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RESEARCH ARTICLE

ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *CASSIA HIRSUTA* (L.) LEAVES

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The antioxidant activity of ethanolic extract of *Cassia hirsuta* (Family-*Caesalpiniaceae*) leaves has been investigated in the present study. The antioxidant activity of the *Cassia hirsuta* leaves ethanolic extract was assessed by DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging method. Extract showed significant DPPH free radical scavenging effect compared with standard antioxidant ascorbic acid. IC_{50} value of ascorbic acid and leaves extract was found 1.25 µg/ml and 200.96 µg/ml, respectively. The value of extract indicated significant antioxidant activity of the plant.

Key words: *Cassia hirsuta,* Antioxidant, DPPH, *Caesalpiniaceae*.

INTRODUCTION

Antioxidants are type of molecules that neutralize harmful free radicals, produced through a chain of reactions 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 (Sies, 1997). 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 studied as oxidative stress might be an important part of many human diseases particularly stroke and neurodegenerative incidents (Joseph *et al* 2010). 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 (Chu *et al* 2000; Koleva *et al* 2002; Mantle *et al* 2000; Oke *et al* 2002) other than well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices (Schuler, 1990) but there is still a demand to find more information on the antioxidant potential of plant species.

Cassia hirsuta Linn. plant (Irwin and Barneby, 1982; Holm *et al* 1979) belongs to the family *Caesalpiniaceae* and commonly called as stinking cassia and hairy senna (**Figure 1**).

Fig. 1. Photograph of *Cassia hirsuta* Linn.

It is a terrestrial perennial, erect shrub up to 150 cm in tall, stem rounded, solid glabrons, flowering period from September to December and fruiting in November to January. It is a native of tropical America and now distributed in Malaysia, Indo-China, Thailand, Asian and African tropics, Laos, Java, Brazil, California, New Mexico and India (Holm *et al* 1979). It is used as a green manure and forage plant. In Africa, it planted as a shade plant in young coffee plantation. Leaves and young pods are eaten, usually steamed or cooked in vegetable or in salads. In Java, the leaves are used medicinally for treating herpes. A decoction of leaves is used against irritation of skin in Thailand. In Laos, the seeds are used as a substitute for coffee. Phytomedicinally, the plant parts or extracts are used for treating illness in man. Plants as gifts of nature have many therapeutic properties combined with much nutritive value, which have made their use in chemotherapy as valuable as the synthetic drugs. Herbal organ of the body are used to feed and restore to health those parts, which have become weakened. It is a medicinal plant widely used for stomach troubles, dysentery, abscesses, rheumatism, fever and other diseases. Seeds contain phytotoxin, tannins and 0.25% chrysarobin. Seeds also contain a water soluble sugars extract as D-galactose and D-mannose in 1:4 molar ratio from hydrolysed compound on paper chromatogram Present manuscript mainly deals with the methylation studies for polysaccharide structure of *Cassia hirsuta* Linn. seeds - galactomannan. It is also useful in dental caries: powdered seeds are used to massage on gums and teeth (Brenan, 1967; Revathi and Parimelazhagan, 2010; Jamir *et al* 2010; Singh *et al* 2007).

Scientists have been proving that all the natural things are not good for health. Different retrospective studies done over the last 20 years indicated that the incidence of deaths occurring due to exposure to plants (as a proportion of total patients poisoned by traditional plant medicine) was about 1.5% in France, 5% in Belgium, 6.5% in Italy, 7.2% in Switzerland and 6% in Turkey (Gaillard *et al* 1999). Therefore, there is a need to have an understanding of the risks posed by herbal medicines so as to ensure that such products could be used safely.

Keeping in view the biopotential of plants and their derived products (Dahiya and Gautam, 2011; Jain *et al* 2011; Zahid Hosen *et al* 2011; Chowdhury *et al* 2012; Emran *et al* 2012), present investigation was directed toward evaluating the antioxidant property of *Cassia hirsuta* leaves extract.

MATERIALS AND METHODS

Collection of plant

The plant *Cassia hirsuta* was collected from the Chittagong University campus. The plant was taxonomically classified and identified scientifically by Professor Dr. Md. Mostafa Kamal Pasha, Taxonomist, Department of Botany, University of Chittagong, Bangladesh with help of direct field study and taxonomic analysis.

Preparation of plant extract

The fresh leaves of *Cassia hirsuta* were washed with water immediately after collection. Then, these were chopped into small pieces, air dried at room temperature for about 10 days and pulverized into powder form and stored in an airtight container. 600 g leaf powder was macerated in 6 litre pure ethanol for 7 days at room temperature. After 7 days, leaf ethanol extract was filtered off through a cotton plug and finally with a Whatman No. 1 filter paper. Then the extract was concentrated under reduced pressure below 50 °C through rotary vacuum evaporator. The concentrated extracts were collected in a petri dish and allowed to air dry for complete evaporation of ethanol. The whole process were repeated three times and finally, 72 g blackish-green colored, concentrated leaf extract was obtained (yield 12% *w/w*) which was kept in refrigerator at 4 °C.

In vitro assay for antioxidant activity of plant extract

The antioxidant activity of *Cassia hirsuta* leaves extract was assessed in comparison to standard antioxidant ascorbic acid (BDH, England) depending on the scavenging effect of 1, 1-diphenyl-2-picrylhydrazyl (DPPH)-free radical. The whole procedure was done according to established procedure (Braca *et al* 2001; Brand-William *et al* 1995). Ascorbic acid solution (5 ml) and different concentrations of extract (20, 40, 60, 80, 100, 200, 400 and 800 μg/ml in methanol) (5 ml) were mixed with 3 ml of 0.4 mM (0.004 %) DPPH solution. The mixtures were kept in dark for 30 min to measure the absorbance at 517 nm using UV-Visible spectrophotometer (UV-1601 Shimadzu, Kyoto, Japan) 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:

$$\text{Scavenging activity (\%)} = [(A - B) / A] \times 100 \quad (1)$$

Where A is absorbance of control (DPPH solution without the sample) and 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 IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis.

RESULTS AND DISCUSSION

Assay for antioxidative activity of Cassia hirsuta

DPPH free radical scavenging activity of *Cassia hirsuta* and ascorbic acid is shown in the **Table 1.** Both ascorbic acid and *Cassia hirsuta* leaves ethanolic extract showed dose dependent activity.

Table 1. DPPH free radical scavenging activity of ascorbic acid and *Cassia hirsuta* leaves extract

Test material	Concentration (*μ*g/ml)	% Scavenging activity	IC_{50} (*μ*g/ml)
Ascorbic acid (Standard)	20	70.02	IC_{50} = 1.25
	40	74.06	
	60	79.25	
	80	84.09	
	100	88.50	
	200	90.75	
	400	94.50	
	800	97.34	
Cassia hirsuta	20	7.04	IC_{50} = 200.96
	40	17.25	
	60	25.70	
	80	27.11	
	100	31.51	
	200	54.40	
	400	63.20	
	800	78.70	

Among the eight different concentrations used in the study (20, 40, 60, 80, 100, 200, 400 and 800 *μ*g/ml) ascorbic acid showed 70.02, 74.06, 79.25, 84.09, 88.50, 90.75, 94.50 and 97.34% scavenging activity where highest scavenging activity was 97.34% at concentration 800 *μ*g/ml. On the other hand, *Cassia hirsuta* leaf ethanol extract showed 7.04, 17.25, 25.70, 27.11, 31.51, 54.40, 63.20 and 78.70% scavenging activity at the above mentioned eight different concentrations where highest scavenging activity of *Cassia hirsuta* leaf ethanol extract was 78.70% at 800 *μ*g/ml.

% of scavenging activity or % of inhibition was plotted against log concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis.

IC_{50} value of ascorbic acid and *Cassia hirsuta* leaves ethanolic extract was found 1.25 and 200.96 *μ*g/ml respectively (**Figure 2**). The IC_{50} value obtained for *Cassia hirsuta* extract and ascorbic acid indicated that *Cassia hirsuta* extract possessed higher efficiency to neutralize free radicals than that of ascorbic acid (**Figure 3**). Different research suggested that most of the plant extracts showing antioxidant activity are due to presence of the phenolic compounds (Ramarathnam *et al* 1997; Velioglu *et al* 1998). Phenolic natural compounds such as flavonoids 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 (Rice-Evans *et al* 1995). The phenolic compounds, identified in the extract might contribute to the antioxidant activity of *Cassia hirsuta* extract.

CONCLUSION

The results of the study demonstrated that the ethanolic extract of *Cassia hirsuta* exhibited very

potential antioxidant effect in experimental models which supported 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.

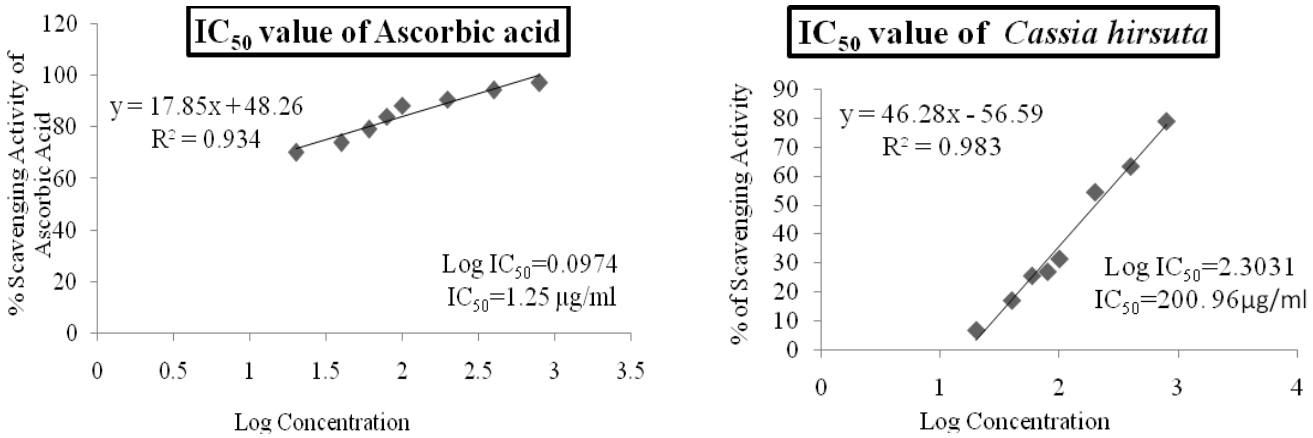

Fig. 2. Comparative IC_{50} values of reference antioxidant and studied plant *Cassia hirsuta* leaf extract: (a) ascorbic acid (b) plant extract

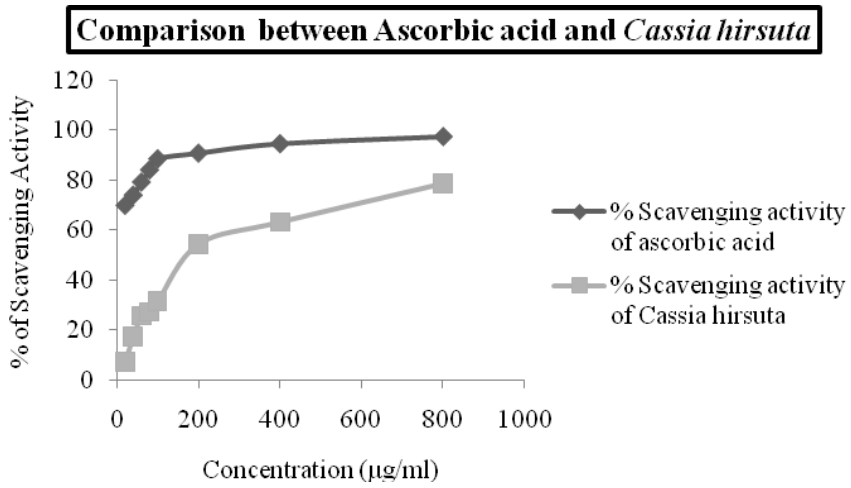

Fig. 3. Comparative % scavenging activities of *Cassia hirsuta* extract and ascorbic acid

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