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Short Communication

Antioxidant and Cytotoxic Properties of the Methanol Extract of *Argyreia argentea* Stem

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Abstract

The antioxidant potential of *Argyreia argentea* methanol *stem* extract was evaluated by 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method. The extract showed very significant effect as antioxidant in comparison to ascorbic acid, a reference antioxidant. The radical scavenging activity showed by the extract was a dose dependent phenomenon. The extract at the concentration of 400 µg/ml among five different concentrations (10, 50, 100, 200, 400 µg/ml) showed the highest scavenging activity, 94.86%. The stem extract was also evaluated for its cytotoxic properties by measuring significant LC50 value in a Brine-Shrimp lethality bioassay. LC50 value of *Argyreia argentea* stem extract was found 939.18 µg/ml, at a 95% confidence limit, giving the lower and upper limits 608.98 and 2043.9 µg/ml, respectively.

Keywords: *Argyreia argentea;* DPPH; Ascorbic acid; Scavenging activity; Gallic acid.

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1. Introduction

Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions [1], that damage living cells, spoil foods, degrade materials such as rubber, gasoline, lubricating oil. Antioxidants terminate these chain reactions through the removal of free radical intermediates and inhibition of other oxidation reactions [2]. This is why plants and animals maintain complex systems of multiple antioxidants, such as glutathione, vitamin C, and vitamin E along with some enzymes like catalase, superoxide dismutase and various peroxidases. The use of antioxidants in pharmacology is intensively

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studied as oxidative stress might be an important part of many human diseases particularly stroke and neurodegenerative incidents [1]. Antioxidants, therefore, are routinely added to meals, oils, foodstuffs, and other materials to prevent free radical damage. 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. A lot of new plant species have been investigated in the search for novel antioxidants [3-6] other than well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices [7] but there is still a demand to find more information on the antioxidant potential of plant species. Cytotoxicity usually gives a preliminary idea on the anticancer effect of plant extract containing bioactive compounds those are toxic to living body at higher doses and pharmacologically beneficial at lower doses.

Argyreia argentea belonging to the Convolvulaceae family is an evergreen shrub that is mainly found in Chittagong, Jessore, Mymensingh, Noakhali, Sylhet and Tangail of Bangladesh and it is widely distributed in Eastern India, Bhutan and Nepal [8]. It has been used by the tribes of Chittagong Hill tracts in the treatment of various diseases. The plant is locally known as Bitarak Rupar tola ludi by Chakma and Kajinganj, Naiprabong by Marma of Bangladeshi tribal communities. The plant grows in secondary forest and scrub jungles. It gives flower and fruit during July to October. The plant is used in the treatment of Boils, Gastric, Tumour, Marasmus, Paralysis and Spermaforrhoea [8]. Its roots and leaves are used for the treatment of nervous disorder, skin infection, gonorrhea and aphrodisiac. Despite enormous local and traditional uses, *Argyreia argentea* (*A. argentea*) has not been evaluated and analyzed to be documented well. This study interests to evaluate the antioxidant and cytotoxic activity of the crude methanol extract of *A. argentea*.

2. Materials and Methods

Collection of plant

The stems of *A. argentea* were collected from Chittagong Hill tracts, Bangladesh, in the month of January 2009. The plant was taxonomically identified and authenticated by Bangladesh National Herbarium, Mirpur, Dhaka. The specimen is preserved in Bangladesh National Herbarium under the Plant Accession No. 34198.

Preparation of plant extract

The fresh stems of *A. argentea* were washed with distilled water immediately after collection. The collected stems were chopped into small pieces, air dried at room temperature for about 20 days and ground into powder to store in an airtight container. 790 gm powder was macerated in 8 L pure methanol (99% Anal-R) for 7 days at room temperature with occasional stirring. After 7 days, methanol extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. The extract was concentrated under reduced pressure below 50^0C through rotatory vaccum evaporator. The

concentrated extracts were collected in an Eggplant Flask and allow it to air dry for complete evaporation. The whole process was repeated three times and finally 15 gm of greenish extract was obtained (1.89 % w/w) which was preserved at 4^0C.

Preparation of extract solution

A 200 mg of extract was dissolved in 2 ml of methanol to give solution of known concentration (100µg/µl). Methanol was chosen as solvent because of high solubility of crude extract in methanol as well as no inhibitory effect on the cultures.

Assay for Antioxidant Activity

The antioxidant activity of *A. argentea* extract was assessed in comparison to standard antioxidant ascorbic acid (BDH, England) depending on the scavenging effect of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The whole procedure was administrated according to established procedure by Braca *et al.* [9]. Ascorbic acid solution (1ml) and different concentrations of extract (10, 50, 100, 200, 400 µg/ml) solution (1ml) were mixed with 3 ml of 0.4 mM DPPH solution. The mixtures were kept in dark for 30 minutes to measure the absorbance at 517 nm using UV-Visible Spectrophotometer (Cintra, Australia) and ascorbic acid was used as a positive control. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. The degree of decolorization of DPPH from purple to yellow indicated the scavenging efficiency of the extract. The scavenging activity against DPPH was calculated using the following equation:

$$\text{Scavenging activity (\%)} = [(A - B) / A] \times 100$$

where *A* is absorbance of control (DPPH solution without the sample), *B* is the absorbance of DPPH solution in the presence of the sample (extract/ ascorbic acid).

The scavenging activity (%) or % inhibition was then plotted against log concentration and from the graph IC50 (Inhibition concentration 50) value was calculated by linear regression analysis.

Assay for cytotoxicity

Cytotoxic activity of plant extract was determined by Brine-Shrimp lethality bioassay as described by Meyer *et al.* [10]. Shrimp eggs were added to the artificial "sea water" (25g salt per liter water) in the larger compartment of an unequally dividend tank which was darkened by covering it with Aluminum foil [11]. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam. 20 shrimp larvae were added to 5 ml of sea water in 5 test tubes and 1000, 800, 600, 400, 200,100, 50, 25µg/ml solutions of extracts, prepared from 500mg of crude through serial dilution, were added to these test tubes. Each concentration was tested in triplicate. A

control containing 5 ml of DMSO solvent was used for each solvent. The test tubes were maintained under illumination. After 24 hours have elapsed, survivors were counted with the aid of a 3X magnifying glass. From the % lethality of brine shrimp, the probits were calculated for each concentration by using computer software "BioStat-2007". Probits were then plotted against corresponding log concentration of stem extract to get LC50 (lethal concentration 50) value through regression analysis.

Phytochemical analysis

Alkaloid test: Crude methanol extract of *A. argentea* stem was subjected to analyze for a qualitative screening of the occurrence and existence of alkaloid in the plant. Extract (0.5 g) was neutralized by adding 1 or 2 drop of dilute H_2SO_4. The resulting solution was treated with a very small amount of Mayer's reagent (potassiomercuric iodide solution), Wagner's reagent (Iodopotassium iodide) and Hager's reagent (1% picric acid solution). The color of precipitates formed in each case was noted.
Flavonoid test: To a small amount of the extracts, a few drops of concentrated hydrochloric acid were added and immediate color development was observed keenly.

3. Results and Discussion

The DPPH free radical scavenging activity of the *A. argentea* stem extract and ascorbic acid is shown in Table 1. Stem extract showed higher free radical scavenging effect compared to the standard antioxidant, ascorbic acid. Among eight different concentrations used in the study (10 to 1000 µg/ml) stem extract showed highest scavenging activity 95.96% at the concentration of 1000 µg/ml. On the other hand, ascorbic acid showed 50.75%, 73.62%, 84.27%, 90.13%, 92.52%, 94.52%, 97.07% and 98.66% activity at 10, 50,100, 200, 400, 600, 800, 1000 µg/ml, respectively (Tables 1 and 2).

Table 1. DPPH free radical scavenging activity of *A. argentea* extract and ascorbic acid.

Conc. (µg/ml)	log conc.	Absorbance		% scavenging activity		IC50 µg/ml	
		Plant extract	Ascorbic acid	Plant extract	Ascorbic acid	Plant extract	Ascorbic acid
Control	-	0.8146	0.8146	-	-		
10	1	0.3696	0.4012	54.63	50.74	3.9	7.6
50	1.7	0.1297	0.2149	84.08	73.61		
100	2	0.072	0.12815	91.16	84.26		
200	2.3	0.057	0.0804	93.00	90.13		
400	2.6	0.0419	0.0609	94.86	92.52		

Table 2. Brine shrimp cytotoxicity of methanol extract of *A. argentea.*

Dose (µg/ml)	Log dose	Total	Alive	Death	% lethality	Actual %	Probit
25	1.40	20	20	0	0.0	0.01	2.442
50	1.70	20	19	1	5.0	0.05	3.365
100	2.00	20	18	2	10.0	0.10	3.720
00	2.30	20	15	5	25.0	0.25	4.353
400	2.60	20	15	5	25.0	0.25	4.333
600	2.78	20	14	6	30.0	0.30	4.487
800	2.90	20	11	9	45.0	0.450	4.874
1000	3.00	20	8	12	60.0	0.60	5.251

Here, Actual percent and probit were calculated using statistical software "Biostat 2007"

*Actual % = Actual formulas (*n* is the number of animals in a group): For the 0% dead, 100 (0.25/*n*), for the 100% dead, 100 (*n*- 0.25) /*n*

Percent (%) scavenging activity or % inhibition was plotted against log concentration and from the graph IC50 (inhibition concentration 50) value was calculated by linear regression analysis (Fig. 1). Regression analysis from the plot of (%) scavenging activity against log concentration showed that the IC50 value of ascorbic acid and stem extract was found 7.6 µg/ml and 3.9 µg/ml, respectively (Fig. 1).

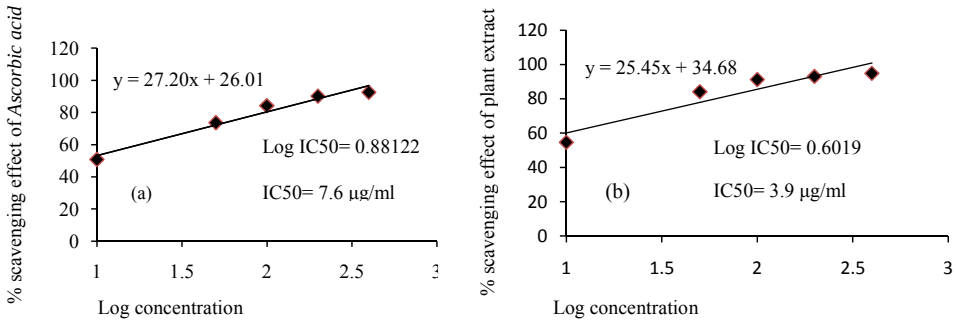

Fig. 1. Comparative IC50 values of reference antioxidant and studied plant *A.argentea* stem extract: (a) ascorbic acid, (b) plant extract.

A simple method utilizing the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical has been developed to determine the antioxidant activity of natural products. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. That turns to yellow as the molar absorption of the DPPH radical at 517 nm reduces from 9660 to 1640 when the odd electron of DPPH radical becomes paired

with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric with respect to number of electrons captured. IC50 value denotes the concentration of sample required to scavenge 50% of the DPPH free radical [12].

The IC50 value obtained for *A. argentea* stem extract and ascorbic acid (Fig. 1) indicates that *A. argentea* stem extract possess efficiency to neutralize free radicals higher than ascorbic acid (Fig. 2). Different research suggests that most of the plant extracts showing antioxidant activity are due to the presence of phenolic compounds [13, 14]. Phenolic natural compounds such as flavonoids (Table 4) possess antioxidant activity due to their redox properties which allow them to act as reducing agents and singlet oxygen quencher. In addition, they have metal chelating potentials [15]. The phenolic compounds, identified in the extract might contribute to the antioxidant activity of *A. argentea* stem extract.

Table 4. Observation on phytochemical group tests.

Test	Reagent	Change of colors	Indication
Alkaloid	Mayer's reagent	White or creamy white precipitate	Alkaloid present
	Wagner's reagent	Brown or deep brown precipitate	"
	Hager's reagent	yellow crystalline precipitate	"
Flavonoid	Conc. HCl	Development of color	Flavonoid present

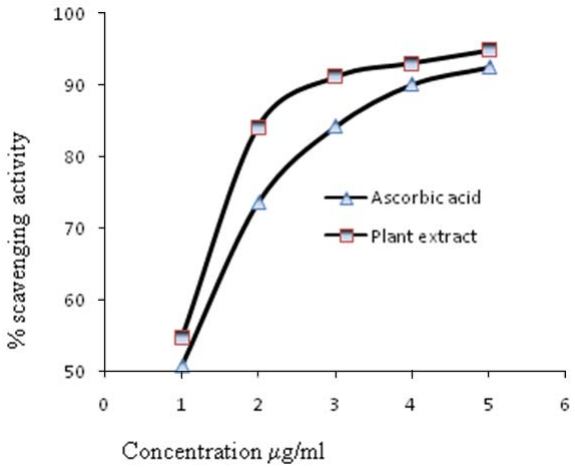

Fig. 2. Comparative % scavenging activities of *A. argentea* stem extract and ascorbic acid.

Assay of cytotoxic activity in Brine Shrimp Lethality Bioassay, percent mortality of brine shrimp at five different concentrations (10 to 200 µg/ml) of *A. argentea* stem extract has been presented in Table 2. Extract showed lethality in a dose dependent manner. More specifically, 0.01 %, 0.05 %, 0.10 %, 0.25 %, 0.25 %, 0.30 %, 0.450 % and 0.60 % mortality was observed at concentration of 25, 50, 100, 200, 400, 600, 800, 1000 µg/ml, respectively (Table 2). From the % lethality of brine shrimp, probits were plotted against corresponding log concentration of stem extract (Fig. 3). From this plot LC_{50} (lethal concentration 50) value was calculated by regression analysis (Table 3). LC_{50} value of *A. argentea* stem extract was found 939.18µg/ml at 95% confidence limit where the lower and upper limits were 608.98 and 2043.9µg/ml (Table 3). Brine shrimp lethality assay [10] is an indication of cytotoxicity, antibacterial activities, pesticidal effects and various pharmacologic actions [16]. In cytotoxicity, the LC_{50} value obtained for *A. argentea* indicates that the extract has high pharmacological actions [17]. It also indicates that the plant might have the potentiality to kill cancer cells [16].

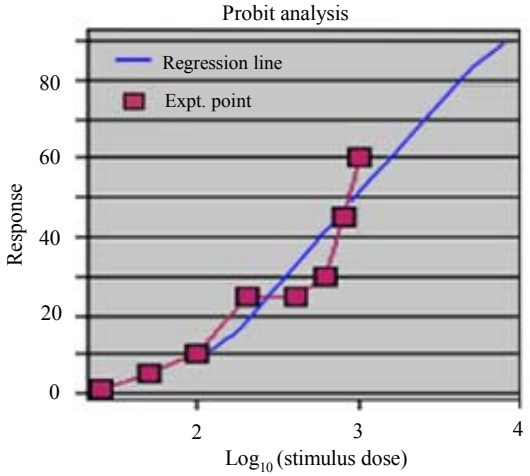

Fig. 3. Regression line for determining the LC50 value of methanol extract of *A. argentea.*

Qualitative secondary metabolite tests (Table 4) showed that *A. argentea* possess both alkaloid and flavonoids. Plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack. Many of these compounds have been used in the form of whole plants or plant extracts for food or medical applications in man [18] because plants are the natural reservoir of many antimicrobial, anticancer agents, analgesics, anti-diarrheal, antifungal as well as various therapeutic activities [19]. Presence of such alkaloid and flavonoids might have some role in showing antioxidant and cytotoxic properties of the *A. argentea.*

4. Conclusion

The results of the study demonstrate that the methanol extract of *A. argentea stem* exhibits very potential antioxidant and cytotoxic effect in experimental models which support the claims by traditional medicine practitioners. These results can be strong scientific evidence to use this plant as a useful source of antioxidant references. However, further studies are still necessary to elucidate a mechanistic way how the plant contributes in these pharmacologic properties. Phytochemical investigation is also proposed in order to isolate the active fraction and eventually the pure compound.

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