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GC-MS analysis of bioactive constituents of *Aristolochia bracteolata* Linn with *in-vitro* antioxidant properties

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ABSTRACT

The aim of this study was to evaluate the antioxidant activity of hexane, ethyl acetate and methanol extracts of *Aristolochia bracteolata* Linn. Hexane, ethyl acetate and methanol extract were prepared using soxhlet extraction. DPPH and GC-MS were also performed. Methanol extract showed increased scavenging activity on 2,2-diphenyl-picrylhydrazyl (DPPH) (IC_{50} 270.12 ±1.12 µg/ml), hydroxyl (IC_{50} 450.04 ± 0.71 µg/ml), nitric oxide (IC_{50} 640.06 ± 1.24 µg/ml) and hydrogen peroxide (IC_{50} 300.51 ± 0.67 µg/ml) radicals, as well as high reducing power. Methanol extract also showed strong suppressive effect on lipid peroxidation (IC_{50} 400.01±1.23) and metal chelating ability of methanol extract showed moderate effect (IC_{50} 680.09 ± 0.33) when compared to the reference standard. In the present study the methanol extract of *Aristolochia bracteolata* has been subjected to GC-MS (Gas Chromatography-Mass Spectrometery) analysis. Sixty chemical constituents have been identified, The major peaks from GC-MS results revealed the presence of seven different phytochemical constituents like dodecane (7.50%), tetradecane (7.31%), tridecane (6.59%), undecane (6.29%), pentadecane (3.99%), hexadecane (2.69%) and decane (1.49%) whereas some other minor peaks corresponds to the presence of other phytoconstituents with medicinal properties. The results so obtained in the present study clearly indicate that methanol extract has a significant potential and can be used as an antioxidant agent. The presence of various phytochemical constituents may further pave a way for finding a novel drug.

INTRODUCTION

Plants have been used as a source of traditional medicine since years. Many medicinal plants contain potent antioxidant activities and many are eminent sources for phytochemicals. Free radicals are pivotal cause of aging, coronary heart disease, inflammation, cancer, stroke, diabetes mellitus (Cheng *et al*., 2003; Slater, 1984). During oxidative stress, reactive oxygen molecules (ROS) suchas hydroxyl (OH), superoxide (O_2^-, OOH), and peroxyl (ROO) radicals are produced. The ROS play its major role in the pathogenesis of neurodegenerative disorders, cancer, cardiovascular diseases, cataracts, atherosclerosis, and inflammation (Aruoma, 1998).

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In the cells, Reactive oxygen species (ROS) are produced by exogenous environmental agents and cellular metabolism. ROS are produced by redox cycling (Halliwell and Gutteridge, 1999). Overproduction of ROS can damagenucleic acids, carbohydrates, proteins, lipids and enzymes leading to various diseases. To overcome deleterious affects of several damages, living systems do have specific pathways but sometimes these repair mechanisms fail to keep pace with such deleterious effects (Halliwell, 1995; Nilsson, 2004). Antioxidants play a major role in scavenging free radicals. This in turn reduces the risk of cardiovascular diseases and cancer. Many plant extracts and phytochemicals have antioxidant activity (Al-Saikhan *et al*., 1995, Bergman *et al*., 2001; Cao *et al*., 1996; Oomah *et al*., 1994; Wang *et al*., 1996; Yen and Duh, 1995). Therefore search for novel natural antioxidants of plant origin has increased. The present work evaluates the possible antioxidative effects of hexane, ethyl acetate and methanol extract of whole plant of *Aristolochia bracteolata*.

Many natural antioxidants have already been isolated such as oilseeds, cereal crops, leaves, spices, vegetables, roots, and herbs (Ramarathnam *et al*., 1995). Phenolic compounds are reported to quench oxygen-derived free radicals (Wanasundara and Shahidi, 1996; Yuting *et al*., 1990). Also, phenolic compounds neutralize free radicals in various model systems (Ruch *et al*., 1989; Silva*et al*., 1991; Zhang *et al*., 1996). Nowadays the most commonly used antioxidants are butylated hydroxyanisole (BHA), tert-butyl hydroquinone, butylated hydroxytoluene (BHT) and propyl gallate. However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Whysner *etal*., 1994). Therefore, there is an increasing interest in natural and safer antioxidants. In this study, we evaluated the antioxidant activity by employing various *in vitro* assay systems, such as the DPPH/superoxide/nitric oxide radical scavenging, reducing power and iron ion chelation, in order to understand the usefulness of this plant as a food stuff as well as in medicine.

Scientific classification

Kingdom: Plantae Order: Piperales Family: Aristolochiaceae Subfamily: Aristolochioideae Genus: *Aristolochia* Species: *bracteolata Aristolochia bracteolata* has been reported to be used for inflammatory diseases, fever and insect bites. The whole plant is very bitter and used as abortifacient alterative, purgative, antiperiodic, emmenagogue, anthelmintic, antipyretic, anti-inflammatory agents. The stem and the root contain the alkaloid aristolochic acid. It should be used with great caution since the plant can be toxic to mammals. The dried, powdered root has been shown to increase the contractions of the uterus during labor. The leaves and roots are used to rid the body of Guinea worm (a parasitic infection caused by a nematode). Externally, its juice is applied to foul and neglected ulcers to destroy insect larvae. It is also used to treat scorpion bites.

MATERIALS AND METHODS

Collection of plant material

Plants were obtained from places near Coimbatore, South India. The plant specimen *Aristolochia bracteolata* was identified, certified and the voucher specimen number (LCH110) was deposited at Loyola college herbarium in the department of Plant Biology and Biotechnology, Chennai, India.

Preparation of the extract

The solvents which were used for preparation of extract were ethyl acetate, hexane, and methanol. Whole plant powder (100g) was taken and the extract was prepared by soxhlet extraction method using 300 ml of each solvent (low polarity to high polarity). The extract was then filtered using ordinary filter paper. Then the obtained filtrate were concentrated under reduced pressure at 40^0C using rotary evaporator and stored in a refrigerator at 2 to 8^0C for further experiments.

Chemicals and reagents

DPPH (1,1-diphenyl,2-picrylhydrazyl), PMS (phenazine methosulphate), NBT (nitro- blue tetrazolium), ferric chloride, NADH (nicotinamide adenine dinucleotide phosphate reduced), TCA (trichloroacetic acid) and BHT (butylated hydroxyltoluene) were obtained from Sigma chemical co., USA. Ascorbic acid was obtained from SD fine chem Ltd, Biosar, India. ferrozine, folin phenol reagent and Tween 40 were purchased from Hi-Media Pvt.Ltd. Mumbai, India. All the other chemicals were of analytical grade.

Reducing power activity

The reducing power of *Aristolochia bracteolata* hexane, ethyl acetate and methanol extracts were evaluated according to the method of Oyaizu (Oyaizu, 1986). Extracts with different concentration (200–1000 g/ml) were suspended in distilled water and mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium ferricyanide and was incubated at 50 ºC for 20 min. It was then centrifuged at 3000 rpm for 10 min by adding 2.5 ml of 10 % trichloroacetic acid. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). Absorbance was read at 700 nm. Butylated hydroxytoluene (BHT) was used as standard.

DPPH radical scavenging assay

DPPH quenching ability of solvent extract was measured according to Hanato *et al*. (Hanato *et al*., 1988). Extracts (ethyl acetate, hexane, and methanol) with different concentration ranging (200–1000 µg/ml) were mixed with DPPH solution (0.15%) in methanol. Then it was incubated at darkfor 10 min and the absorbance was read at 515 nm. The antiradical activity was expressed as IC_{50} (µg/ml), (the antiradical dose required to cause a 50% inhibition). Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)}=[(A_0 - A_1/A_0)\times 100] \quad \ldots(1)$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of sample or the standard sample.

Hydroxyl radical scavenging activity

The hydroxyl scavenging assay was performed as described by the method of Elizabeth and Rao (Elizabeth and Rao, 1990) with slight changes. The reaction mixture contained 100 µl of 28 mM 2-deoxy-2-ribose (dissolved in phosphate buffer, pH 7.4), extracts of various concentrations hexane, ethyl acetate and methanol (200–1000 µg/ml),100 µl H2O2 (1 mM) and 100 µl ascorbic acid (1 mM), 200 µl of lM ferric chloride and 1.04 Mm Ethylenediaminetetraacetic acid (EDTA) (1:1 v/v). The mixture was incubated for 1 h at 37°C. The absorbance was read at 532 nm against the blank solution. Vitamin C was used as a positive control. The scavenging activity was calculated using formula (1).

Nitric oxide scavenging activity

It can be estimated by the use of GriessIllosvoy reaction (Garratt, 1964). Here, GriessIllosvoy reagent was modified using N-(1-Naphthyl) ethylenediamine dihydrochloride (0.1% w/v). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and different concentration of hexane, ethyl acetate and methanol extract (200–1000 µg/ml) was incubated at 25 °C for 150 min.

After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted and mixed with 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min. Then, 1 ml of N-(1-Naphthyl) ethylenediamine dihydrochloride (1%) was added and allowed to stand for 30 min. The absorbance was measured at 540 nm. Ascorbic acid was used as standard. The scavenging activity was calculated using the formula (1).

Hydrogen peroxide radical scavenging assay

The ability of the extract to scavenge hydrogen peroxide was determined according to the method of Ruch *et al.*, 1989. A solution of hydrogen peroxide (2 mmol/l) was prepared in phosphate buffer (pH 7.4). All the extracts with concentrations (200–1000 µg/ml) were added to hydrogen peroxide solution (0.6 ml). After 10 min, absorbance was read at 230 nm against blank solution and compared with ascorbic acid, the standard.

Lipid peroxidation in rat liver homogenate

The peroxidation effect of the extracts on lipid peroxidation was determined according to the thiobarbituric acid method. Ferric chloride– hydrogen peroxide was used to produce the liver homogenate peroxidation (Yen and Hsieh, 1998). The reaction mixture containing 0.2 ml of all the three extracts (200–1000 µg/ ml) with 1.0 ml of 1% liver homogenate (each 100 ml homogenate solution contains 1.0 g rat liver), then 50 µl of ferric chloride (0.5 mM) and hydrogen peroxide (0.5 mM). The mixture was incubated at 37˚C for 60 min.

After that, 1.0 ml of thiobarbituric acid (0.67%) and trichloroacetic acid (15%) was added and heated in boiled water for 15 min. The absorbance was read at 532 nm. Ascorbic acid was used as the positive control. The percentage of lipid peroxidation effect was calculated according to formula (1).

Metal Chelating activity

The chelation of ferrous ions byhexane, ethyl acetate and methanol extract of Aristolochia bracteolata were estimated by the method of Dinis *et al.* (1994). The different concentrations of extract (200–1000 µg/ml) were added to 2 mM $FeCl_2$ (0.05 ml). Then it was added with 5 mMferrozine (0.2 ml), this mixture was shaken vigorously and left to stand at room temperature for 10 min. Absorbance of the solution was read at 562 nm. EDTA was used as a positive control. The percentage inhibition of ferrozine-Fe^{2+} complex formation was calculated using the formula (1).

Statistical analysis

The data for physiological and biochemical parameters were analyzed and expressed as meanof triplets of the same experiments ± SD. The IC_{50} values were calculated from linear regression analysis. Results were processed by Microsoft Excel (2007).

GC – MS analysis

Two micro liter of sample was injected into the gas chromatograph HP-6890(GC) equipped and coupled to a mass detector MS (HP 5972). Separation of compound was achieved using RTX volatile capillary column (60 m × 0.32 mm × 0.5 µ). Injection port temperature was ensured as 200^{o}C with 250^{o}C as transfer temperature. The oven temperature was elevated from 40^{0}C (2 min) to 220^{0}C at 15^0 C/min with final hold 5 min. The ionization voltage was 70eV. The carrier gas helium was passed through at a flow rate of 1ml/min. The compounds were identified with the help of NIST Libraries based on their molecular mass.

Interpretation of mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having many patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS

***In vitro* antioxidant assays**

Reducing power activity

Fig. 1(a) shows the reductive capabilities of hexane, ethyl acetate and methanol extracts of *Aristolochia bracteolata* compared to butylated hydroxytoluene. The reducing power of methanol extract was very potent when compared to hexane and ethyl acetate extracts and the reducing power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe^{3+} ions, which had a lesser reductive activity than the standard of butylatedhydroxy toluene.

DPPH radical scavenging assay

The methanol extract exhibited a significant dose dependent inhibition of DPPH activity compared to ethyl acetate and hexane extract with a 50% inhibition (IC_{50}) at a concentration of 270.12 ± 1.12, 350.01 ± 0.85 and 600.23 ± 1.20 µg/ml respectively. The results are presented in Fig. 1(b). The IC_{50} value of vitamin C was 220.11 ± 0.63 µg/ml.

Hydroxyl radical scavenging activity

The results for hydroxyl scavenging assay is shown in Fig.1(c). The concentrations for 50% inhibition for methanol, ethyl acetate and hexane extract were found to be 450.04 ± 0.71, 530.12 ± 1.98 and 690.22 ±1.16 µg/ml respectively. The IC_{50} value of vitamin C was 220.05 ± 0.97 µg/ml.

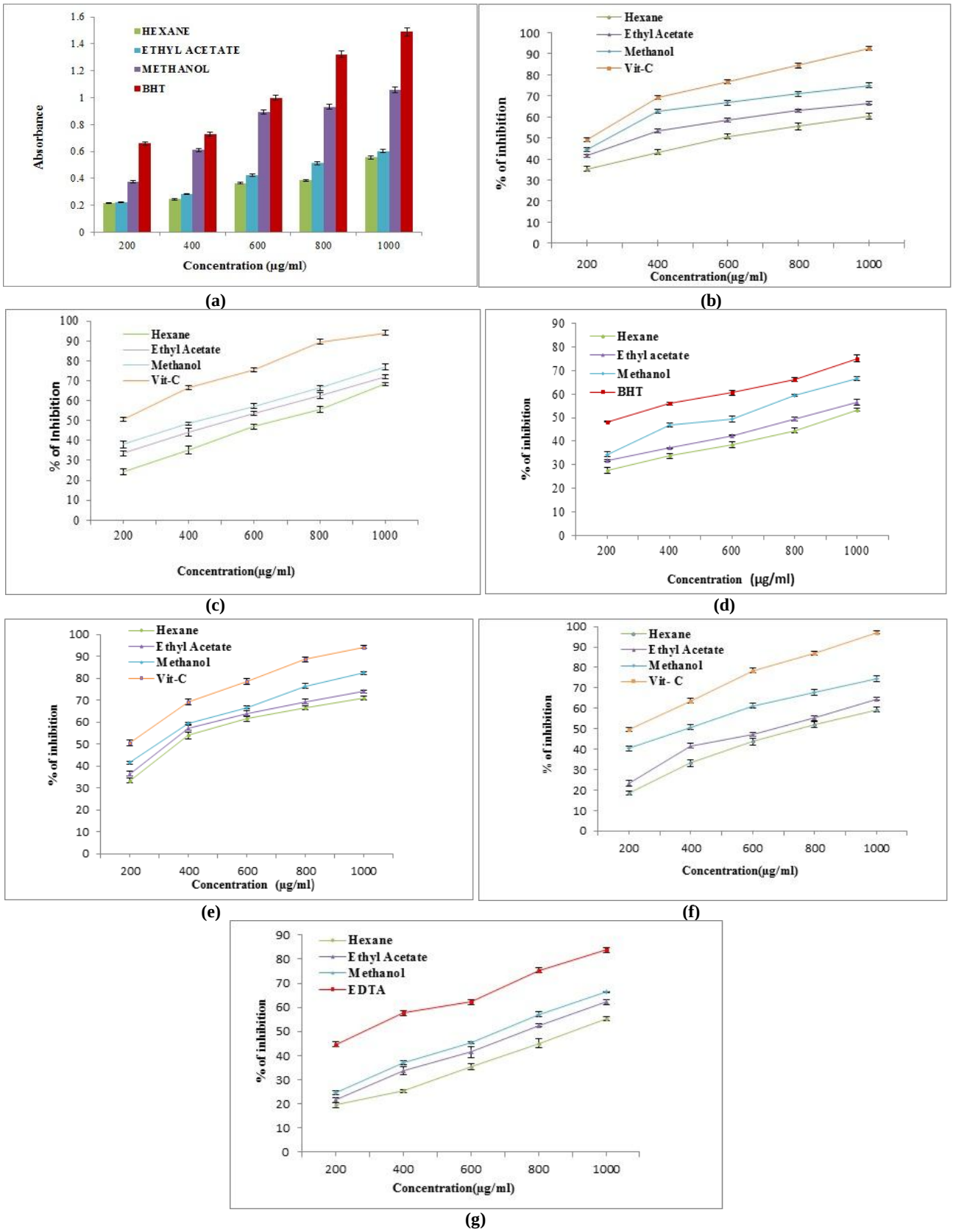

Fig. 1: (a) Reductive ability of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata* hexane, ethyl acetate, methanol extracts and BHT. **(b)** DPPH scavenging effect of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata* hexane, ethyl acetate, methanol extracts and vitamin C. **(c)** Hydroxyl radical scavenging effect of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata*hexane, ethyl acetate, methanol extracts and vitamin C. **(d)** Nitric oxide scavenging effect of different concentrations (200–1000 µg/ ml) of *Aristolochia bracteolata* hexane, ethyl acetate, methanol extracts and vitamin C. **(e)** Hydrogen peroxide scavenging effect of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata*hexane, ethyl acetate, methanol extracts and vitamin C. **(f)** Antilipid peroxidation effect of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata* hexane, ethyl acetate, methanol extracts and vitamin C. **(g)** Metal chelating effects of different concentrations (200–1000 µg/ml) of *Aristolochia bracteolata* hexane, ethyl acetate, methanol extracts and vitamin C. Each value represents the mean ± SEM of triplicate experiments.

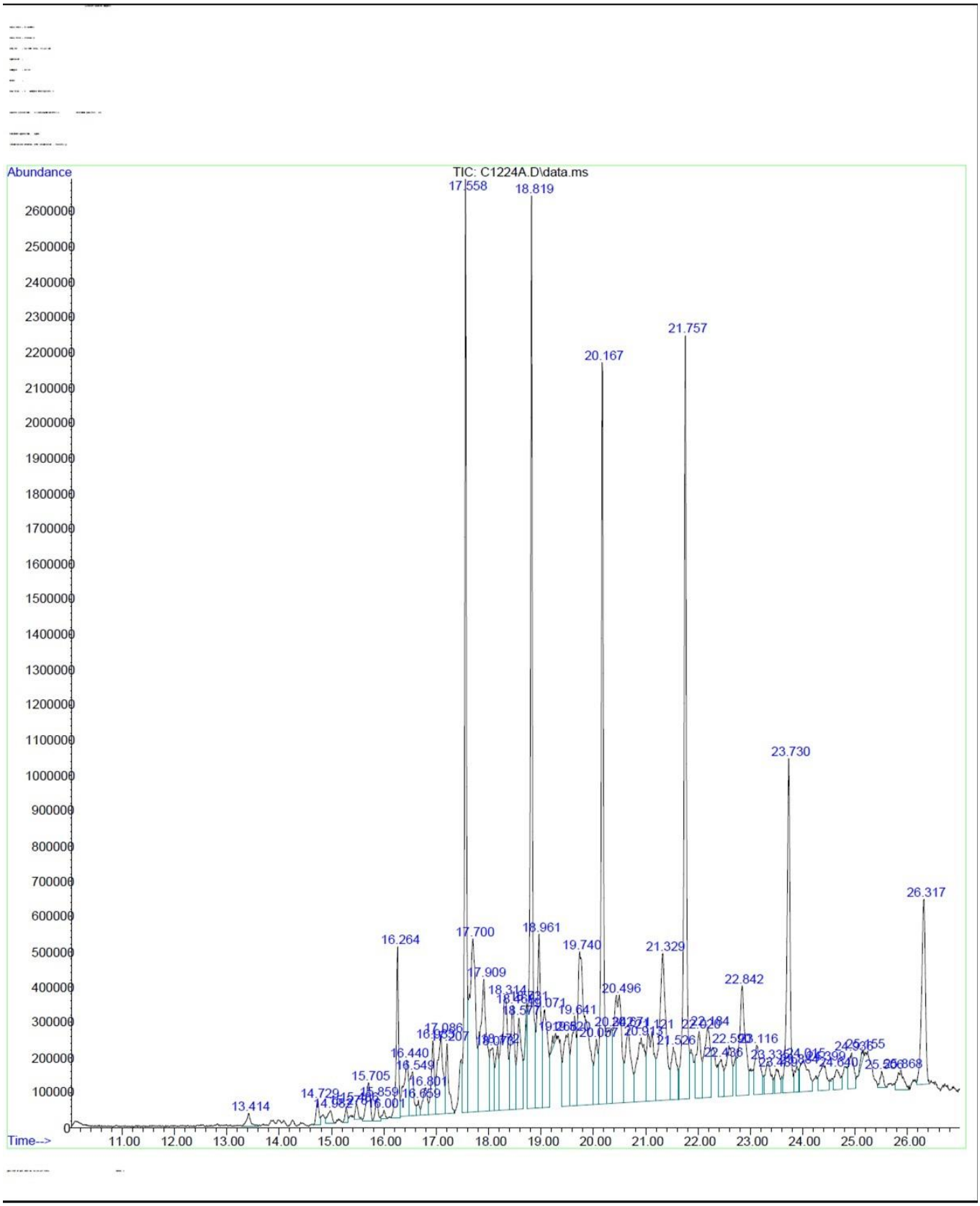

Fig 2: GC-MS analysis of *Aristolochia bracteolata*.

Nitric oxide scavenging activity

The scavenging of nitric oxide by methanol extract was increased in a dose-dependent manner as illustrated in Fig. 1(d). At concentration of 640.06 ± 1.24, 830.13 ± 0.92, 930.32 ± 0.81 µg/ml of methanol, ethyl acetate and hexane extract, 50% of nitric oxide generated during incubation was scavenged. The IC_{50} value of vitamin C was 250.15±0.23µg/ml.

Hydrogen peroxide scavenging activity

Hydrogen peroxide anion scavenging activities of all the three extracts are given in Fig. 1(e). The 50% of hydrogen peroxide anion radical generation was scavenged at the concentration of 300.51 ± 0.67, 330.23 ± 0.97 and 370.71 ± 1.25 µg/ml for methanol, ethyl acetate and hexane extracts respectively. The IC_{50} value of vitamin C was 200.02 ± 1.39 µg/ml.

Lipid peroxidation in rat liver homogenate

Activity of all the extracts on lipid peroxidation is shown in Fig. 1(f). The methanol, ethyl acetate and hexane extract showed 50 % inhibition of lipid peroxidation at the concentration of 400.01 ± 1.23, 680.17 ± 0.95 and 760.11 ± 1.55 µg/ml respectively. The IC_{50} value of BHT was 220.21 ± 1.16 µg/ml.

Metal Chelating activity

The activity of all the three extracts on chelating the ferrous ions is shown in Fig. 1(g). Methanol, ethyl acetate and hexane extract showed 50% of chelation generation at the concentration of 680.09 ± 0.33, 760.17 ± 0.57 and 900.54 ± 1.12 µg /ml respectively. The IC_{50} value of EDTA was 300.13 ± 1.15 µg/ml.

GC – MS analysis

The GC-MS analysis of methanolic extract clearly showed the presence of sixty phytoconstituents. The GC-MS chromatogram of the compounds with their peaks was shown in Fig. 2. The phytoconstituents with their retention time (RT), molecular weight, molecular formula (MW) and concentration (peak area%) are presented in Table 1.

Table 1: Phytocomponents identified in the *Aristolochia bracteolata*.

S. No.	Rt	Name Of The Phytocomponent	Peak Area
1.	13.414	Ethylene 1,2-dichloro	0.20
2.	14.729	Nonane	0.28
3.	14.982	p-Xylene	0.22
4.	15.278	Nonane, 3-methyl-	0.14
5.	15.486	Octane 4,5-dipropyl-	0.16
6.	15.705	Octane 2,3-dimethyl-	0.60
7.	15.859	Nonane 3-methyl-	0.25
8.	16.001	2-methyl-4-octenal	0.08
9.	16.264	Decane	1.49
10.	16.440	Benzene(1-methylethyl)	1.10
11.	16.549	Decane 4 –methyl-	0.65
12.	16.659	Butanoic acid, decyl ester	0.14
13.	16.801	Hexane 3,3-dimethyl-	0.48
14.	16.933	Benzene 1-ethyl-2-methyl-	0.91
15.	17.086	Propanoic acid, 3-mercapto-, 2-ethylhexylester	1.60
16.	17.207	Decane 3-methyl-	0.80
17.	17.558	Undecane	6.29
18.	17.700	Benzene 1,4-diethyl-	4.12
19.	17.909	1-Butene 1,4-dichloro-	3.25
20.	18.073	o-Cymene	0.89
21	18.172	Benzene 4-ethyl-1,2-dimethyl-	0.82
22.	18.314	Nonane, 5-(1-methylpropyl)-	2.72
23.	18.468	Decane, 1,1'-oxybis-	1.68
24.	18.577	3-Octyne	1.52
25.	18.731	1,3-Cyclopentadiene, 1,2,3,4-tetra	0.15
26.	18.819	Dodecane	7.50
27.	18.961	Undecane, 2,6-dimethyl-	2.54
28.	19.071	2-Piperidinone, N-[4-bromo-n-butyl]-	1.79
29.	19.268	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	0.13
30.	19.520	3-Octyne, 6-methyl-	1.65
31.	19.641	2-Isopropyl-5-methyl-1-heptanol	1.65
32.	19.740	Heptane, 2,6-dimethyl-	4.84
33.	20.057	Benzene, (3-chloro-1-methyl-1-propenyl)-	1.00
34.	20.167	Tridecane	6.59
35.	20.342	2,8-Decadiyne	1.38
36.	20.496	Diazoprogesterone	3.17
37.	20.671	5-Tetradecene, (E)-	1.67
38.	20.913	Oxalic acid, 3,5-difluorophenyl undecyl ester	1.88
39.	21.121	5-Butyl-5-ethylheptadecane	1.92
40.	21.329	Dodecane, 2,6,10-trimethyl-	3.57
41.	21.526	Stigmasterol	1.08
42.	21.757	Tetradecane	7.31
43.	22.020	1,5-Heptadiyne	1.14
44.	22.184	Benzene, 1-ethenyl-4-methyl-	1.63
45.	22.436	2-Cyclopenten-1-one, 3-bromo-	0.62
46.	22.590	Cyclododecanol, 1-ethenyl-	1.15
47.	22.842	Dodecane, 2,6,11-trimethyl-	2.52
48.	23.116	Butanoic acid, 4-(1,1-dimethylethoxy)-3-hydroxy-2-methyl-, ethyl ester, [r-(R*,S*)]-	1.03
49.	23.335	9-Methyltricyclo[4.2.1.1(2,5)]deca-3,7-diene-9,10-diol	0.70
50.	23.489	Cyclododecanol, 1-ethenyl-	0.44
51.	23.730	Pentadecane	3.99
52.	23.884	11-Tetradecyn-1-ol acetate	0.34
53.	24.015	Naphthalene, 1,2,3,4-tetrahydro-5, 6-dimethyl-	0.95
54.	24.399	9,12-Octadecadienoic acid (Z,Z)-,2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl ester	0.69
55.	24.640	Benzene, 2,5-cyclohexadien-1-yl-	0.45
56.	24.936	Dodecane, 2-methyl-	0.66
57.	25.155	Dispiro[2.2.2.0]octane, 4,5-cis-diphenyl-	0.14
58.	25.506	Sulfurous acid, butyl tetradecyl ester	0.20
59.	25.868	Citronellol	0.47
60.	26.317	Hexadecane	2.69

DISCUSSION

In the present study, three extracts of *Aristolochia bracteolata* were studied for *in vitro* antioxidant activity using standard methods. The methanol extracts have shown more antioxidant activity as compared to other two extracts. The variations in activity may be due to the fact that diversity in the basic chemical structure of phytoconstituents possesses different degree of antioxidant activity against different free radicals. Plant phenolics are known to exhibit potent antioxidant activity (Jaggi and Kapoor, 1999). Hence, the observed antioxidant activity of the extracts of *Aristolochia bracteolata* may be due to the presence of these constituents.

For the measurements of the reductive ability, we studied the Fe^{3+} to Fe^{2+} transformation in the presence of *Aristolochia*

bracteolata extracts, using the method of Oyaizu (Oyaizu, 1986). The reducing power increased with increasing concentration of the extract and thus methanol extract with increasing concentration of 1000 µg/ml shows absorbance at 1.05.The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Meir *et al.*,1995). In previous studies, *Aristolochia 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).

DPPH test is usually used to evaluate antioxidative activity of antioxidants Oyaizu (Oyaizu, 1986). This method is based on the formation of DPPH-H by the reaction due to reduction of alcoholic DPPH solution (Williams *et al*., 1995). In the present study, methanol extract proved to be a best DPPH scavenger (which reduces the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine) as compared to hexane and ethyl acetate extract. Hydroxyl radical scavenging capacity of an extract is directly related to its antioxidant activity(Babu *et al*., 2001).

Previous studies have shown that the plant *Aristolochia bracteolata* acted as a free radical scavenger against nitric oxide (Somashekar and Shirwaikar, 2003). In the present study, Methanol extract was found best to scavenge nitric oxide as compared to hexane and ethyl acetate extract. Nitric oxide plays an important role in various types of inflammatory processes in the animal body. Nitric oxide radical inhibition study showed that the extract was a potent scavenger of nitric oxide. The extract inhibited nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide competed with oxygen leading to reduced production of nitric oxide (Marcocci *et al*., 1994). In the PMS–NADH–NBT system, superoxide anion derived from the dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease in the absorbance at 560 nm with antioxidants thus indicates the consumption of the generated superoxide anion in the reaction.

Methanol extract inhibited free radical mediated deoxyribose damage remarkably. Lipid peroxidation is an oxidative alteration of polyunsaturated fatty acids in the cell membranes that generates a number of degradation products. Malondialdehyde (MDA), one of the products of lipid peroxidation, has been studied widely as an index of lipid peroxidation and as a marker of oxidative stress (Janero, 1990). Methanol extract showed a strong inhibition of lipid peroxidation.

The chelating of ferrous ions by the extract was estimated by the method of Dinis *et al*. (Dinis *et al*., 1994). Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complexes decreases. Measurement of the rate of color reduction therefore allows estimation of the chelating activity of the coexisting chelator (Yamaguchi *et al*., 2000). In our study methanol extract reveals effective capacity for iron binding, suggesting that action as an antioxidant may be related to its iron binding capacity.

In the present study, methanol extract was given for GC-MS analysis where it showed around sixty phytocomponents. Some of the major peaks correspond to decane, undecane, dodecane, tridecane, tetradecane, pentadecane, hexadecane etc whereas some useful phyto components with medicinal applications comprising minor peaks were stigmasterol, 2-piperidinone, cymene etc. Rests of the phytocomponents are also enlisted in table 1. The gas chromatography shows various heights of the peak which indicate the relative concentrations of the components present in the plant. The nature and structure of the compounds are identified by using the mass spectrometer. It also analyzes the compounds which are eluted at different times. These mass spectra act as fingerprint of that compound which is identified from the data library. In a previous study, 13 compounds were identified by GC-MS analysis from *Aristolochia krysagathra* (Jegadeeswari *et al*., 2012).

CONCLUSION

From the above results, it is concluded that the methanol extract of *Aristolochia bracteolate* has significant and promising antioxidant activity. GC-MS analysis is the first step towards understanding the nature of active principles in this medicinal plant. The methanol extract of this plant showed presence of sixty phyto components with various applications which merits further investigation for isolation of individual phytochemical constituents which may pave a way for finding a novel drug.

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