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*Short Communication*

# Free radical scavenging activity and phenolic content of *Cassia sophera* L.

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**Dried methanol extract of leaves of *Cassia sophera* L. was dissolved in distilled water, and then fractioned by re-extracting with n-hexane, chloroform, and ethyl acetate, subsequently. The free radical scavenging activity (FRSA) of methanol extract and various fractions of methanol extract was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. The FRSA of ethyl acetate fraction was superior to all other fractions ($IC_{50}$ = 15.42 µg/ml), which was higher than synthetic antioxidant butylated hydroxyanisole, BHA (18.25 µg/ml). The amount of total phenolic compounds was further determined. The total phenolic content in EtOAc fraction (13.25%) was the highest as compared to other extracts. The results of this study suggest that this plant could serve as a source of natural antioxidants and preservative agents with potential applications in food industries.**

**Key words**: *Cassia sophera* L., radical scavenging activity, phenolic content.

## INTRODUCTION

Since ancient times the plant extracts have been in use for many purposes, such as food, drugs and perfumery. Many medicinal plants contain large amounts of antioxidants such as polyphenols, which have been widely used as additives to avoid the degradation of foods. Also, antioxidants have an important role in preventing a variety of stress-related diseases and aging because these are closely related to the active oxygen and lipid peroxidation (Noguchi and Niki, 1999). Antioxidants have been used for the prevention and treatment of free radical-related disorders (Middleton et al., 2000). However, there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis (Barlow, 1990). There is a scientific interest to find naturally occurring antioxidants for use in foods to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Velioglu et al., 1998).

*Cassia sophera* L. (Caesalpiniaceae) is a medicinal plant of Bangladesh and Indian sub continent, which is widely used as folk medicine for the treatment of many diseases. According to the physicians of Unani medicine, three plants viz., *Cassia occidentalis* Linn., *C. sophera* Linn. and *C. sophera* Linn. var. Purpurea Roxb. are the varieties of 'Kasondi' and are invariably conditions (Lubhaya, 1975; Awan, 1984; Ahmad-Billal et al., 2005). 'Kasondi' is described in Unani literature to be repulsive of morbid humors (specially phlegm), resolvent, blood purifier, carminative, purgative, digestive, diaphoretic (Lubhaya, 1975; Kareem, 1979; Awan, 1984). In ethno botanical literature, it is mentioned to be effective in the treatment of pityriasis, psoriasis, asthma, acute bronchitis, cough, diabetes and convulsions of children (Chopra et al., 1956; Agharkar, 1991; Dutt, 1995; Kirtikar

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**Table 1.** Free radical scavenging activity (DPPH) and total phenolic compounds (mg/g dry weight) of leaves of *Cassia sophera* L.

| Sample | $IC_{50}$ (µg/ml) | Total phenolic (mg GAE/g dw) |
|---|---|---|
| Methanol extract | 33.21 ± 2.1 | 82.33 ± 3.2 |
| Hexane fraction | 152.34 ± 5.2 | 30.15 ± 2.5 |
| Chloroform fraction | 55.12 ± 1.5 | 52.11 ± 1.8 |
| Ethyl acetate fraction | 15.42 ± 1.2 | 132.52 ± 4.5 |
| Ascorbic acid (Control) | 6.54 ± 0.9 | na |
| BHA (Control) | 18.25 ± 1.3 | na |

Values are given as mean ± S.D. of triplicate experiments.
BHA: butylated hydroxyanisole.
na: Not applicable.

and Basu, 2000).

Owing to its various ethnopharmacological properties, no report available in the literature on the screening of different solvent extracts of *C. sophera* L. leaves for their antioxidant properties. Therefore, the aim of this study is to investigate the antioxidant property of *C. sophera* L. with the possible use of natural antioxidants as food preservatives.

## Material and methods

### Preparation of methanol extracts

The air-dried leaves of *C. sophera* L. (Caesalpiniaceae) was powdered in a blender and 30 g of it extracted with MeOH (160 ml × 3 times) at room temperature. The extract was then filtered and evaporated on a rotary vacuum evaporator to give a solid MeOH extract (2.73 g). This MeOH extract (2.42 g) was then suspended in water (60 ml) and partitioned successively with n-hexane, chloroform ($CHCl_3$) and ethyl acetate (EtOAc) to yield hexane (0.680 g), $CHCl_3$ (0.092 g), EtOAc (0.158 g) and residual MeOH (0.652 g) sub-fractions separately. Solvents (analytical grade) for extraction were obtained from commercial sources.

### Free radical scavenging capacity

The antioxidant activity of the methanol extract and its derived sub-fractions including hexane, ethyl acetate, chloroform and residual methanol was measured on the basis of the scavenging activities of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO) free radical (Cuendet et al., 1997). Various concentrations of 100 µl of test samples were added to 3 ml of a 0.004% (w/v) methanol solution of DPPH. After 30 min of incubation period in the dark at room temperature, the absorbance was measured against a blank at 517 nm. Inhibition of free radical DPPH in percent (%) was calculated by the formula:

$$\text{Percentage inhibition (\%)} = [(A_{blank} - A_{sample}) / A_{blank})] \times 100$$

Where, $A_{blank}$ is the absorbance of the control reaction (containing all reagents except test compound), and $A_{sample}$ is the absorbance of the test compound. $IC_{50}$ values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation, prepared from the concentration of the samples and percentage inhibition of free radical formation/ percentage inhibition DPPH was assayed. Synthetic antioxidant reagents, butylated hydroxyanisole (BHA) and L-ascorbic acid (each from Sigma-Aldrich, St. Louis, MO), were used as positive controls and all tests were carried out in triplicate.

### Determination of total phenolics

Total phenolic constituents of the aforementioned extracts were determined by Folin-Ciocalteu reagent (Sigma–Aldrich, St. Louis, MO) in alkaline medium (Lister and Wilson, 2001) and was expressed as gallic acid equivalents (GAE). Different concentrations of gallic acid were prepared in 80% methanol. 100 µl test sample (from a range of concentrations) was taken in a cuvette, then 1 ml of distilled water and 500 µl (1/10 dilution) of the Folin–Ciocalteu reagent was added, and cuvette was shaken thoroughly. After 1 min, 1500 µl of 20% sodium carbonate ($Na_2CO_3$) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of samples was measured at 760 nm and the results were expressed in mg of gallic acid per g (GAE) of dry weight of samples.

## Results and discussion

Free radical scavenging activity of methanol extract and its sub-fractions, measured by DPPH assay, is shown in Table 1. Their activity of the plant extracts is concentration- dependent and lower $IC_{50}$ value reflects better protective action. The $IC_{50}$ values of methanol extract and its various organic sub-fractions were recorded in the range of 15.42 to 152.34 µg/ml. Polar sub-fractions of methanol extract (e.g., EtOAc fraction) exhibited stronger DPPH scavenging activity than non-polar fraction. The free radical scavenging activity of ethyl acetate fraction ($IC_{50}$ = 15.42 µg/ml) was superior to all other fractions. The $IC_{50}$ value of ethyl acetate fraction ($IC_{50}$ = 15.42 µg/ml) was lower than synthetic antioxidant, butylated hydroxyanisole (BHA) ($IC_{50}$ = 18.25 µg/ml). The strongest activity of ethyl acetate fraction may be related to its higher phenolic content (132.52 mg GAE/g) as measured by gallic acid test (Table 1).

The total phenolic contents of MeOH extract and its fractions were found in the range from 30.15 to 132.52 mg GAE/g of dry sample (Table 1). The total phenolic

content of MeOH extract was 82.33 mg GAE/g and its sub-fractions including hexane, chloroform and ethyl acetate were 3.01, 5.21 and 13.25 % of dry sample, respectively. These results showed that the total phenolic content in EtOAc fraction (13.25 %) was the highest as compared to other extracts. This may be due to the presence of high bioactive compounds in EtOAc fraction. In particular, the EtOAc fraction showed the strongest DPPH activity. Therefore, we assume that this high activity is related to the presence of bioactive compounds such as phenolic compounds in this fraction (Table 1).

Leaves extracts of *C. sophera* L. showed good anti-oxidant activity. In particular, polar extract or fractions were more active than non-polar faction. This activity is due to most bioactive compounds such as polyphenols including tannins, flavonoid existed in higher polar fraction (Karamanoli, 2002). The strongest activity of polar fraction such as ethyl acetate fraction may be related to its higher phenolic content as measured by gallic acid test (Table 1) suggesting that activity is mostly related to its water-soluble phenolic compounds. Poly-phenols are one of the major plant compounds with antioxidant activity. The antioxidant activity of phenolic compounds is reported to be mainly due to their redox properties (Galato et al., 2001), which can play an impor-tant role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. In recent years, the biological and pharma-cological effects of the phenolic compounds have attracted increasing interest for their potential beneficial effects on human health. They occur ubiquitously in plant origin and have shown to contain potent antioxidant, anti-inflammatory and cancer-preventive activities (Ghiselli et al., 1998; Visioli et al., 1998; Fabiani et al., 2006). Because numerous studies have clearly demonstrated that phenolic compounds exhibit important quality proper-ties (Tomás-Lorente et al., 1992; Suárez-Valles et al., 1994), the determination and quantification of phenolic compounds is of particular interest.

The antioxidative property of methanol extract and its different fractions associated with *C. sophera* L. proved that they can be used as a source of natural antioxidants with potential application to reduce oxidative stress with health benefits. Our findings could introduce a unique natural source possesses strong antioxidants.

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