL of 70% ethanol, 100 µL of ammonium thiocyanate (300 mg/mL distilled water) and 100 µL of ferrous chloride (2.45 mg/mL in 3.5% hydrochloric acid). Final reaction solution was mixed and incubated at room temperature for 3 min. Absorbance was measured at 500 nm. Linoleic acid emulsion without extract served as control. Inhibition of linoleic acid oxidation was calculated by using the following formula:

% Inhibition = [(control OD – sample OD)/control OD)] X 100.

DPPH radical scavenging activity

2,2-Diphenyl picrylhydrazyl (DPPH) free radicals scavenging activity was assessed according to Blois (1958), with a slight modification. 500 µL of extract solution was mixed with 1 mL of 0.1 mM DPPH in ethanol solution and 450 µL of 50 mM Tris-HCl buffer (pH 7.4) was added. The solution was incubated at 37ºC for 30 min and reduction of DPPH free radicals was measured by reading the absorbance at 517 nm. Control was maintained. Ascorbic acid solution was used for comparison. This activity is given as % DPPH scavenging and calculated according to the following equation:

% DPPH scavenging = [(control OD – sample OD) / (control OD)] X 100

Reducing power

Reducing power assay was carried out as described by Yildirim et al. (2001). 500 µL of the extract was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The reaction mixture was incubated at 50ºC for 30 min. Afterwards, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. 2.5 mL supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride. Absorbance was measured at 700 nm. Ascorbic acid solution was used for comparison. Increased absorbance of the reaction mixture indicated increased reducing power.

Determination of amount of total phenolic compounds

The amounts of total phenolic content of the extracts were determined by the method described by Singleton et al. (1999). 500 µl the extract was transferred to a 100 mL Erlenmeyer flask and the final volume was adjusted to 46 mL by addition of distilled water. 1 mL of Folin-Ciocalteau reactive solution was added and incubated at room temperature for 3 min. 3 mL of 2% sodium carbonate solution was added and the mixture was shaken on a shaker for 2 h at room temperature. The absorbance was measured at 760 nm. Gallic acid was used as the standard for a calibration curve. The phenolic compound content was expressed as gallic acid equivalent.

Statistical analysis

Each of the experiment was performed in triplicate. The results shown are the means of these measurements (Table 3, 4, 5, 6). Data were analyzed with SPSS software to determine the correlation between antioxidant properties in an extract. Pearson parametric correlation analysis was carried out using SPSS software (Version 10).

RESULTS AND DISCUSSION

In *Aristolochiaceae*, the aristolochic acids I and II are predominant. Aristolochic acid I, IV and terpenoids have been characterized from stems of *A. manshuriensis* (Nakanishi et al., 1982). Extensive investigations on the

Table 3. Results of ammonium thiocyanate assay (%) of the Aristolochiaceae species.

Plant	Petroleum ether			Chloroform			Ethyl acetate		
	10	50	100	10	50	100	10	50	100
A. brasiliensis	6.7	20.28	10.68	19.38	17.02	1.63	36.14	21.37	3.62
A. bracteolata	49	26.17	9.05	35.68	18.47	11.32	57.88	17.3	9.32
A. indica	35.13	29.17	19.38	21.55	9.78	3.89	40.21	26.08	19.38
A. siliquosa	45.65	35.5	9.42	29.34	18.84	11.14	35.14	19.2	9.14
A. tagala	57.42	36.23	21.01	12.31	8.87	0	56.15	47.14	33.78

Table 4. DPPH radical scavenging activity of the Aristolochiaceae species.

Plant	Petroleum ether			Chloroform			Ethyl acetate		
	10	50	100	10	50	100	10	50	100
A. brasiliensis	16.51	14.23	13.5	16.74	13.05	13.5	45.34	14.23	13.05
A. bracteolata	55.67	18.37	14.23	59.98	13.5	36.67	19.36	15.52	17.84
A. indica	39.42	16.15	15.52	20.29	18.36	15.11	22.11	16.22	35.81
A. siliquosa	58.62	14.23	11.18	35.91	20.29	11.98	11.98	11.98	12
A. tagala	71.2	37.5	16.15	15.52	11.98	12	46.5	34.98	13.5

Table 5. Reducing power activity of the Aristolochiaceae species.

Plant	Petroleum ether			Chloroform			Ethyl acetate		
	10	50	100	10	50	100	10	50	100
A. brasiliensis	0.52	0.58	0.61	0.46	0.76	1.01	0.77	0.95	1.19
A. bracteolata	0.60	0.72	0.94	0.56	0.73	0.98	0.69	0.97	1.25
A. indica	0.61	0.77	0.89	0.64	0.78	0.90	0.85	0.97	1.01
A. siliquosa	0.66	0.74	0.87	0.69	0.72	0.84	0.86	0.95	1.05
A. tagala	0.73	0.84	0.26	0.71	0.81	0.87	0.92	1.09	1.28

Table 6. Total poly phenols (µg) of the Aristolochiaceae species.

Plant	Petroleum ether			Chloroform			Ethyl acetate		
	10	50	100	10	50	100	10	50	100
A. brasiliensis	18	27	40	18	22	22	28	34	44
A. bracteolata	22	36	49	20	25	37	25	28	33
A. indica	20	26	33	20	31	40	24	31	44
A. siliquosa	21	27	30	18	22	22	34	42	44
A. tagala	20	39	34	30	41	50	19	24	30

terpenoids of *Aristolochia* genus have been carried out since 1935 and more than 200 terpenoids have been isolated and characterized by research groups in Rio de Janeiro, Araraquara (Brazil), Nanjing, Beijing (China), Tainan (Taiwan), Bombay (India) and Bonn (Germany) (Tian-Shung et al., 2004).

All the extracts exhibited antioxidant properties. The most effective free radical scavenging activity was shown by all three solvent extracts of *A. tagala*, the least activity was observed in *A. brasiliensis* (Tables 3 - 6). The most effective antioxidant and DPPH radical scavenging potential was shown by petroleum ether and ethyl acetate extracts of *A. tagala*, the least activity was shown by *A. brasiliensis* (Tables 3 and 4). Reducing power activity and amount of total polyphenols were high in chloroform extract of *A. indica* and ethyl acetate extract of *A. tagala*. The least activity was shown by *A. brasiliensis* (Tables 5 and 6).

Data were analyzed with SPSS software showed a correlation between antioxidant properties in an extract

Table 7. P-values of correlation analysis *A. brasiliensis* petroleum ether (I), chloroform (II) and ethyly acetate (III) extract.

I.

	DPPH	RP	APC
AA	0.632	0.695	0.883
DPPH		0.063	0.251
RP			0.188

II.

	DPPH	RP	APC
AA	0.660	0.289	0.589
DPPH		0.371	0.071
RP			0.300

III.

	DPPH	RP	APC
AA	0.346	0.019	0.058
DPPH		0.365	0.404
RP			0.039

AA, Antioxidant activity; DPPH activity; RP, reducing power activity; APC, amount of phenoloic compounds.

Table 8. P-values of correlation analysis *A. bracteolata* petroleum ether (I), chloroform (II) and ethyly acetate (III) extract.

I.

	DPPH	RP	APC
AA	0.223	0.159	0.039
DPPH		0.383	0.262
RP			0.121

II.

	DPPH	RP	APC
AA	0.517	0.219	0.298
DPPH		0.735	0.814
RP			0.079

III.

	DPPH	RP	APC
AA	0.507	0.235	0.327
DPPH		0.743	0.834
RP			0.091

AA, Antioxidant activity; DPPH activity; RP, reducing power activity; APC, amount of phenoloic compounds.

Table 9. P-values of correlation analysis *A. indica* petroleum ether (I), chloroform (II) and ethyly acetate (III) extract.

I.

	DPPH	RP	APC
AA	0.407	0.141	0.061
DPPH		0.266	0.347
RP			0.081

II.

	DPPH	RP	APC
AA	0.214	0.093	0.084
DPPH		0.121	0.130
RP			0.008

III.

	DPPH	RP	APC
AA	0.652	0.050	0.238
DPPH		0.702	0.413
RP			0.288

AA, Antioxidant activity; DPPH activity; RP, reducing power activity; APC, amount of phenoloic compounds.

Table 10. P-values of correlation analysis *A. siliquosa* petroleum ether (I), chloroform (II) and ethyly acetate (III) extract.

I.

	DPPH	RP	APC
AA	0.455	0.071	0.279
DPPH		0.384	0.176
RP			0.208

II.

	DPPH	RP	APC
AA	0.055	0.269	0.277
DPPH		0.323	0.222
RP			0.546

III.

	DPPH	RP	APC
AA	0.498	0.184	0.048
DPPH		0.314	0.546
RP			0.232

AA, Antioxidant activity; DPPH activity; RP, reducing power activity; APC, amount of phenoloic compounds.

exists (Tables 7 to 11). From these tables, we suggest that antioxidant activity may be affected by different parameters, such as DPPH free radicals scavenging activity, reducing power and amount of phenolic compounds. Effects of these parameters are changeable. For example, there is a correlation between antioxidant and DPPH activity in petroleum ether and ethyl acetate extracts of *A. tagala* (Table 11). In *A. bracteolata* antioxidant investigations of the ethanol extract along with its two successive fractions using nitric oxide and (DPPH)-induced free radical assay methods showed good free radical scavenging activity (Shirwaikar and Somashekar, 2003). Like antioxidant activity and DPPH there are sta-

Table 11. P-values of correlation analysis *A. tagala* petroleum ether (I), chloroform (II) and ethyly acetate (III) extract.

I.

	DPPH	RP	APC
AA	0.022	0.508	0.437
DPPH		0.530	0.415
RP			0.944

II.

	DPPH	RP	APC
AA	0.495	0.250	0.195
DPPH		0.245	0.300
RP			0.055

III.

	DPPH	RP	APC
AA	0.039	0.051	0.038
DPPH		0.089	0.076
RP			0.013

AA, Antioxidant activity; DPPH activity; RP, reducing power activity; APC, amount of phenoloic compounds.

tistically sign ificant correlations between reducing power and amount of total phenolic compounds in chloroform and ethyl acetate extracts of *A. indica* and *A. tagala* (Tables 9 and 11, respectively). Yildrim et al. (2001) have suggested that there may be relationship between phenolic com-pounds and reducing powers. Presence of phenolic compounds might be the reason for reducing power.

As mentioned above, these plants contain aristolochic acid and terpenoids. The antioxidative effect is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes (Shahidi et al., 1992). The antioxi-dant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994). In *A. longa* the total phenolics were esti-mated as 1.47 mg and free radical scavenging activity of these phenols were estimated by Djeridane et al. (2006). Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol, in the phosphor-lipid bilayer of lipoprotein particles, back to its active antioxidant form. They are also known to inhibit various types of oxidizing enzymes. These potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search for health-beneficial phytochemicals (Halliwell and Gutteridge, 1989; Hall and Cuppett, 1997).

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