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Short communication

Antioxidant properties of methanolic extracts of the leaves of seven Egyptian *Cassia* species

MAHER MAHMOUD EL-HASHASH[1]
MAHFOUZ MOHAMED ABDEL-GAWAD[2]
MORTADA MOHAMED EL-SAYED[2]
WAFFA AHMED SABRY[2]
EL-SAYED SALEH ABDEL-HAMEED[2]
EZZAT EL-SAYED ABDEL-LATEEF[2,*]

[1] *Chemistry Department, Faculty of Science, Ain Shams University Egypt*

[2] *Laboratory of Medicinal Chemistry Theodor Bilharz Research Institute Warrak El-Hader Giza, Egypt*

In the present study, antioxidant activity of methanolic extracts of the leaves of seven Egyptian *Cassia* species was investigated using two methods, the phosphomolybdate method and 1,1 diphenyl-2-picrylhydrazyl radical ($DPPH^{\cdot}$) scavenging activity method. The results revealed that *C. glauca* is the most potent species and that the activity of other plant species decreases in the following order: *C. grandis* > *C. nodosa* > *C. fistula* > *C. didymobtrya* > *C. occidentalis* > *C. sophera*.

Defatted methanolic extract of the most active plant *C. glauca* was subjected to fractionation using different organic solvents such as $CHCl_3$, EtOAc and BuOH. Antixidant activities of the fractions were investigated and the results showed that ethyl acetate fraction possessed high activity. Total phenolic and flavonoid concentrations of each plant extract were determined using the Folin-Ciocalteu reagent and aluminum chloride. Correlation between radical scavenging capacities of extracts and total phenolic concentration was observed.

Keywords: *Cassia* species, antioxidant, phenolics, flavonoids, DPPH

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Reactive oxygen species (ROS) or oxygen free radicals can cause damage to cells and tissues during infections and various degenerative disorders such as cardiovascular diseases, aging and neurodegenerative diseases, like Alzheimer's disease, mutations and cancer (1, 2). The most widely used synthetic antioxidants, butylated hydroxyanisole (BHA) and butylated hydroxytoulene (BHT) have been restricted because of serious concerns about their carcinogenic potential (2, 3).

Natural antioxidants, especially phenolics and flavonoids, are safe; they protect the human body from free radicals and retard the progress of many chronic diseases as well as lipid oxidative rancidity in foods (4). Numerous studies were carried out on plants with antioxidant properties (3–5). However, there is still great interest in finding new antioxidants from natural sources.

* Correspondence; e-mail: ezzat_ea@yahoo.com

Cassia genus (family *Leguminosae*) represents one of the largest and diverse groups of flowering plants, including herbs to trees. Plants of the genus *Cassia* are widely distributed in most tropical and subtropical countries. *Cassia* species have biological and medical activities such as hepatoprotective, antibacterial, antifungal, antioxidant, antitumor, antidiabetic and antiparasitic (6). Species of the genus *Cassia* have been also used as laxative, purgative, antipyretic, antiviral, as well as anti-inflammatory agents. Phytochemical analysis of certain *Cassia* species led to the isolation of flavonoids, anthraquinones, proanthocyanidins and condensed tannins (7–9). The aim of this study was to determine the total phenolic concentration and antioxidant properties of defatted methanolic extracts of seven Egyptian *Cassia* species by using the phosphomolybdate and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging methods.

EXPERIMENTAL

Chemicals

DPPH was purchased from Fluka (Germany). Aluminum chloride, sodium carbonate, sodium phosphate, ammonium molybdate, ascorbic acid and gallic acid were purchased from Aldrich Chemicals (USA). The Folin-Ciocalteu reagent (FCR) was freshly prepared according to the method described by Huang and Prior (10). All other chemicals and solvents used were of analytical grade and purchased from either Sigma or Merck, Germany. Absorbance measurements were recorded using the ultraviolet-visible spectrophotometer, Spectronic 601Milton Roy (USA).

Plant material

The leaves of seven *Cassia* species under investigation, *Cassia didymobtrya*, *C. fistula*, *C. glauca*, *C. grandis*, *C. nodosa*, *C. occidentalis* and *C. sophera*, were collected in March 2007 from the Giza Zoo, El-Orman Botanical Garden, El-Zohrea Garden and the Garden of the Faculty of Agriculture, Giza, Egypt. The plants were kindly identified by Mrs. Traes Labib of the El-Orman Botanical Garden, Giza, Egypt. Voucher specimens were deposited in the Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Giza, Egypt. The plants were dried in shade, finely powdered with an electric mill and kept for the extraction process.

Extraction and fractionation

A hundred grams of fine powdered leaves of each species were soaked in 300 mL 85 % methanol for one week at room temperature, with shaking day by day followed by filtration and further extraction for three times. The solvent was removed in vacuum using a rotatory evaporator, affording a known mass of each methanolic extract. Each plant extract was defatted with petroleum ether and the remaining defatted methanol extract was dried and became ready for chemical investigation.

Ten grams of the most active methanolic extract of *C. glauca* was dissolved in 40 mL distilled water and then successively partitioned with chloroform, ethyl acetate and finally with *n*-butanol (4 x 50 mL solvent), affording a known mass of each respective fraction.

DPPH free radical scavenging activity

The free radical scavenging activity of the different extracts was measured according to the procedure described by Shirwaikar *et al.* (11). Various concentrations of each extract (2 mL) were added to 2 mL solution of 0.1 mmol L^{-1} DPPH. An equal volume of methanol and DPPH served as a control. After 20 min of incubation at 37 °C in the dark, the absorbance was recorded at 517 nm. The experiment was performed in triplicate. The DPPH radical scavenging activity was calculated and the SC_{50} (concentration of sample required to scavenge 50 % of DPPH radicals) value was evaluated. Decrease of the DPPH solution absorbance indicates an increase of the DPPH radical scavenging activity.

Total antioxidant capacity by the phosphomolybdate method

The total antioxidant capacity of the extract was evaluated by the phosphomolybdate method (12). The assay is based on the reduction of Mo(VI) to Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Each sample solution (0.3 mL) and ascorbic acid (100 μg mL^{-1}) were combined with 3 mL of reagent (0.6 mol L^{-1} sulfuric acid, 28 mmol L^{-1} sodium phosphate and 4 mmol L^{-1} ammonium molybdate). A typical blank solution contained 3 mL of reagent solution and an appropriate volume of the solvent used for the sample. All tubes were capped and incubated in a boiling water bath at 95 °C for 90 min. After the samples were cooled to room temperature, the absorbance of the solution of each sample was measured at 695 nm against the blank. The experiment was performed in triplicate. The antioxidant activity is expressed as equivalents of ascorbic acid (AAE).

Phytochemical screening

Identification of the major chemical constituents of defatted methanolic extracts of *Cassia* species was carried out using the standard procedures previously described by Edeoga *et al*. (13).

Total phenolic concentration

The concentration of total phenolics of each plant extract was determined according to the method described by Kumar *et al*. (14). Gallic acid was used as standard. Briefly, a mixture of 100 μL of plant extract (100 μg mL^{-1}), 500 μL of Folin-Ciocalteu reagent and 1.5 mL of Na_2CO_3 (20 %) was shaken and diluted up to 10 mL with water. After 2 hours, the absorbance was measured at 765 nm. All determinations were carried out in triplicate. The total phenolic concentration was expressed as gallic acid equivalents (GAE).

Total flavonoid concentration

Total flavonoid concentration of each plant extract was determined according to the reported procedure by Kumaran and Karunakaran (4). 100 μL of plant extract (10 mg mL^{-1}) in methanol was mixed with 100 μL of 20 % $AlCl_3$ in methanol and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorbance was measured at 415 nm after 40 min against the blank. The blank consisted of all reagents and solvent without

$AlCl_3$. All determinations were carried out in triplicate. The total flavonoid concentration was expressed as rutin equivalents (RE).

Statistical analysis

All data were presented as mean ± SD. The SC_{50} values were calculated using the SPSS 13.0 program by probit-graphic interpolation for six concentration levels.

RESULTS AND DISCUSSION

The results in Table I reveal that all methanolic extracts of *Cassia sp*. showed a good inhibitory activity against DPPH radical. SC_{50} values ranged from 19 to 226 μg mL^{-1}, with the extract of *C. glauca* showing the highest antiradical activity with SC_{50} of 18.53 μg mL^{-1} while the lowest activity showed *C. sophera* with SC_{50} of 225.88 μg mL^{-1}.

Total antioxidant capacity of the seven species using the phosphomolybdate method are displayed in Table I, showing that the methanolic extract of *C. glauca* has the highest antioxidant activity of 533.85 mg AAE g^{-1} extract and drops to that of *C. occidentalis* with 134.50 mg AAE g^{-1} extract.

As it is expected and seen in Table II the methanolic extract of *C. glauca* has the highest concentration of phenolics and flavonoids (206.76 mg GAE g^{-1} and 71.36 mg RE g^{-1}).

To the best of our knowledge, there is no information available in the literature on the antioxidant activity of the leaves of the seven *Cassia* species under investigation, except for *C. fistula*. The high activity of *C. glauca* prompted us to select it for the fractionation process using three organic solvents such as $CHCl_3$, EtOAc and BuOH. Results in Table III show that the EtOAc fraction had the highest antiradical activity toward $DPPH^{\cdot}$ with SC_{50} of 5.43 μg mL^{-1}, followed by BuOH and $CHCl_3$ fractions with SC_{50} of 40.96

Table I. Yield, free radical scavenging activity and total antioxidant capacity of methanolic extracts of Cassia *species*

Species	Yield (%)	DPPH free radical scavenging activity SC_{50} (μg mL^{-1})[a]	Total antioxidant capacity (mg AAE g^{-1} extract)[b]
C. didymobtrya	18.9	202.75	184.01
C. fistula	20.2	132.86	198.55
C. glauca	27.9	18.53	533.85
C. grandis	23.5	50.37	240.00
C. nodosa	26.7	75.15	210.51
C. occidentalis	22.4	213.48	134.50
C. sophera	21.8	225.88	136.53

[a] SC_{50} – concentration in μg mL^{-1} required for scavenging the DPPH radical (100 μg mL^{-1}) by 50 %. Mean value, $n = 2$.
[b] Antioxidant capacity monitored by the phosphomolybdate method. Mean value, $n = 2$.

Table II. Total amount of phenolic and flavonoid compounds of methanolic extracts of Cassia *species*

Species	Total phenols (mg GAE g^{-1} extract)[a]	Total flavonoids (mg RE g^{-1} extract)[a]
C. didymobtrya	122.76 ± 2.85	24.83 ± 1.05
C. fistula	138.63 ± 0.79	45.53 ± 0.81
C. glauca	206.76 ± 2.00	71.36 ± 7.20
C. grandis	169.73 ± 2.90	39.03 ± 1.00
C. nodosa	161.60 ± 1.90	47.76 ± 1.13
C. occidentalis	117.13 ± 2.19	24.66 ± 2.34
C. sophera	101.46 ± 1.85	22.56 ± 1.15

[a] Mean ± SD, $n = 3$.

Table III. Yield, free radical scavenging activity and total antioxidant activity of Cassia glauca *fractions*

Fraction	Yield (%)	DPPH free radical scavenging activity SC_{50} (μg mL^{-1})[a]	Total antioxidant capacity (mg AAE g^{-1} extract)[a]
Chloroform	0.3	44.07 ± 0.64	338.32 ± 7.48
Ethyl acetate	3.2	5.43 ± 0.30	818.68 ± 0.30
Butanol	4.0	40.96 ± 3.55	443.35 ± 2.66
Ascorbic acid	–	7.90 ± 0.21	–

[a] Mean ± SD, $n = 3$.

and 44.07 μg mL^{-1}, respectively. Also, the antioxidant capacity monitored using the phosphomolybdate method showed that the EtOAc fraction had the highest antioxidant activity (818.6 mg AAE g^{-1} extract), followed by BuOH and $CHCl_3$ fractions (443.3 and 338.3 mg AAE g^{-1} extract, respectively).

Concentrations of total phenolics and flavonoids are presented in Table IV. It is evident that ethyl acetate fraction has the highest total phenolic content (366.50 mg GAE g^{-1}) whereas $CHCl_3$ and BuOH fractions have 136.53 and 174.15 mg GAE g^{-1}, respectively. Total flavonoid concentrations of the three fractions as shown in Table IV are equivalent to 28.50, 52.06 and 69.10 mg RE g^{-1}, respectively.

There is a linear correlation between the total phenolic concentration of methanol extracts of seven *Cassia* species and their total antioxidant capacity and free radical scavenging activity, with R^2 of 0.789 and 0.937, resp. Also, positive correlation has been recorded between total phenolic concentration of the $CHCl_3$, EtOAc and BuOH fractions of *C. glauca* and their total antioxidant and free radical scavenging activity. These results are in full agreement with previous studies, which proved that there is a linear correlation between the total phenolic concentration and the antioxidant capacity of some medicinal plants (2–5).

Table IV. Total concentrations of phenolic and flavonoid compounds from different fractions of Cassia glauca

Fraction	Total phenols (mg GAE g^{-1} plant extract)[a]	Total flavonoids (mg RE g^{-1} plant extract)[a]
Chloroform	136.50 ± 3.89	28.50 ± 1.52
Ethyl acetate	366.50 ± 1.90	52.06 ± 0.13
Butanol	174.15 ± 2.83	69.10 ± 0.07

[a] Mean ± SD, $n = 3$.

CONCLUSIONS

Free radical scavenging activity and total antioxidant capacity of methanolic extracts of the seven *Cassia* species increases with increasing the total phenolic concentration, *C. glauca* having the most potent antioxidant activity. Therefore, chromatographic separation and identification of the chemically active constituents of these plants are already underway.

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S A Ž E T A K

Antioksidativno djelovanje metanolnih ekstrakata listova sedam egipatskih vrsta roda *Cassia*

MAHER MAHMOUD EL-HASHASH, MAHFOUZ MOHAMED ABDEL-GAWAD, MORTADA MOHAMED EL-SAYED, WAFFA AHMED SABRY, EL-SAYED SALEH ABDEL-HAMEED i EZZAT EL-SAYED ABDEL-LATEEF

U radu je ispitano antioksidativno djelovanje metanolnih ekstrakata listova sedam egipatskih vrsta roda *Cassia* koristeći fosfomolibdatnu metodu i metodu vezanja slobodnih 1,1-difenil-2-pikrilhidrazil radikala (DPPH). Rezultati pokazuju da *C. glauca* ima najveću aktivnost te da se djelovanje smanjuje sljedećim redom: *C. grandis* > *C. nodosa* > *C. fistula* > *C. didymobtrya* > *C. occidentalis* > *C. sophera*. Odmašćeni metanolni ekstrakt najaktivnije biljke *C. glauca* frakcioniran je pomoću različitih organskih otapala kao što su $CHCl_3$, EtOAc i BuOH. Ispitivanje antioksidativnog djelovanja pojedinih frakcija pokazuje da je etil-acetatna frakcija najaktivnija. Pomoću Folin-Ciocaltuovog reagensa i aluminijevog klorida određena je ukupna koncentracija fenola i flavonoida svakog pojedinog ekstrakta. Uočena je korelacija između sposobnosti hvatanja slobodnih radikala i ukupnog sadržaja fenola.

Ključne riječi: Cassia vrste, antioksidans, fenoli, flavonoidi, DPPH

Chemistry Department, Faculty of Science, Ain Shams University, Egypt

Laboratory of Medicinal Chemistry, Theodor Bilharz Research Institute, Warrak El-Hader, Giza, Egypt