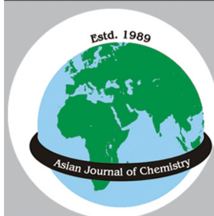

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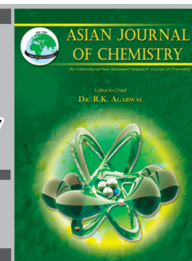

Cassia surattensis: An Indispensable Source for Natural Antioxidants

AMBREEN FATIMA[1], AZIZ-UR-REHMAN[1,*], TAUHEEDA RIAZ[1], NADIA ABBAS[1],
MUHAMMAD ATHAR ABBASI[1], SABAHAT ZAHRA SIDDIQUI[1] and ZAHEER-UD-DIN KHAN[2]

[1]Department of Chemistry, Government College University, Lahore-54000, Pakistan
[2]Department of Botany, Government College University, Lahore-54000, Pakistan

*Corresponding author: Tel: +92 42 111000010, Ext. 449; E-mail: azizryk@hotmail.com

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In this study, phytochemical screening and antioxidant capacity of aqueous and organic fractions of aerial parts of *Cassia surattensis* L. was investigated. Phytochemical tests showed that many secondary metabolites including flavonides, steroids, alkaloids, saponins, tannins and anthraquinones *etc*., were present in the different plant extracts like crude methanolic extract, *n*-hexane, chloroform, ethyl acetate, *n*-butanol soluble fractions and remaining water fraction. First of all the antioxidant components were extracted in methanol and then partitioning was done in solvents of different polarity. Evaluation of the antioxidant activity and radical scavenging activity of these fractions was done by using different antioxidant assays such as total phenolic contents (TPC), 2,2-diphenyl-1-picrylhydrazil (DPPH) scavenging and the trolox equivalent antioxidant capacity (TEAC) of all the studied fractions was evaluated by FRAP and ABTS (radical cation scavenging activity). All the fractions exhibited significant antioxidant potential. The results showed that highest % inhibition of DPPH was shown by ethyl acetate soluble fraction with IC_{50} 4.00 ± 1.7 µg/mL of butylated hydroxytoluene (BHT) equivalents. This fraction also contained highest total phenolic contents (348.83 ± 1.5 mg of gallic acid equivalents) and highest TEAC value (0.99 ± 0.9) measured by ABTS assay and methanolic crude extract exhibited highest total TEAC value (158.5 ± 1.2) by FRAP method.

Key Words: *Cassia surattensis* L., Phytochemical screening, DPPH, Total phenolic, Organic fractions.

INTRODUCTION

Although, the oxygen is the symbol of life, but it also generates reactive oxygen species (ROS) in living organisms and the increasing level of ROS in the body can cause many degenerative diseases like diabetes, cancer and heart disease *etc*.[1-6]. Defence mechanism collectively known as antioxidant defence system (ADS) which acts to regulate oxidative reactions. This system contains antioxidants and enzymes. Antioxidants may be scavenger antioxidants and prevention antioxidants that control the oxidative damage and enzymes are used to repair the oxidative damage. Toxic effects due to the production of free radicals and peroxides are produced because of the disturbances in the normal redox state. The free radicals have adverse effects on the cell components like lipids, proteins and DNA which cause many degenerative disorders[7]. A large number of natural antioxidants perform many biochemical activities like inhibition of reactive oxygen species generation, scavenging of free radical and alteration of intracellular redox potential *etc*.[8]. It has been reported that a large number of secondary metabolites like terpenoids, flavonoides, anthocyanidines *etc*., are effective against the free radicals[9,10]. According to the reports of World Health Organization (WHO), *ca.* 80 % of the world's people rely on plant-based natural and traditional medicines for their primary healthcare needs[11]. Plants used for traditional medicines contain a broad range of chemical constituents that can be used to treat infectious as well as chronic diseases[12]. The development in the exploration of herbal drugs is in progress all over the world. To isolate and develop better herbal drugs botanist, scientists, chemists and pharmacist are researching on the herbal drugs in the field of phytochemistry, chemistry and pharmacology[13].

Cassia surattensis L. (syn. *Senna surattensis*) is indigenous to South East Asia, Malay Peninsula, Sumatra and Java. *Cassia surattensis* L. is cultivated in the gardens of Lahore, Hyderabad and Karachi districts of Pakistan[14]. It is reported that a biopolymer made up of D-manonose and D-galactose has been isolated and characterized in molar ratio of 3:1 from the seeds of *Cassia surattensis*[15]. Antimicrobial activity of *Cassia surattensis* L. and *Cassia fistula* has been evaluated which showed that methanolic extracts have been used against several pathogenic microbes. The results showed that *Cassia surattensis* contained antimicrobial phytochemicals which were found active against a wide array of pathogens of human[16]. Two anthraquinones

namely chrysophanol and physcion were isolated from ethyl acetate extracts of *Senna surattensis*[17]. Podes of *Senna surattensis* led to the isolation of a flavonol glycoside namely 5,7-dihydroxy-4'-methoxyflavonol-3-O-β-D-galactopyranoside, along with physcion, chrysophanol, kaempferide and quercetin[18]. To the best of our knowledge, no salient antioxidant studies have been carried out on *Cassia surattensis* L. therefore, in the present investigation, the authors describe the comparative *in vitro* antioxidant potential and the phytochemical compositions of aqueous and organic fractions of this plant.

EXPERIMENTAL

Plant collection: The plant *Cassia surattensis* L. of the family Leguminosae was collected from the botanical garden of G.C. University, Lahore and authenticated by Dr. Zaheer-ud-Din Khan, Head of the Botany Department, G.C. University, Lahore Pakistan. A voucher specimen GC. Herb. Bot. 736 of the plant was deposited in the herbarium of the same department.

Extraction and fractionation of antioxidants: Cleanliness of the collected fresh aerial parts (stem, leaves and flowers) of *Cassia surattensis* L. was done to remove the dust and shade dried and then ground into 60 mesh size Willy mill for solvent extraction. First of all methanolic extraction of ground plant material was done with methanol three times separately and then it was evaporated in a rotary evaporator under vacuum. This crude methanolic extract was partitioned by using equal volumes of *n*-hexane, chloroform, ethylacetate and *n*-butanol successively. These organic fractions were evaporated on rotary, dried and weighed to calculate the percentage yield of each fraction. These organic fractions as well as remaining aqueous were studied for the determination of phyto-constituents total phenolic contents and antioxidant potential.

Folin-ciocalteu reagent, 1,1-diphenyl-2-picryl hydrazyl (DPPH), sodium acetate, 2,4,6-triphenyl pyridyl-*s*-triazine (TPTZ), ABTS [2,2'-azinobis (3-ethyl benzothiazoline-6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid, $FeCl_3 \cdot 6H_2O$, dipotassium hydrogen phosphate (K_2HPO_4), potassium hydrogen phosphate (KH_2PO_4), linoleic acid, gallic acid, Tween-20 and ammonium thiocyanate were purchased from Aldrich Chemical Co. (Gillingham, Dorset, UK). Other solvents and chemical reagents were of analytical grade and purchased from either Sigma or Merck representatives. Mayer's reagent, Wagner's reagent, Hagner's reagent, Dragon's reagent, 0.5 M HCl, 1 % $AlCl_3$ solution, LiberMann-Burchard reagent by taking appropriate portion of powdered plant material.

Phytochemical tests of plant material: Phytochemical screening of plant material was performed for the detection of secondary metabolites like lipids, proteins, carbohydrates, alkaloids, flavonoids, steroids, tannins, saponins and anthraquinones *etc*., by using different reported methods[19-21].

Test for flavonids: The plant sample (5 g) was extracted with 20 mL of absolute ethanol for 5 min. The mixture was filtered in the 5 mL of filtrate solution 2 mL of 1 % $AlCl_3$ was added. The presence of yellow precipitates in the solution mixture was an indication of the presence of flavonids.

Test for alkaloids: The plant sample 0.5 g was boiled in a beaker with 40 mL ethanol and then 4 mL of conc. H_2SO_4 was added and further boiled for 5 min and then mixture was filtered. The filtrate was allowed to cool down to room temperature. Then few grams of lead acetate were added to in order to discharge the colour of the filtrate. Filtration was done again and four portions of the solution were made. These parts were tested for alkaloids with the help of Mayer's reagent, Wagner's reagent and Hager's reagent. The presence of turbidity or precipitation in the solution mixture was an indication for alkaloids.

Test for tannins: The plant sample (50 g) was extracted with 20 mL of distilled water for 5 min. The mixture was filtered in 5 mL of filtrate solution few drops of ferric chloride solution were added. The blue-green colour or blue-green precipitates were indicative for the presence of tannins.

Test for steroids: 2 mL of Lieber Mann-Burchard reagent was added to 5 mL water extract. The appearance of violet colour was indicative of steroids presence.

Test for anthraquinones: 5 g plant sample were extracted with 20 mL of absolute ethanol for 5 min. The mixture was filtered, in 5 mL of filtrate 1 mL of N,N-dimethyl aniline solution was added. Red coloration in the solution mixture was indicative of the presence of anthraquinones.

Test for saponnins: 0.2 g plant sample was heated to boiling and let it to boil for 10 min. The hot mixture was filtered and the filtrate was allowed to cool to room temperature. 5 mL of filtrate was diluted to 15 mL with distilled water and shaken vigorously till the appearance of persistence forth which was indicative of the presence of saponins. To test solution, 4 mL dilute H_2SO_4 was added in the 2 mL of above mentioned solution and boiled for 5 min the appearance of green colour was indicative of the presence of saponins.

Antioxidant assays

Determination of total phenolic contents (TPC): Amount of the total phenolic compounds in the aerial parts of *Cassia surattensis* were determined by using the reported procedure of Makkar *et al.*[22]. For this estimation 0.1 mL (0.5 mg/g) of the individual fraction of plant was mixed with the 2.8 mL of 10 % Na_2CO_3 solution and 0.1 mL of 2 N Folin-Ciocalteu reagent. Then absorbance at 725 nm was measured after 40 min by UV-visible spectrophotometer. Standard calibration curve was constructed for different concentrations of gallic acid. Total phenolic contents were determined as milligrams of gallic acid equivalents/g of sample by computing with standard calibration curve. Results are shown as gallic acid equivalents (GAE) in mg/g.

DPPH radical scavenging activity: Lee *et al.*[23] method was used for the estimation of DPPH radical scavenging activity of the samples relative to the butylated hydroxytoluene (BHT), a known antioxidant. Different concentrations of the sample (500, 250, 125, 50, 40, 20 and 10 μg/mL) were combined with the 3 mL of methanolic solution of DPPH (0.1 mM). Reaction was carried out at room temperature for an hour. Then in the UV-visible spectrophotometer absorbance was taken at 517 nm taking methanol as a blank. Sample with lower absorbance contained higher free radical scavenging activity.

FRAP assay: FRAP Assay means ferric reducing antioxidant power assay which was performed based on Benzie and Strain[24] method. 300 mM acetate buffer (3.1 g $CH_3COONa\cdot 3H_2O$ and 16 mL CH_3COOH) was taken as stock solution with pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3\cdot 6H_2O$ solution. Fresh solution was prepared by combining 2.5 mL TPTZ solution, 2.5 mL $FeCl_3\cdot 6H_2O$ solution and 25mL acetate buffer. This solution was warmed at 37 ºC before use. Both the solutions of sample and trolox were formed in methanol (1 mg/mL). 10 µL of each of sample solution and BHT solution were taken and mixed with 2990 µL of FRAP solution. That reaction mixture was kept in the dark for 0.5 h. Then absorbance of that coloured product was measured at 593 nm. Standard calibration curve was constructed for different concentrations of trolox which was compared with FRAP values as micromoles of trolox equivalents per mL of samples. Results were shown in TE µM/mL.

$ABTS^{\hat{}+}$ (radical cation decolorization) assay: $ABTS^{\hat{}+}$ Decolourization assay protocol[25] was used for the estimation of Trolox Equivalents Antioxidant Capacity (TEAC) of the samples. This assay was done as follows. First of all a mixture of ABTS and potassium persulphate (7 mM and 2.45 mM final concentration, respectively was prepared to produce ABTS radical cation, this reaction mixture was kept in the dark at room temperature for 12-16 h before using it. ABTS stock solution was diluted with PBS buffer (pH 7. 4) for the estimation of antioxidant activity of the sample and standard antioxidant to an absorbance of 0.70 ± 0.02 at 734 nm and equilibrated at 30 ºC then added 10 µL of sample or standard antioxidant to 2.99 mL of diluted $ABTS^{\hat{}+}$ solution to an absorbance of (0.700 ± 0.02) again at 30 ºC, absorbance was measured for 6 min with 1 min interval against blank in each assay. Following formula is used for the calculation of percentage inhibition.

$$\text{Inhibition (\%)} = \left(\frac{I - I_f}{I_o} \times 100\right)$$

I_o = Absorbance of radical cation solution before the addition of sample/std. antioxidant. I_f = Absorbance of radical cation solution after the addition of sample/std. antioxidant.

The results were shown as a plot between this concentration of antioxidants and that of trolox as standard reference curve.

RESULTS AND DISCUSSION

The phytochemical screening indicated that crude methanolic extract contained tannins, saponins, flavonoids, steroids, alkaloids as well as anthraquinones. The *n*-hexane soluble fraction contained only alkaloids. In the ethyl acetate soluble fraction flavonoids, steroids, alkaloids were present and no tannins, saponins and anthraquinones were detected. Tannins, flavonoids, steroids, alkaloids and anthraquinoes were present in chloroform soluble fraction but only saponins were absent. The *n*-butanol soluble fraction contained flavonoids, steroids, alkaloids and anthraquinones but no tannins and saponins. In the remaining aqueous fraction only flavonoids and steroids were present (Table-1).

Total phenolic contents: The plant fractions were evaluated by using Follin-Ciocalteu's reagent known as FC reagent for the determination of total phenolic contents. When the phenolic compounds are reduced by different hydroxyl species then the colour of FC reagent changes from yellow to blue according to the amount of phenolic compounds present in the plant extracts. At 725 nm spectrophotometrically, this change was observed. TPC value of ethyl acetate fraction of the plant material was highest (348.83 ± 1.5 mg/g) followed by MeOH fraction (136.0 ± 2.0 mg/g), *n*-hexane soluble fraction (120.5 ± 1.3 mg/g), chloroform soluble fraction (87.33 ± 0.9 mg/g), *n*-butanol soluble fraction (87.166 ± 0.4 mg/g) and least phenolic contents were in remaining water fraction (38.5 ± 2.0 mg/g). Higher the phenolic compounds in the samples higher the TEAC values. The phenolic moieties alone do not represent the total reducing charge of sample but there are also some synergistic interactions amongst different antioxidative compounds present in the sample which are considered to play part in the non-significant correlation between phenolic moieties and TEAC values.

DPPH free radical scavenging activity: This assay was used for the determination of the DPPH free radical scavenging capacity of the plant samples (Table-2). Antioxidant compounds present in the plant samples provides the hydrogen atom which reduces the single electron in DPPH which was monitored spectrophotometrically at 517 nm. Rapid decrease in the absorbance showed that the extract contained greater antioxidant activity. IC_{50} values were calculated and have been shown in Table-3 in the µg/mL of different samples of the plant which indicated that the ethyl acetate soluble fraction contained highest radical scavenging with IC_{50} of 4.00 ± 1.7 µg/mL, then decrease in the order was observed as follows: *n*-butanol soluble fraction with IC_{50} of 16.97 ± 1.5 µg/mL < crude methanolic extract with IC_{50} of 18.47 ± 0.7 µg/mL < chloroform soluble fraction with IC_{50} of 27.06 ± 1.3 µg/mL < remaining water fraction with IC_{50} of 83. 13 ± 0.4 µg/mL and at the end *n*-hexane soluble fraction with IC_{50} of 124.72 ± 0.9 µg/mL (Table-2). The results were compared with BHT, known as a standard antioxidant having IC_{50} of 12.54 ± 0.89 µg/mL.

TABLE-1
SECONDARY METABOLITES PRESENT IN DIFFERENT FRACTIONS OF *C. surattensis* L.

Plant sample	Tannins	Saponins	Flavonoids	Steroids	Alkaloids	Anthraquinones
Methanolic crude extract	+	+	+	+	+	+
n-Hexane soluble fraction	–	–	–	–	+	–
Chloroform soluble fraction	+	–	+	+	+	+
Ethylacetate soluble fraction	–	–	+	+	+	–
n-Butanol soluble fraction	–	+	+	+	+	+
Remaining aqueous fraction	–	–	+	+	–	–

Note: (+) shows presence and (–) shows absence.

TABLE-2
FREE RADICAL SCAVENGING ACTIVITY OF VARIOUS FRACTIONS OF *C. surattensis* L. BY DPPH ASSAY

S. No.	Plant sample	Conc. in (µg/mL)	Percentage scavenging of DPPH radical ± SEM[a]
1	Crude methanolic extract	50	67.47 ± 0.7
		40	57.19 ± 1.0
		20	46.44 ± 0.8
2	*n*-Hexane soluble fraction	500	93.17 ± 0.7
		250	75.60 ± 1.3
		125	51.40 ± 1.8
		50	34.01 ± 1.2
3	Chloroform soluble fraction	250	92.61 ± 1.1
		125	65.56 ± 1.0
		50	46.72 ± 1.4
4	Ethyl acetate soluble fraction	40	84.95 ± 1.0
		20	68.78 ± 2.0
		10	59.05 ± 1.8
		8	49.05 ± 1.6
5	*n*-Butanol soluble fraction	50	71.89 ± 1.3
		40	60.01 ± 1.4
		20	53.27 ± 0.9
		10	47.38 ± 0.5
6	Remaining aqueous fraction	500	95.04 ± 2.0
		250	76.16 ± 1.5
		125	55.04 ± 1.3
		50	42.24 ± 1.1
7	BHT[b]	500	95.51 ± 1.9
		250	95.42 ± 1.5
		125	94.95 ± 1.1
		50	79.43 ± 0.8

[a]Standard mean error of three assays. [b]Standard antioxidant.

FRAP Assay: FRAP assay is used for the determination of the reducing power of various samples which is shown by the colour change of the test solution from yellow to blue and green in proportionate to the reducing power of various samples. In a redox- linked colorimetric method antioxidants are used as reductants in FRAP assay and in stoichiometric excess it provides an easy reduced oxidant system[26]. Ferric form in a ferric tripyridyl triazine complex changes to ferrous form showing intense blue colour. This change was observed by measuring the absorption at 593 nm. In the reaction mixture the absorption change was linked directly with the total reducing power of electron donating antioxidants which reduced the ferric form (ferric cyanide complex) to the ferrous form. This study on *C. surattensis* L. sowed that highest FRAP value was of crude methanolic extract (158.5 ± 1.2 TEµM/mL) which was followed by *n*-butanol soluble fraction (55 ± 1.8 TEµM/mL), ethylacetate soluble fraction (9.0 ± 1.9 TEµM/mL), chloroform soluble fraction (7.5 ± 1.4 TEµM/mL), *n*-hexane soluble fraction (6.5 ± 1.1 TEµM/mL) and the least value was shown by remaining aqueous fraction (3 ± 0.9 TEµM/mL).

ABTS$^{\hat{}+}$ scavenging activity: *In vitro* radical scavenging activity of plant samples of *C. surattensis* was evaluated by ABTS decolorization assay. Hydroxyl radical produced *in vivo* during the stress and metabolic conditions has comparable reduction potential to the ABTS radical cation produced *in vitro*. Antioxidant components having lower reduction potential values than ABTS radical cation reduces the ABTS radical cation to its native form. By comparing the % inhibition values of samples with the trolox standard curve TEAC values were obtained. Greater TEAC value obtained for ethyl acetate soluble fraction (0.99 ± 0.9) was due to the greater solubility of antioxidant components in the ethyl acetate. Similarly small TEAC value for less polar solvent like *n*-hexane (0.12 ± 1.5) showed low solubility of phenolic and other antioxidant components.

Conclusion

These results lead to the conclusion that ethyl acetate and *n*-butanol soluble fractions of *Cassia surattensis* L. contained higher antioxidant potential which indicated that most of the active constituents of this plant were polar phenolic and flavonoid components and powerful antioxidant components were present in this plant as the results of different assays showed that ethylacetate fraction contained highest total phenolic contents (348.83 ± 1.5 mg of gallic acid equivalents) and also contained highest TEAC value (0.99 ± 0.9 mg) measured by ABTS assay. The highest % inhibition of DPPH was shown by ethyl acetate fraction with IC_{50} 4.00 ± 1.7 µg/mL of butylated hydroxytoluene (BHT) equivalents. Methanolic soluble fraction exhibited highest total TEAC value (158.5 ± 1.2) by FRAP method. Hence on the basis of aforesaid results this plant seems an overall indispensable source of natural antioxidants and can be helpful in controlling many degenerative diseases.

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TABLE-3
IC_{50} TOTAL PHENOLICS, TOTAL ANTIOXIDANT ACTIVITY, FRAP VALUES AND INHIBITION OF LIPID PEROXIDATION VALUES OF DIFFERENT SAMPLES OF *C. surattensis* L

S. No.	Plant samples	FRAP value TE (µM/mL) ± SEM[a]	TPC (GAE mg/g of extract) ± SEM[a]	ABTs (TEAC µg) ± SEM[a]	IC_{50} value (µg/mL) ± SEM[a]
1	Crude methanolic extract	158.5 ± 1.2	136.0 ± 2.0	0.37 ± 1.1	18.47 ± 0.7
2	Chloroform soluble fraction	7.5 ± 1.4	87.33 ± 0.9	0.93 ± 1.4	27.06 ± 1.3
3	Ethyl acetate soluble fraction	9.0 ± 1.9	348.83 ± 1.5	0.99 ± 0.9	4.00 ± 1.7
4	*n*-Butanol soluble fraction	55 ± 1.8	120.5 ± 1.3	0.43 ± 1.1	16.97 ± 1.5
5	*n*-Hexane soluble fraction	6.5 ± 1.1	87.166 ± 0.4	0.12 ± 1.5	124.72 ± 0.9
6	Remaining aqueous fraction	3 ± 0.9	38.5 ± 2.0	0.16 ± 1.7	83.13 ± 0.4

[a]Standard mean error of three assays.

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