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Free radical scavenging activity of aqueous root extract of *Argyreia nervosa* (Burm.f.) Boj. (Convolvulaceae)

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Abstract

Aqueous extract of *Argyreia nervosa*, which is widely used in the indigenous system of medicine, was studied for its *in vitro* scavenging activity by different methods viz. DPPH radical scavenging, ABTS radical scavenging, lipid peroxidation, iron chelating activity, superoxide scavenging, total antioxidant capacity, Nitric oxide scavenging and Non-enzymatic Glycosylation of Haemoglobin assay. The results were analyzed statistically by regression method. Its antioxidant activity was estimated by IC_{50} value and the values are 88.86 µg/ml for DPPH radical scavenging, 66.64 µg/ml for ABTS radical scavenging, 31.57 µg/ml for lipid peroxidation, 8.0 µg/ml for iron chelating activity, 113.20 µg/ml for Nitric oxide scavenging and 7.51 µg/ml for superoxide scavenging. Total antioxidant capacity was found to be 22.0 µg/ml. In Haemoglobin glycosylation the % scavenging was found to be 43.77% and 64.42% for concentration 0.5 mg/ml and 1.0 mg/ml respectively. In all the methods, the extract showed its ability to scavenge free radicals in a concentration dependent manner. The results indicate that *Argyreia nervosa* has significant antioxidant activity.

Keywords: *Argyreia nervosa*, ABTS, Haemoglobin glycosylation, superoxide scavenging, lipid peroxidation.

1. Introduction

Though oxygen is essential for the aerobic process, cells under aerobic condition are threatened with the insult of reactive oxygen metabolites, a threat which is efficiently taken care of by the powerful antioxidant system in human body. Aerobic life is characterized as the continuous production of free radicals balanced by an equivalent synthesis of antioxidants. The free radicals are capable of independent existence and cause oxidative tissue damage. The non-radical oxidants like hydrogen peroxide and hypochlorous acid, which do not possess unpaired electrons, are also capable of inciting oxidative tissue damage. The improper balance between reactive oxygen metabolite production and antioxidant defence result in "oxidative stress",

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which deregulates the cellular function leading to various pathological conditions. Antioxidant principles from natural sources possess multifacetedness in their multitude of activities and provide enormous scope in correcting the imbalance. Therefore, much attention is being directed to harness and harvest the antioxidant principles from natural sources [1].

Argyreia nervosa, belonging to family Convolvulaceae, is a very large climbing shrub with woody, white, tomentose stems; leaves simple, large ovate, acute, base cordate, glabrous above, white tomentose beneath; flowers large, purple, silky-pubscent without in long penduncled cymes, corolla tubular-infundiulifom; fruits dry, globose, apiculate. It is distributed throughout India, in areas upto 900 m elevation [2]. The roots are acrid, bitter, astringent, sweet, emollient, thermogenic, appetiser, digestive, carminative, aperient, cardiotonic, anti-inflammatory, expectorant, diuretic, aphrodisiac, rejuvenating, intellect promoting, brain tonic and nervine tonic [2].

2. Materials and methods

2.1 Chemicals and Instruments

All chemicals and solvents used in the study were analytical grade. DPPH (1, 1- Diphenyl-2-Picryl Hydrazyl) and ABTS (2, 2-Azino bis (3-ethyl Benzo Thiazoline-6-Sulphonic acid) were obtained from Sigma Chemicals (St.louis, Mo, USA). Sodium nitroprusside, ferrous sulphate, trichloroacetic acid, dimethyl sulphoxide (DMSO), ethylene diamine tetra acetic acid (EDTA), sodium hydroxide, potassium chloride and sulphanilamide were obtained from Ranbaxy Fine Chemicals Ltd. India. NBT (Nitro blue tetrazolium chloride) and thiobarbituric acid were obtained from Himedia Laboratories Ltd. Mumbai, India. UV spectrophotometer (Shimadzu 1650 pc), Micro plate reader (Biotek Instruments, ELx 800 and pH meter (Elico Ltd., India) were the instruments used for the study.

2.2 Plant Material

The roots of *Argyreia nervosa* were collected at the river side of Manipal, Udupi district, Karnataka, India in November 2007 and was authenticated by Dr. Gopalakrishna Bhat, Professor, Department of Botany, Poorna Prajna College, Udupi, Karnataka, India. A voucher specimen has been deposited at the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India.

2.3 Preparation of Aqueous extract

500 g of the root powder was taken and extracted with chloroform water (1:1000) by maceration. The extract evaporated under vacuum gave a dry extract (yield 40 g) and was stored in a desiccator for future use.

2.4 Preparation of Argyreia nervosa stock solution

Argyreia nervosa stock solution was prepared in distilled water in the concentration of 1000 µg/ml. From this stock solution, different concentration viz. 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/ml were prepared using distilled water and used for antioxidant studies.

2.5 Preparation of stock solution (Standard drugs)

Ascorbic acid was used as the standard. Ascorbic acid stock solution was prepared by dissolving the sample in distilled water to get concentration of 1000 µg/ml. From the stock solution, various dilutions viz 2, 4, 8, 16, 32, 64, 128, 256, 512 and 1000 µg/ml were prepared in distilled water and used for antioxidant studies. Vitamin E (Tocopherol) was used as standard for Antioxidant haemoglobin glycosylation method and lipid peroxidation assay. The stock solution and various dilutions were prepared in methanol in similar manner as stated in ascorbic acid.

Table 1. Comparison of IC_{50} values of extract with standard

Sl. No.	Model	IC_{50} value of aqueous extract (µg/ml)	IC_{50} value of ascorbic acid (µg/ml)
1.	DPPH radical scavenging activity	88.86	9.9982
2.	ABTS radical scavenging activity	63.64	36.42
3.	Iron chelating activity	8.0	2.017
4.	Lipid peroxidation assay	31.57	30.67
5.	Nitric oxide radical scavenging activity	113.20	38.68
6.	Superoxide scavenging activity	7.51	15.79
7.	Total antioxidant capacity	10 mg/ml aqueous extract of *Argyreia nervosa* is equivalent to 22 µg/ml of ascorbic acid.	

Table 2. Antioxidant study by Non-enzymatic haemoglobin glycosylation assay

Sl.No.	Extract/ standard	Concentration (µg/ml)	% scavenging
1	Aqueous extract	0.5	43.77
		1	64.42
2	Vitamin -E	0.5	61.53
		1	86.68

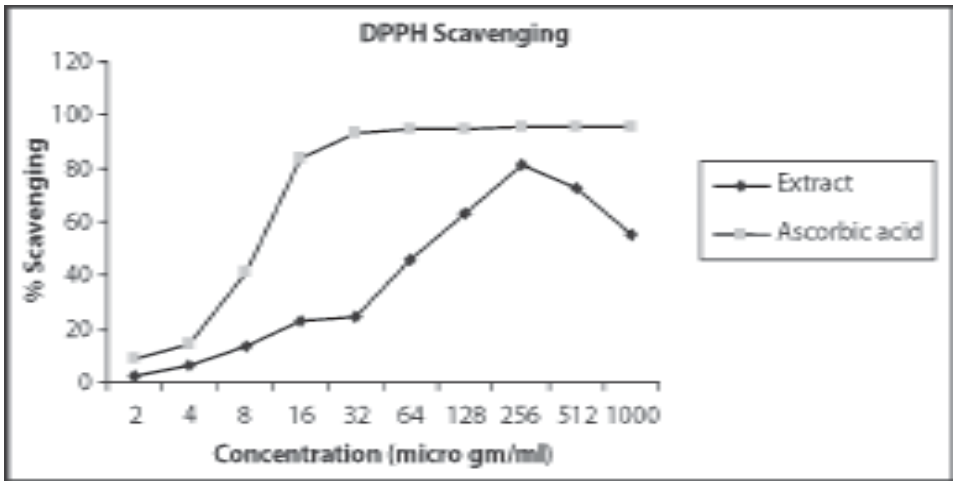

Fig. 1. DPPH radical scavenging activity of different concentrations of *Argyreia nervosa* and ascorbic acid. Each value represents mean ± S.E.M.

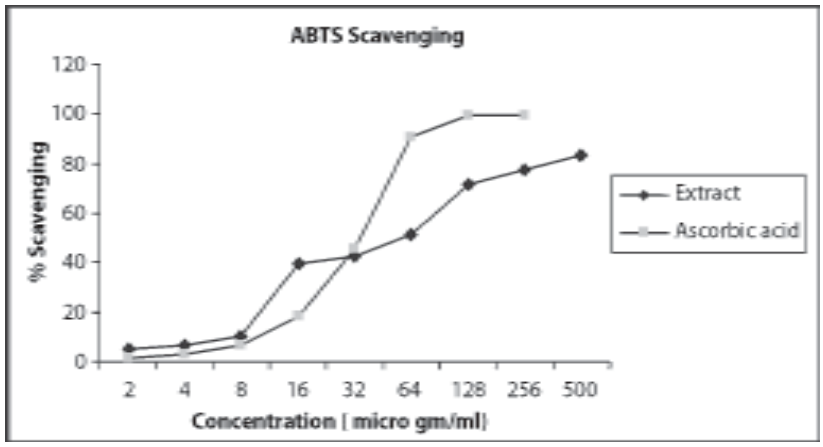

Fig.2. ABTS radical scavenging activity of different concentrations of *Argyreia nervosa* and ascorbic acid. Each value represents mean ± S.E.M.

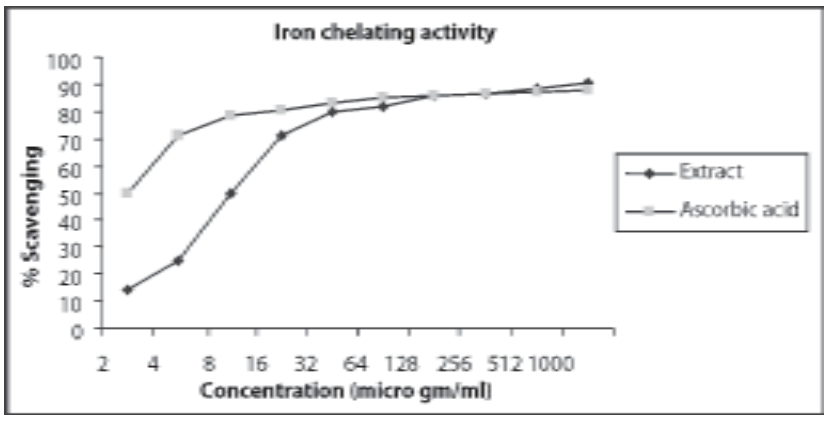

Fig.3. Iron chelating activity of different concentrations of *Argyreia nervosa* and ascorbic acid. Each value represents mean ±S.E.M.

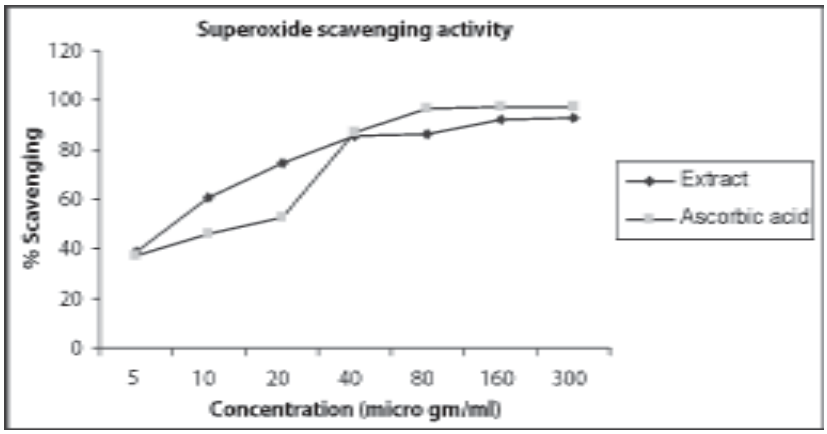

Fig.4. Superoxide scavenging activity of different concentrations of *Argyreia nervosa* and ascorbic acid. Each value represents mean ±S.E.M.

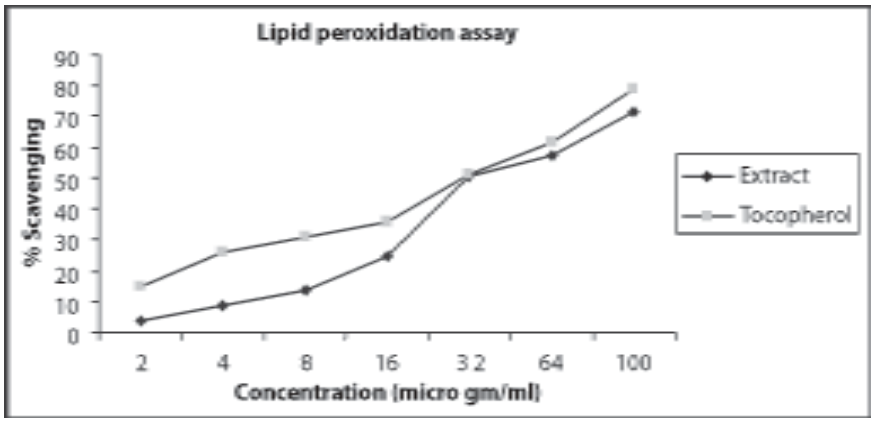

Fig.5. Lipid peroxidation assay of different concentrations of *Argyreia nervosa* and tocopherol. Each value represents mean ±S.E.M.

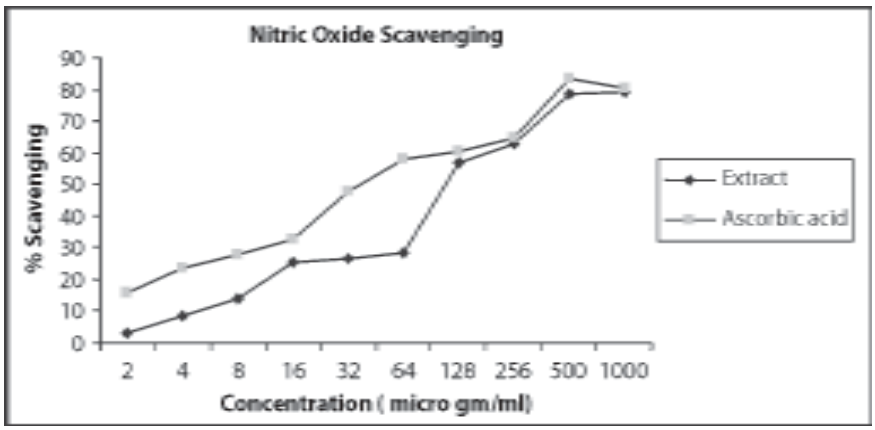

Fig.6. Nitric oxide scavenging activity of different concentrations of *Argyreia nervosa* and ascorbic acid. Each value represents mean ±S.E.M.

2.6 Antioxidant in vitro methods

2.6.1 DPPH Radical Scavenging Activity

To 1 ml of various concentrations of aqueous extract, 1 ml of solution of DPPH 0.1 mM (0.39 mg in 10 ml methanol) was added. An equal amount of water and DPPH was added to the control. Ascorbic acid was used as the standard for comparison. After 20 minutes incubation in the dark, absorbance was recorded at 517 nm. Experiment was performed in triplicate (Fig.1). [3, 4].

2.6.2 ABTS Radical Scavenging activity

ABTS radical cation preparation: ABTS 2 mM (0.0548 g in 50 ml) was prepared in distilled water. Potassium per sulphate 70 mM (0.0189 g in 1 ml) was prepared in distilled water. 200 ml of Potassium per sulphate and 50 ml of ABTS were mixed and used after 2 hrs. This solution is called as ABTS radical cation, which was used for the assay. To the 0.5 ml of various concentration of extract, 0.3 ml of ABTS radical cation and 1.7 ml of Phosphate buffer, pH 7.4

was added. For control, instead of extract water was taken. The absorbance was measured at 734 nm. The experiment was performed in triplicate (Fig.2) [5, 6].

2.6.3 Iron Chelating Activity

The reaction mixture containing 1 ml Ortho-Phenanthroline (0.005 g in 10 ml methanol), 2 ml ferric chloride 200 mM (3.24 mg in 100 ml distilled water) & 2 ml of various concentrations of the extract. The mixture was incubated at ambient temperature for 10 min, and then the absorbance of the same was measured at 510 nm. The experiment was performed in triplicate (Fig.3) [7, 8,].

2.6.4 Superoxide Scavenging Activity

To the 0.5 ml of different concentration of extract, 1 ml alkaline DMSO and 0.2 ml NBT 20 mM (50 mg in 10ml phosphate buffer pH 7.4) was added. The absorbance was measured at 560 nm. The experiment was performed in triplicate (Fig.4) [9].

2.6.5 Lipid Peroxidation Assay

Phospatidylcholine (20 mg) in chloroform (2 ml) was dried under vacuum in a rotary evaporator to give a thin homogenous film and further dispersed in normal saline (5 mL) with a vortex mixer. Lipid peroxidation was initiated by adding 0.05 mM ascorbic acid into a mixture containing liposome (0.1 mL), 150 mM potassium chloride, 0.2 mM ferric chloride, extract (2 to100 µg/ml). The reaction mixture was incubated at 37°C for 40 min. After incubation, the reaction was stopped by adding 1 ml of ice-cold 0.25 M sodium hydroxide containing 20 % TCA (w/v), 0.4 % TBA (w/v), and 0.05 % BHT (w/v). After incubating in a boiling water bath for 20 min, the samples were cooled to room temperature. The blank was prepared in the same manner, but without extract. The pink chromogen was extracted with 1mL of methanol. The absorbance was read at 532 nm (Fig. 5) [10].

2.6.6 Nitric oxide Radical Scavenging Activity

Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4. To 1 ml of various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25°C for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylene diamine was read at 546 nm. The experiment was performed in triplicate (Fig.6) [11, 12].

2.6.7 Total Antioxidant Capacity

0.1 ml of extract (10 mg/ml) dissolved in water was combined in eppendorf tube with 1 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated at 95°C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm. The antioxidant activity was expressed as the number of equivalents of ascorbic acid [13, 14].

2.6.8 Non-enzymatic Glycosylation of Haemoglobin Assay

The antioxidant activity of extract was investigated by estimating degree of non-enzymatic haemoglobin glycosylation, measured colorimetrically. The assay was performed by adding 1 ml of glucose solution, 1 ml of haemoglobin solution, and 1 ml of gentamycin (20 mg/ 100 ml), in 0.01 M phosphate buffer (pH 7.4). The mixture was incubated in dark at room temperature for 72 h. The degree of glycosylation of haemoglobin in the presence of different concentration of extract and their absence were measured colorimetrically at 520 nm (Table 2) [15].

2.7 Statistical Analysis

All results are expressed as mean ± S.E.M. Linear regression (Origin 6.0 version) analysis was used to calculate the IC_{50} values.

3. Results

Concentrations ranging from 2-1000 µg/ml of the aqueous extract of *Argyreia nervosa* were tested for their antioxidant activity in different *in vitro* models. It was observed that free radicals were scavenged by the extract in concentration dependent manner up to the given concentration in all the models. The percentage scavenging and IC_{50} values (Table 1) were calculated for all the models. In DPPH method, the maximum scavenging activity was found at a concentration 256 µg/ml and the minimum scavenging activity at a concentration of 2 µg/ml. IC_{50} value was found to be 88.86 µg/ml. In ABTS scavenging method, maximum activity was exhibited at 500 µg/ml with 83.37% scavenging. Iron chelating activity, maximum scavenging was found at 1000 µg/ml with IC_{50} of 8.0 µg/ml. In superoxide scavenging, the maximum scavenging activity was observed at 300 µg/ml with scavenging of 93.09%. IC_{50} value for nitric oxide scavenging model was found to be 113.20 µg/ml Maximum scavenging activity was observed at100 µg/ml with an IC_{50} value of 31.57 µg/ml in lipid peroxidation. Antioxidant haemoglobin glycosylation, the percentage scavenging was found to be 43.77% and 64.42% for concentration 0.5 mg/ml and 1.0 mg/ml of extract respectively. Total antioxidant capacity of aqueous extract of *Argyreia nervosa* (10 mg/ml) is equivalent to 22.0 µg/ml of ascorbic acid.

4. Discussion

There is an extensive evidence to implicate free radicals in the development of degenerative diseases such as diabetes, liver cirrhosis, nephrotoxicity, cancer etc. Reactive oxygen species such as superoxide anions, hydroxyl radical, and nitric oxide inactivate enzymes and damage important cellular components causing tissue injury through covalent binding and lipid peroxidation [16-18]. DPPH is a stable free radical. When antioxidant reacts with this stable radical, the electron becomes paired off and bleaching of the colour stochiometrically depends on the number of electrons taken up [19-20].

O-phenonthroline quantitatively forms complexes with Fe^{2+}, which get disrupted in the presence of chelating agents. The aqueous extract interfered with the formation of ferrous-o-phenonthroline complex, thereby suggesting that the extract has metal chelating activity. [21-25].

The antioxidant activity of the extract by ABTS radical assay implies that the action may be either inhibiting or scavenging radicals since both inhibition and scavenging properties of antioxidant towards this radical have been reported in earlier studies [26].

In the Nitric oxide assay, the *Argyreia nervosa* extract scavenged the free radicals produced *in vitro* by using sodium nitroprusside. This may be due to the antioxidant principles in the extract, which compete with oxygen to react with nitric oxide [27-28]. Superoxide is a highly reactive molecule that can cause oxidation or reduction of solutes depending on their reduction potential. In our study, alkaline DMSO used for superoxide generation indicates that *Argyreia nervosa* is a potent superoxide scavenger [29].

Malondialdehyde and other aldehydes have been identified as a product of lipid peroxidation that reacts with the thiobarbituric acid to give pink coloured species. The decrease in the concentration of the malondialdehyde level with the increase in the concentration of *Argyreia nervosa* extract indicate the antioxidant role of the extract [30]. Total antioxidant capacity of the extract was calculated based on the

formation of phosphomolybdenum complex [31]. With the above experiment, it is evident that the plant *Argyreia nervosa* possess significant scavenging activity.

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