INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 4, p. 76-84, 2024

RESEARCH PAPER OPEN ACCESS

# *Cassia sophera* L.: Antioxidant potential profiling of different extracts of leaf, flower, seed and stem

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**Key words:** *Cassia sophera* L., Antioxidant activity, DPPH, Calsalpiniaceae

http://dx.doi.org/10.12692/ijb/25.4.76-84 Article published on October 04, 2024

## Abstract

*Cassia sophera* L. is a predominant traditional medicinal shrub as well as considered as a remedy in folk arena due to its antidysenteric, antidiarrhoeal, antipyretic, antinociceptive, anthelmintic and antioxidant effect. In the present study, the antioxidant effect of n-hexane, chloroform, ethyl acetate and methanol extract of leaf, flower, seed and stem of *C. sophera* L. was evaluated by DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The assay of free radical scavenging activity of four different solvent extracts revealed that the ethyl acetate extract of leaf and stem exhibited good antioxidant activity with $IC_{50}$ of 61.296 and 121.289 µg/ml respectively as compared to ascorbic acid considered as positive control. In addition, the antioxidant potential of the solvent extracts of leaf, flower, seed and stem of *C. sophera* L. is firstly reported in the present study except methanolic extract of leaf.

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## Introduction

Plants are a great repository of phytochemicals rendering with health aid and development due to a large area of therapeutic actions as medicines from primitive period till today. Besides, modern analytical technology has opened a large window of tremendous investigations on the plants and herbs used in folk medicine all over the world (Mulaudzi *et al.,* 2022; Mehari *et al.,* 2021; Anokwuru *et al.,* 2022; Ahmed *et al.,* 2022**)**. In fact, bioactive compounds obtained from herbs are beneficial against the undesirable side effects of chemotherapy and promote general health and longevity (Yuan *et al.,* 2022). This is why an intense inclination on the investigation of the medicinal potential of plants is currently growing. In connection to this, one of the most significant potential phytochemicals is natural antioxidants like ascorbic acid, tocopherol, flavonoids, phytoestrogens, phenolic acids, phytic acids, etc (Bendich and Langseth *et al.,* 1995) which show magnificent therapeutic efficacy to lessen and combat the risk of chronic diseases, cancers, and heart diseases caused by reactive oxygen species (ROS) in the body (George *et al.,* 2021**;** Majumder and Afia *et al.,* 2019; Miller *et et al.,* 2000).

*Cassia sophera* L. originates from the subfamily of Caesalpinioideae under Leguminosae family spreading from Africa to India and South East Asia. *C. sophera* which is assumed as an indigenous species of South America is a shrub (Gulzar *et al.,* 2014). In Bangladesh the plains and hills of Chittagong, Sylhet are the abundantly growing place of *C. sophera* which is locally known as Kalkasunda, Kasaundi, Kasunda and Baner (Yusuf *et al.,* 1994). Moreover, it also grows at a large extent in India as well as other subtropical and tropical Asian countries such as Pakistan, Sri Lanka and Myanmar. Consequently its use is evidently found as traditional medicines in Japan, Korea and China as well as the Indian subcontinent (Drever *et al.,* 2008; Guo *et al.,* 1998). It can grow more than three meters tall. Local people sometimes consume the soft leaves and the pods. The Ayurveda and Unani traditions reported its potential and effective use for treating piles, jaundice, psoriasis, skin diseases, eye inflammation and even snake bites (Drever *et al.,* 2008**;** Bilal *et al.,* 2005; Arijit *et al.,* 2012; Aminabee and Lakshmana *et al.,* 2012). The seed, flower, root and bark of *C. sophera* are well known for its astringency, laxative and carminative (Drever *et al.,* 2008; Kirtikar and Basu *et al.,* 2006; Lee *et al.,* 2001**;** Gupta *et al.,* 2010). In addition the ethnobotanical literature states that the leaves are used medicinally for its anti-inflammatory, anti-rheumatic, laxative properties. The leaves were also considered as expectorant for coughs, colds, bronchitis, asthma and also effective for liver disorder. Previous studies showed that analgesic, anticonvulsants, antidiabetic, herbicidal, bactericidal activity of seed were investigated (Majumder and Afia *et al.,* 2019). Moreover alcoholic extract obtained from leaves is a potential relaxant for intestinal and bronchial muscle. On the other hand, phytochemical investigation revealed that a flavone glycoside and senna glycoside from leaves and anthraquinones, chrysophanol, physcion and beta-sitosterol from root were reported (Bilal *et al.,* 2005). It is also noted that a good number of bioactive compounds such as 1,2,7-trihydroxy-3-methylanthraquinone, sopheranin, beta-sitosterol, chrysophanol, physcion, emodin, 1-octadecanol and quercetin were isolated from heartwood (Khare *et al.,* 2009).

Although several studies have been performed on *C. sophera* so far, extensive research on the phytochemical and pharmacological aspects of *C. sophera* cultivated in Bangladesh is inadequate. Therefore, a deeper attention should be given to evaluate several biological and chemical potential of *C. sophera*. This is why, we have assessed and compared the antioxidant activities of n-hexane, chloroform, ethyl acetate and methanol extract of leaf, flower, seed and stem of *C. sophera* by using standard *in vitro* assays for substantiating its ethnobotanical medicinal use in the present study for the first time except methanolic extract of leaf.

## Materials and methods

Fully matured plant parts like fresh leaves, stems, flowers and seeds of *C. sophera* were collected from

the hilly areas of Sylhet district in Bangladesh. The specimen of the samples was authenticated by the taxonomist of the Bangladesh national Herbarium, Dhaka where a voucher (No.43734) has been deposited.

The leaves, stems, flowers and seeds were separately dried in an oven at 38°C. These dried samples of leaves, stem, flower and seed were ground using a Wiley mill up to a particle size of 20 mesh. Under the cold extraction process, the powdered material of leaves (250 g) was extracted with n-hexane for 5 days at room temperature. Afterwards the solid residue obtained from the first extraction step was successively soaked with chloroform, ethyl acetate and methanol. In this process the polarity of the solvent was gradually increased. The similar cold extraction process applied on the powdered material of leaves was also implemented for the solvent extraction of the powdered material of flower (175 g), seed (110 g) and stem (100 g) respectively. Each extract obtained from the respective solvent was subjected to evaporation by Rotary evaporator under reduced pressure. Consequently, a gummy mass of n-hexane extract (9 g), chloroform extract (7.5 g), ethyl acetate extract (7 g) and methanol extract (6 g) was obtained.

UV-Visible Spectrophotometer (Perkin Elmer Shelton, CT 06484 USA, Lambda 25) was used to measure UV absorbance measurements. In order to evaporate solvents, a rotary evaporator (BUCHI, Rotavapor R-210, Switzerland) was used. All the solvents used in this experiment were analytical grade (Sigma-Aldrich, St. Louis, MO, USA).

The DPPH method is a facile spectrophotometric technique for the determination of antioxidant activity (Braca *et al.,* 2001; Angeli *et al.,* 2021). An unpaired electron on the DPPH (2, 2-diphenyl-1-picrylhydrazyl) free radical imparts an intense violet due to its solution. The free radical scavenging activity was easily measured by the reduction of absorbance in the 517 nm region using a spectrophotometer. In the procedure of DPPH method, firstly 1 ml of each of ascorbic acid solutions of different strengths in methanol (5, 10, 25, 50, 100, 200, 400 µg/ml in methanol) was mixed with 3 ml of 0.4 mM DPPH solution. Afterwards, 1 ml of each of the extracts was also mixed with 3 ml of 0.4 mM DPPH solution. As per the standard procedure, the mixture was kept totally enclosed for protection from exposure to light for 30 minutes to measure the absorbance at 517 nm under the UV-Visible Spectrophotometer. In this study, ascorbic acid solution was used as a positive control. The higher the free radical terminating potential of the sample, the lower the absorbance obtained. Under the action of the antioxidants, the DPPH solution turned to yellow from purple and the degree of this transformation indicates the radical quenching power of the analyte.

The free radical scavenging potential against DPPH was computed according to the formula:
[(A-B)/A] x100.Where “A” is the absorbance of the reference (DPPH solution free from any antioxidant) and “B” is the absorbance of the free radical source solution in the presence of a sample (DPPH plus extract/ascorbic acid). The radical quenching power (in percent) was then plotted against concentration and from the graph, the $IC_{50}$ (Concentration at 50% inhibition) value was estimated through linear regression analysis.

For the assessment of antioxidant activity, each experiment was repeated in triplicate and the mean value of the results was calculated.

**Results and discussion**

The antioxidant activity of the various extracts collected from leaf, flower, seed and stem of the plant was assayed by using the DPPH method.

The n-hexane extracts of stem, flower, leaf and seed showed diminutive free radical scavenging effect on DPPH radicals where these four parts exhibited a range of $IC_{50}$ from 368.175 to 1342.413 µg/ml shown in Table 1.

**Table 1.** $IC_{50}$ values of solvent extracts of leaf, flower, seed and stem of *Cassia sophera* L.

| Plant parts | $IC_{50}$(µg/ml) | | | |
|---|---|---|---|---|
| | n-Hexane | Chloroform | Ethyl acetate | Methanol |
| Flower | 884.595 ± 4.47 | 304.98 ± 1.175 | 152.224 ± 3.30 | 476.95 ± 1.99 |
| Leaf | 937.048 ± 2.32 | 681.915 ± 1.91 | 61.296 ± 1.55 | 313.99 ± 1.03 |
| Seed | 1342.413 ± 3.08 | 333.724±2.22 | 305.26 ± 1.25 | 415.787 ± 2.50 |
| Stem | 368.175 ± 2.53 | 590.972 ± 3.29 | 121.289 ± 0.67 | 170.45 ± 1.59 |
| Ascorbic acid | 6.344±0.0175 | | | |

Values are the means ± standard deviations (n = 3).

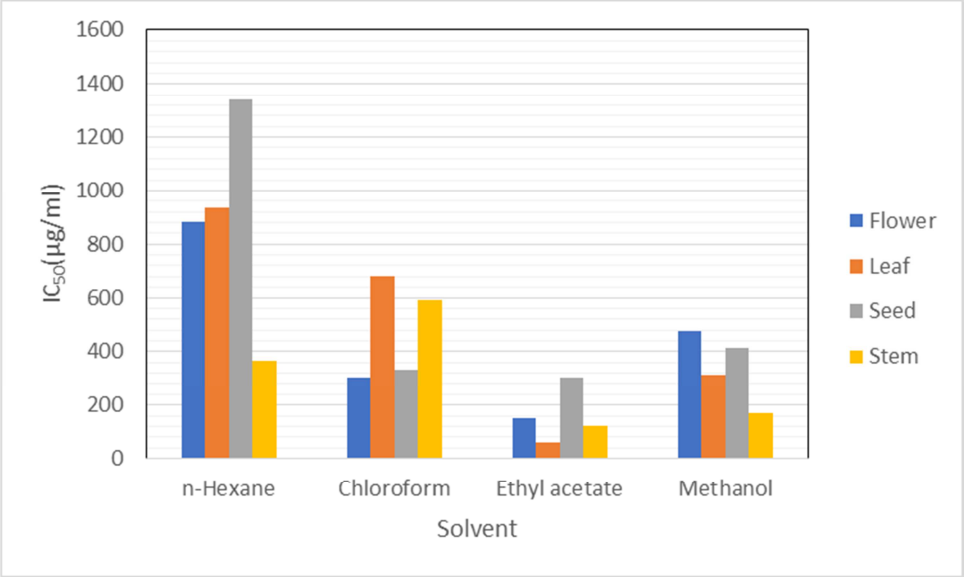


**Fig. 1.** $IC_{50}$ values of solvent extracts of four different parts of *Cassia sophera* L.

In addition, Table 4 shows that the lowest free radical scavenging effect obtained from n-hexane extract of seed was 32.83% inhibition at a concentration of 400 µg/ml whereas the highest effect found from the extract of stem was 51.40% inhibition at the same concentration compared to positive control given in the Table 5.

Moreover, from the Table 1, it is seen that the chloroform extract of leaf, flower, seed and stem showed moderate scavenging effect on DPPH radicals and their activity based on $IC_{50}$ was in the range from 304.98 to 681.915 µg/ml.

In assessment of chloroform extract, the leaf extract had lowest free radical scavenging effect that was 40.26% inhibition at a concentration of 400 µg/ml given in the Table 3 while the flower extract had the highest effect was 56.59% inhibition at the same concentration compared to standard ascorbic acid shown in the Table 2.

**Table 2.** Free radical scavenging activity of different solvent extracts of flower

| Extracts | Concentration (µg/ml) | Absorbance | %Inhibition |
|---|---|---|---|
| n-Hexane | 5 | 1.053 | 23.25 |
| | 10 | 1.041 | 24.12 |
| | 25 | 1.033 | 24.70 |
| | 50 | 1.017 | 25.87 |
| | 100 | 0.997 | 27.33 |
| | 200 | 0.956 | 30.32 |
| | 400 | 0.888 | 35.27 |
| | blank | 1.372 | |
| Chloroform | 5 | 0.852 | 27.05 |
| | 10 | 0.851 | 27.14 |
| | 25 | 0.841 | 27.99 |
| | 50 | 0.820 | 29.79 |
| | 100 | 0.764 | 34.58 |
| | 200 | 0.665 | 43.06 |
| | 400 | 0.507 | 56.59 |
| | blank | 1.168 | |
| Ethyl acetate | 5 | 0.846 | 27.56 |
| | 10 | 0.825 | 29.36 |
| | 25 | 0.804 | 31.16 |
| | 50 | 0.726 | 37.84 |
| | 100 | 0.620 | 46.91 |
| | 200 | 0.430 | 63.18 |
| | 400 | 0.245 | 79.02 |

| | | | |
|---|---|---|---|
| | blank | 1.168 | |
| Methanol | 5 | 0.983 | 27.13 |
| | 10 | 0.974 | 27.79 |
| | 25 | 0.958 | 28.98 |
| | 50 | 0.943 | 30.09 |
| | 100 | 0.898 | 33.43 |
| | 200 | 0.847 | 37.21 |
| | 400 | 0.728 | 46.03 |
| | blank | 1.349 | |

Furthermore, the Table 1 & Fig. 1 reveals that the ethyl acetate extract leaf manifested the most significant free radical scavenging at $IC_{50}$ of 61.296 µg/ml while the stem and flower extract showed moderate antioxidant activity at $IC_{50}$ of 121.289 and 152.224 µg/ml respectively. On the other hand, the lowest free radical scavenging effect at $IC_{50}$ of 305.26µg/ml was obtained from ethyl acetate extract of seed shown in the Table 3.

**Table 3.** Free radical scavenging activity of different solvent extracts of Leaf

| Extracts | Concentration (µg/ml) | Absorbance | %Inhibition |
|---|---|---|---|
| n-Hexane | 5 | 0.883 | 26.84 |
| | 10 | 0.878 | 27.25 |
| | 25 | 0.873 | 27.67 |
| | 50 | 0.864 | 28.41 |
| | 100 | 0.851 | 29.49 |
| | 200 | 0.804 | 33.38 |
| | 400 | 0.769 | 36.28 |
| | blank | 1.207 | |
| Chloroform | 5 | 0.879 | 27.17 |
| | 10 | 0.871 | 27.83 |
| | 25 | 0.864 | 28.41 |
| | 50 | 0.847 | 29.82 |
| | 100 | 0.827 | 31.48 |
| | 200 | 0.783 | 35.12 |
| | 400 | 0.741 | 40.26 |
| | blank | 1.207 | |
| Ethyl acetate | 5 | 0.669 | 44.57 |
| | 10 | 0.652 | 45.98 |
| | 25 | 0.643 | 46.72 |
| | 50 | 0.615 | 49.04 |
| | 100 | 0.552 | 54.26 |
| | 200 | 0.445 | 63.13 |
| | 400 | 0.334 | 72.32 |
| | blank | 1.207 | |
| Methanol | 5 | 0.869 | 28.66 |
| | 10 | 0.853 | 29.32 |
| | 25 | 0.834 | 30.90 |
| | 50 | 0.775 | 35.79 |
| | 100 | 0.732 | 39.35 |
| | 200 | 0.682 | 43.49 |
| | 400 | 0.554 | 54.10 |
| | blank | 1.207 | |

**Table 4.** Free radical scavenging activity of different solvent extracts of seed

| Extractives | Concentration (µg/ml) | Absorbance | %Inhibition |
|---|---|---|---|
| n-Hexane | 5 | 0.846 | 25.91 |
| | 10 | 0.849 | 25.65 |
| | 25 | 0.849 | 25.65 |
| | 50 | 0.838 | 26.61 |
| | 100 | 0.827 | 27.58 |
| | 200 | 0.808 | 29.24 |
| | 400 | 0.767 | 32.83 |
| | blank | 1.142 | |
| Chloroform | 5 | 0.840 | 28.44 |
| | 10 | 0.829 | 29.38 |
| | 25 | 0.814 | 30.66 |
| | 50 | 0.795 | 32.28 |
| | 100 | 0.730 | 37.81 |
| | 200 | 0.663 | 43.52 |
| | 400 | 0.554 | 52.81 |
| | blank | 1.174 | |
| Ethyl acetate | 5 | 0.849 | 27.68 |
| | 10 | 0.843 | 28.19 |
| | 25 | 0.828 | 29.47 |
| | 50 | 0.801 | 31.77 |
| | 100 | 0.754 | 35.77 |
| | 200 | 0.677 | 42.33 |
| | 400 | 0.509 | 56.64 |
| | blank | 1.174 | |
| Methanol | 5 | 0.835 | 28.87 |
| | 10 | 0.822 | 29.98 |
| | 25 | 0.826 | 29.64 |
| | 50 | 0.803 | 31.60 |
| | 100 | 0.774 | 34.07 |
| | 200 | 0.717 | 38.92 |
| | 400 | 0.598 | 49.06 |
| | blank | 1.174 | |

From the deeper studies of other researchers on different plants, we can predict that this significant antioxidant potential may be due to the phenolic phytochemical species extracted in this polar fraction (Semwal *et al.,* 2015**;** Cosa *et al.,* 2019**;** Yao *et al.*, 2013). It is clearly noted that phenolic acids and flavonoids widely occurred in the plant kingdom, especially in fruits and vegetables, are the chief categories of phenolic forming an important category of phenolic phyto-compounds that manifest antioxidant activity (Wojdyło *et al.,* 2007).

Moreover the medicinal and physiological function of the phenolic phyto-compounds possess benevolent promises for human well-being. Besides being radical scavengers, these are known to be able to prevent inflammations and cancers (Ghiselli *et al.,* 1998;

Visioli *et al.*, 1998). The origin of the antioxidant property of phenolic phytochemicals is their oxidation-reduction property which impart to exterminate free radicals, quench reactive oxygen (singlet and triplet), or to neutralize peroxy-compounds (Galalto *et al.*, 2001).

In addition, Table 1 also shows that methanol extracts of stem showed moderate activity with $IC_{50}$ of 170.45µg/ml among its four extracts. On the contrary, the lowest free radical scavenging effect at $IC_{50}$ of 476.95µg/ml was obtained from methanol extract of flower given in the Table 1.

**Table 5.** Free radical scavenging activity of different solvent extracts of stem

| Extractives | Concentration (µg/ml) | Absorbance | %Inhibition |
|---|---|---|---|
| n-Hexane | 5 | 0.898 | 25.53 |
| | 10 | 0.885 | 26.72 |
| | 25 | 0.824 | 29.63 |
| | 50 | 0.787 | 32.79 |
| | 100 | 0.766 | 34.58 |
| | 200 | 0.704 | 39.88 |
| | 400 | 0.589 | 51.40 |
| | blank | 1.171 | |
| Chloroform | 5 | 0.838 | 28.43 |
| | 10 | 0.830 | 29.12 |
| | 25 | 0.825 | 29.54 |
| | 50 | 0.796 | 32.02 |
| | 100 | 0.771 | 34.15 |
| | 200 | 0.736 | 37.14 |
| | 400 | 0.673 | 42.52 |
| | blank | 1.171 | |
| Ethyl acetate | 5 | 0.850 | 27.41 |
| | 10 | 0.819 | 30.05 |
| | 25 | 0.759 | 35.18 |
| | 50 | 0.682 | 41.75 |
| | 100 | 0.594 | 49.27 |
| | 200 | 0.387 | 66.95 |
| | 400 | 0.129 | 88.98 |
| | blank | 1.171 | |
| Methanol | 5 | 0.835 | 28.69 |
| | 10 | 0.823 | 29.71 |
| | 25 | 0.790 | 32.53 |
| | 50 | 0.730 | 37.66 |
| | 100 | 0.643 | 45.08 |
| | 200 | 0.495 | 57.72 |
| | 400 | 0.313 | 73.27 |
| | blank | 1.171 | |

In connection to this point, from previous research of other investigators, we can surmise that these highly polar extracts probably contain phenolic phytochemicals known as anti-tumor nutraceuticals (Ahmed *et al.,* 2022; Li *et al.,* 2012). Reactive oxygen species (ROS) play a significant role in cell differentiation, aging and various other cellular processes related to overall health (Mates *et al.,* 2000). The phyto-compounds in the highly polar extract of the stem may have the potential to fight against the ROS levels in human tissue. Therefore, we can clearly see that the ethyl acetate and methanol fractions of various parts of the plant contained more potent antioxidants in the bioassays employed.

Several investigations in the past ascribe the antioxidant potential of phyto-extracts to the occurrence of flavonoids, phenolics, terpenoids as well as saponins, tannins, alkaloids etc and we believe that different solvent extracts of our study contain similar class of compounds due to the physico-chemical reasons like solvent polarity (Dangles *et al.,* 2000; Moura *et al.,* 2007; Gülçin *et al.,* 2004; Grabmann *et al.,* 2005). The antioxidative property of polar mixtures like the ethyl acetate and the methanol extract of leaf, stem and flower of *C. sophera* L. imply that this traditional herb may impart health benefits in human beings by reducing oxidative stress. Our findings provide the herbal physician another natural source of strong antioxidants for treatment.

It can be summarized that the antioxidant activity of the different extracts of leaf, flower, seed and stem of *C. sophera* L. was found to be consistent with the folk uses of this plant by local people. This is the first report of antioxidant activity of four separate parts of this shrub. In the present study, we have found significant reactive free radical extinguishing potentials in the most highly polar media extractions, namely ethyl acetate and methanol of the four parts of the plant. We strongly believe that deeper studies at the molecular and pharmacological level on the plant may bring greater human benefits.

**Acknowledgements**

We thankfully acknowledge the generous help provided by the Department of Pharmacy, Dhaka

University of Dhaka, for the assessment of antioxidant of the crude extracts of plant materials. We are also thankful to the Director, BCSIR Laboratories, Dhaka, Bangladesh for facilitating the research related procurements.

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