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A comparative study on the antioxidant activity of *Cassia sophera* (L.) leaf and bark extracts

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ABSTRACT

Background: *Cassia sophera* (L.), or kasundi, is an important plant in traditional medicinal system belonging to family caesalpiniaceae. The leaf extract of this plant is known for various pharmacological activities including, anti-inflammatory, anti-rheumatic, purgative property etc.

Objectives: The objective of the present study was to evaluate and compare the antioxidant potential of methanolic leaf and stem bark extracts of *C. sophera* (L.).

Methods: We measured and compared antioxidant potential by using DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging, ferric reducing power antioxidant capacity and total antioxidant capacity assay; and through total phenol and flavonoid content determination.

Results: Preliminary phytochemical study revealed the presence of alkaloid, glycoside, tannin, saponin and flavonoid in both extracts. The extracts showed moderate antioxidant activity in a dose dependent manner. In DPPH radical scavenging assay, the IC_{50} values of the leaf and bark extracts were 204.44 µg/ml and 297.37 µg/ml, respectively, whereas IC_{50} value for the reference ascorbic acid was 19.08 µg/ml. Furthermore, both the extracts showed moderate antioxidant activity in Ferric reducing power and Total antioxidant capacity assay. In addition, the bark extract exhibited higher amount of flavonoid and phenolic content compared to the leaf extract, which were expressed as quercetin and gallic Acid equivalent respectively.

Conclusion: Based on these findings, it can be concluded that both the extracts possess equitable antioxidant potential of which methanolic stem bark extract of *C. sophera* is more promising one and possesses higher antioxidant potential.

Keywords: *Cassia sophera*, *In-vitro*, Antioxidant capacity, DPPH radical, IC_{50}, Reducing power, Flavonoid.

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INTRODUCTION

Oxidative stress occurs due to an imbalance between reactive oxygen species (ROS) and antioxidant defenses. This stress is involved in several acute and chronic pathological processes, such as cardiovascular diseases, acute and chronic kidney disease (CKD), neurodegenerative diseases (NDs), macular degeneration (MD), biliary diseases, cancer etc. An effective antioxidant has the capacity to nullify the oxidative stress by destroying the free radicals at cellular levels. As per increasing risks for human to various deadly diseases worldwide, there has been a trend to use the resourceful medicinal and dietary plants as therapeutic antioxidants. Various plants have been reported to reveal antioxidant activity; many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers as well [1,2].

Cassia sophera (L.), locally known as kasundi, is a medicinally important plant belonging to family caesalpiniaceae. It is distributed throughout Bangladesh, India and in most tropical countries [4,5]. In ethno-botanical literature, the leaves are mentioned to be used for their anti-inflammatory, anti-rheumatic, and purgative property, as an expectorant for cough, cold, bronchitis, asthma, and in liver disorders [5]. Earlier studies have investigated on the pharmacological activities of the seeds of *C. sophera* including analgesic and anticonvulsant [6], antidiabetic [7], inhibition of lipid peroxidation [8], herbicidal [9], and fungicidal [10] effects. Despite the immense ethno-medicinal properties attributed to *C. sophera*, the reported phyto-pharmacological study on variety levels of this plant is relatively infrequent to the best of our knowledge. Therefore, the present study was aimed to evaluate and compare antioxidant activity of the methanolic leaf and stem bark extracts of *C. sophera*, by using classical in-vitro assays for the purpose of validating its ethnomedicinal use.

METHODS AND MATERIALS

Collection of plant material: The fresh green leaves and stem barks of *Cassia sophera* were collected from Dhaka district of Bangladesh, in the month of April 2018. The plant was identified and authenticated by the expert taxonomist from Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. (Accession number of *C. sophera* -46501).

Preparation of plant extract: After cleaning, the leaves and barks were air dried and then grounded into coarse powders with the help of a suitable blender. About 250g of each powdered material was cold extracted with methanol for 10 days with occasional stirring. The crude extracts were then filtered with Whatman No. 1 filter paper and after that the beakers containing extracts were placed in water bath (at 40°C-50°C) to evaporate the solvent from the extracts, resulting in semi-solid extracts.

Preliminary phytochemical screening: Preliminary phytochemical analysis of the methanolic leaf and stem bark extracts of *C. sophera* was carried out based on the standard methods to identify the presence phytochemical constituents [11].

Tests for antioxidant activity:

DPPH free radical scavenging activity: The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described [12]. Plant extracts (0.1 mL) were added to 3 mL of a 0.004 % methanol solution of DPPH. Absorbance at 517 nm was determined after 30 min and the percentage inhibition activity was calculated from $[(A_o-A_1)/ A_o] \times 100$, where A_o was the absorbance of the control (DPPH solution) and A_1 was the absorbance of the extracts/standard. The inhibition curves were prepared and IC_{50} values were calculated.

Ferric reducing antioxidant power (FRAP): The ferric reducing antioxidant power was determined according to the method described [13]. According to this method, the reduction of Fe^{3+} to Fe^{2+} is determined by measuring the absorbance of Perl's Prussian blue complex. Briefly, different concentrations of extracts in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide $[K_3Fe(CN)_6]$ (2.5 mL, 1 %). The mixture was incubated at 50°C for 20 min. An aliquot (2.5 mL) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The supernatant (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1 %) and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as the reference.

Determination of total antioxidant activity: The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure described [14]. A 0.3 mL extract was combined with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling at room temperature. Methanol (0.3 mL) in the place of extracts was used as the blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid.

Determination of total phenolic content: The total phenolic content of plant extract was determined employing the method as described involving Folin-Ciocalteu reagent as oxidizing agent and gallic acid as standard [15]. At first, 0.5 mL of plant extracts or standard of different concentration solutions were taken in test tube and 2.5 mL of Folin – Ciocalteu (diluted 10 times with water) reagent solution was added into these test tubes. Then 2.5 mL of sodium carbonate (7.5%) solution was added and incubated for 20 minutes at 25°c to complete the reaction. Then the absorbance of the solution was measured at 760 nm using a spectrophotometer against blank.

Determination of total flavonoid content: The content of flavonoid compounds in the extract was determined by the method described [16]. Quercetin was used as standard and the flavonoid content of the extract was expressed as mg of quercetin equivalent/gm of dried extract. At first, 1mL of extracts was placed in volumetric flask, and then 5mL of distilled water added followed by 0.3mL of 5% $NaNO_2$. After 5 minutes, 0.6 mL of 10% $AlCl_3$ was added and volume made up with distilled water. The solution was mixed and absorbance was measured at 510 nm.

RESULTS AND DISCUSSION

Phytochemical screening:

Preliminary phytochemical screening revealed the presence of various bioactive components like carbohydrates, alkaloids, glycosides and flavonoids (Table 1).

Table 1: Results of phytochemical screening of different extracts of *C. sophera*.

Extract	Carbohydrate	Alkaloid	Glycoside	Tannin	Flavonoid	Steroid	Saponin
MLCS	+	+	+	+	+	+	+
MBCS	+	+	+	+	+	-	+

MLCS and MBCS denote for methanolic leaf and methanolic stem bark extracts respectively of *C. sophera;* (+): Present; (-): Absent

Antioxidant activity evaluation:

DPPH free radical scavenging assay: In DPPH free radical scavenging assay, Fig 1(a) showed plant extracts exhibited concentration dependent antiradical activity by inhibiting $DPPH^-$ radical. Ascorbic Acid which is a well-known antioxidant, showed higher degree of free radical-scavenging activity than that of the extracts at each concentration points. The IC_{50} values of the leaf and bark extracts shown in Fig 1b were 204.44 µg/ml and 297.37 µg/ml respectively, whereas IC_{50} value for the reference Ascorbic Acid was 19.08 µg/ml. DPPH antioxidant assay is based on the ability of 2, 2- diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts

an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the change in absorbance and percentage of scavenging activity is calculated. At this point, the extracts were able to reduce DPPH radical (visible deep purple color) to the yellow coloured diphenyl picrylhydrazine [17]. Additionally, it has been determined that the antioxidant effect of plant products is mainly due to radical scavenging activity of phenolic compounds such as flavonoids, polyphenols, tannins, and phenolic terpenes [18]. Therefore, one of the possible mechanisms of the good antioxidant activity of the extracts might be the resultant of containing good amount of phenolic compounds, which show antioxidant activity due to their redox properties.

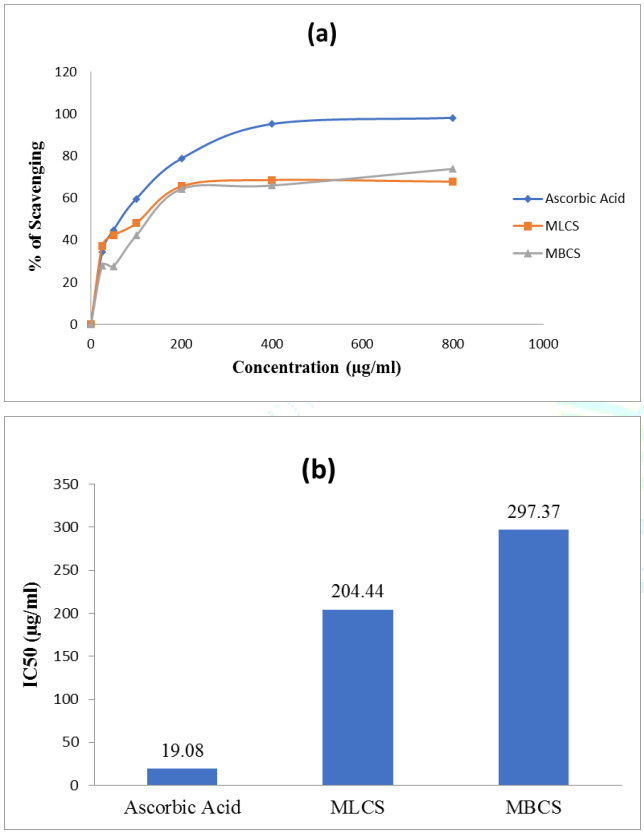

Fig. 1: (a) DPPH radical scavenging activity of methanolic leaf and methanolic stem bark extracts, MLCS and MBCS respectively of *C. sophera* along with Ascorbic Acid (Standard) and (b) IC_{50} (µg/ml) values of MLCS, MBCS and Ascorbic Acid (Standard)

Ferric reducing antioxidant power (FRAP): Fig. 2. showed the reducing power capabilities of the plant extracts compared to ascorbic acid. Both extracts displayed almost similar and moderate reducing power which was found to rise with increasing concentrations of the extracts. In reducing power assays, the presence of antioxidants in the extract can reduce the oxidized form of iron (Fe^{3+}) to its reduced form (Fe^{2+}) by donating an electron. Thus, it can be assumed that the presence of reductants (i.e. antioxidants) in *C. sophera* extracts cause the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the Fe^{2+}complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. A higher absorbance indicates greater reducing power ability [19].

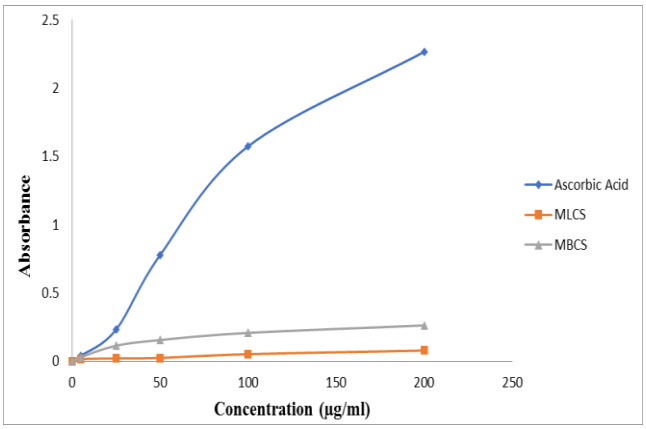

Fig. 2: Ferric reducing antioxidant power of methanolic leaf and methanolic stem bark extracts, MLCS and MBCS respectively of *C. sophera* along with Ascorbic acid (Standard) at different concentrations.

Total antioxidant activity: The assay was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and subsequent formation of a green phosphate/Mo (V) complex at acidic pH with a maximal absorption at 695 nm [13]. The total antioxidant activity of the plant extracts was measured and compared with the reference standard Ascorbic Acid. The high absorbance values indicated that the extracts possessed significant antioxidant activity. The result shown in Fig. 3., revealed that the extracts had good antioxidant activities and the effects increased with increasing concentration.

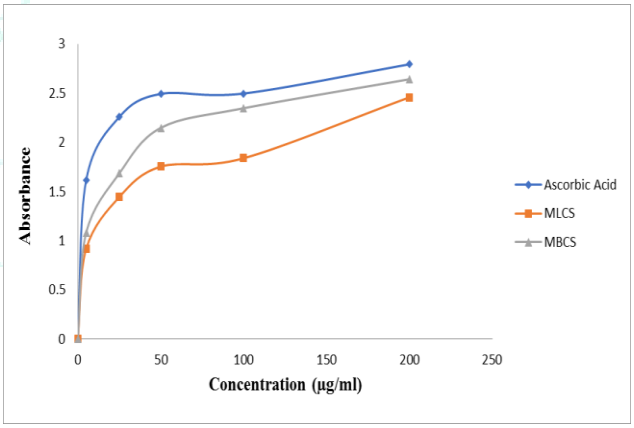

Fig. 3: Total antioxidant activtiy of methanolic leaf and stem bark extracts, MLCS and MBCS respectively of *C. sophera* along with Ascorbic acid (Standard) at different concentrations.

Total phenol and total flavonoid content: Table 2, showed the results of total phenol and total flavonoid content of the methanolic leaf and bark extracts of *C.sophera*. The flavonoid content of the extracts under this investigation correlates with the antioxidant activity; being highest in bark extract (729.82 mg/g QAE), while the leaf extract also showed good result (576.25 mg/g QAE). Among the extracts highest amount of phenol was also found in bark extract (94.32 mg/g GAE).

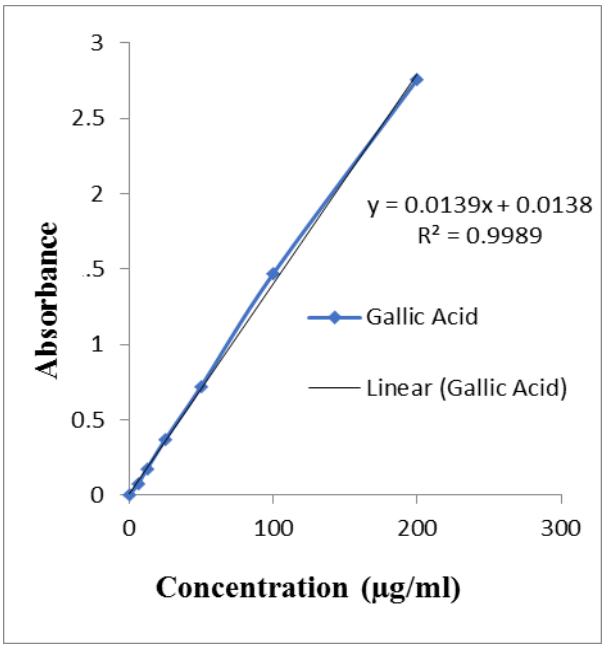

Fig. 4: Standard curve of Gallic Acid for the determination of total phenolic content

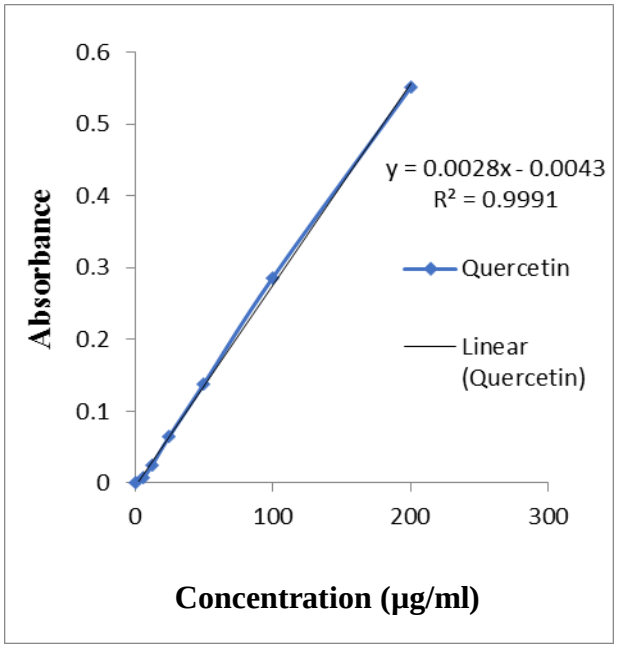

Fig. 5: Standard curve of Quercetin for the determination of total flavonoid content

Table 2: Total amount of phenolics and flavonoid content of different extracts of *C. sophera*.

Plant extracts	Total phenol (In mg/g, Gallic Acid Equivalents)	Total flavonoid (In mg/g, Quercetin Equivalents)
MLCS	55.11	576.25
MBCS	94.32	729.82

MLCS and MBCS denote for methanolic leaf and methanolic stem bark extracts respectively of *C. sophera*

Different studies suggest that different types of polyphenolic compounds (flavonoids, phenolic acids) found in plants have multiple biological effects, including antioxidant activity [20] and present study indicates the presence of polyphenolic compounds in different extracts of *C. sophera*. Moreover, the antioxidant activity of polyphenolic compounds is mainly due to their redox properties, which can play an important role in adsorbing and neutralising free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [21]. Phenolic content of the plant extract was determined by using Folin-Ciocalteu reagent. Phenolic content of the extracts was calculated on the basis of the standard curve for gallic acid in Fig. 4. The flavonoid content of the extracts was calculated on the basis of the standard curve for quercentin in Fig. 5.

CONCLUSION

Considering the results of the conducted study, it can be implicit that the methanolic stem bark extract of *C. sophera* possesses greater antioxidant potential than its methanolic leaf counterpart. This could be a trace to investigate more potential plant parts of *C. sophera* and to find out the rationale behind the use of this plant as folk medicine. However, further studies are warranted to clearly understand the underlying mechanism of the observed actions and to isolate the active phytochemical constituent (s) responsible for such activities.

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CONFLICTS OF INTEREST: Authors declare that they have no conflicts of interest.

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