

Research Article

Phytochemical Studies of *Cassia occidentalis* Linn. Flowers and Seeds in Various Solvent Extracts

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ABSTRACT

In the present study, the phytochemical screening of *Cassia occidentalis* Linn. were performed in petroleum ether, chloroform and methanolic extracts. The chloroform and methanolic extracts of both flower and seed were found to contain flavonoids, alkaloids, phenolics/tannins, steroids, glycosides and anthraquinones. The antioxidant potential of flowers and seeds in different solvent extracts were evaluated by various biochemical assays namely, DPPH (2, 2'-diphenyl-1-picrylhydrazyl) radical scavenging activity, reducing power activity. Their SC50 and EC50 values were determined to evaluate the therapeutic potential, in which seeds were found to have higher antioxidant activity revealed by lower SC50 and EC50 value. The total phenol, flavonoid, flavonol and tannin content were determined for both parts to study the free radical scavenging property. The seeds were found to have higher antioxidant activity when compared to flowers in various solvent extracts indicating their pharmacological property.

Keywords: *Cassia occidentalis* Linn., Phytochemical screening, Phenol and flavonoid content, Radical scavenging, SC50, EC50.

INTRODUCTION

Plants contain a wide variety of free radical scavenging molecules, such as flavonoids, anthocyanins, carotenoids, dietary glutathione, vitamins and endogenous metabolites that are rich in antioxidant activities. These plant derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, peroxide decomposers, enzyme inhibitors and synergists¹. The free radicals induce oxidative damage to biomolecules (lipids, proteins and nucleic acids), the damage which eventually causes atherosclerosis, aging, cancer, diabetes mellitus, inflammation, AIDS and several degenerative diseases in humans².

Flavonoids act as natural antioxidants^{3,19}. Phenolic compounds, including phenolic acid and flavonoids have been recognized as having health-related properties, including anticancer, antiviral and anti-inflammatory activities⁴. Synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole, have been restricted to be used as food as they are suspected to induce adverse side effects and also carcinogenic. Therefore, the importance of search for natural antioxidants has greatly increased in the recent years⁵.

Cassia occidentalis, a native plant of South India, commonly called as Coffee Senna belongs to the family Caesalpiniaceae. Their roots, leaves and seeds were used for fever, menstrual problems, tuberculosis, diuretic, anemic, liver complaints and as a tonic for general weakness and illness⁶. This plant is also used to cure sore

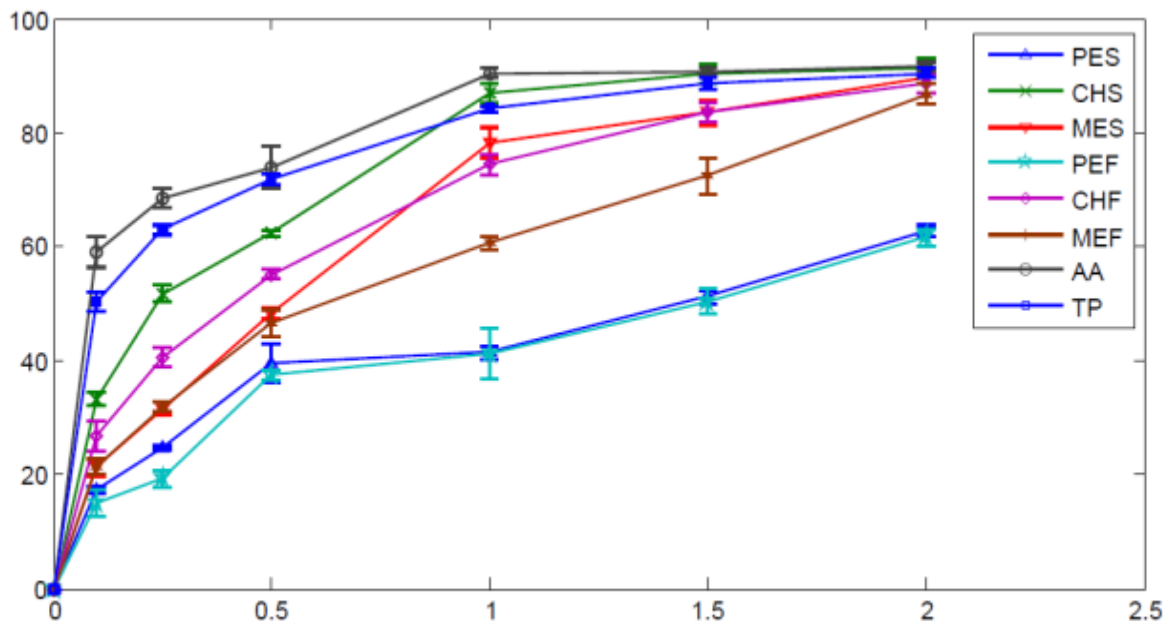
eyes, hematuria, rheumatism, typhoid, asthma, disorder of haemoglobin, leprosy⁷. The seeds are brewed into a coffee like beverage for asthma, malaria, fevers and stomach complaint. Aqueous extract of *Cassia occidentalis* exhibited significant antihyperglycemic activity in normal and alloxan induced diabetic rats⁸. In Ayurveda their leaves, bark and seeds were used in the treatment of diabetes, inflammation, ulcers and leprosy. It is bitter, sweet and also used as a purgative⁹. The chemical constituents isolated from *Cassia occidentalis* leaves are alkaloids, flavonoids, tannins, phlobatannins, chrysophanol, emodin, physcion, tetrahydroanthracene, derivative, germichryson, occidentalins A and B¹⁰. Seeds were found to contain galactomannan gum, tannic acid, oils, amodiene, toxalbumin and crisarobin¹¹. The aim of the present study is to evaluate the antioxidative properties present in flower and seeds of *Cassia occidentalis*.

MATERIALS AND METHODS

Chemicals: Folin-Ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Potassium ferricyanide, Trichloroacetic acid (TCA), Ferric chloride, Gallic acid, (±)-Catechin, Rutin, Aluminium chloride, α-Tocopherol, Ascorbic acid and all solvents used were of analytical grade and purchased from Sigma Chemical Co. (St. Louis, MO, USA).

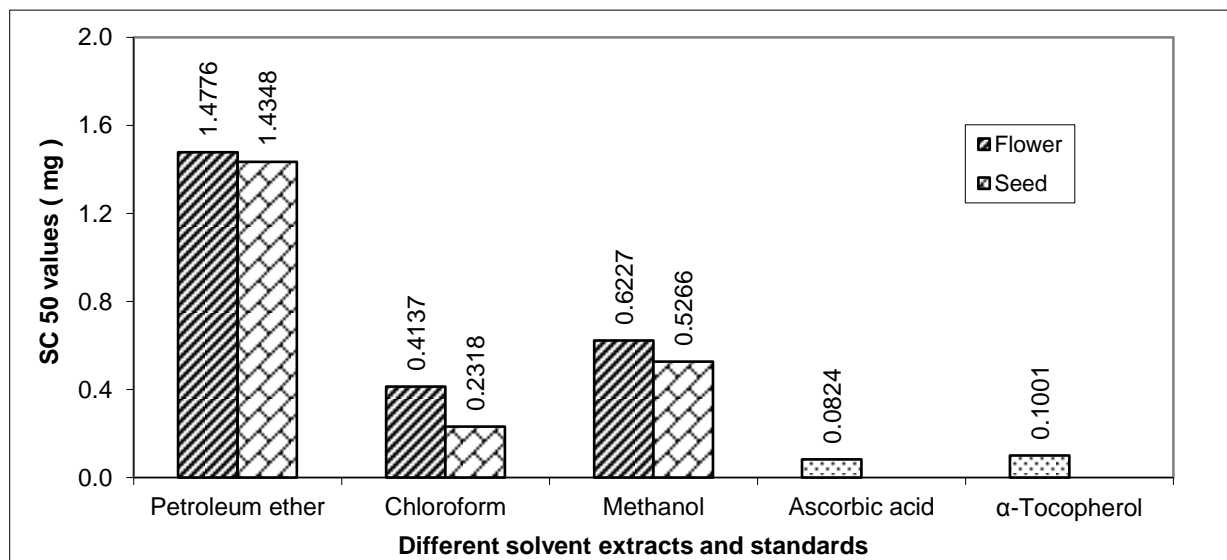
Plant Collection and Extraction: *Cassia occidentalis* flowers and seeds were collected from Periyar University

Figure 1. DPPH radical scavenging activities of *Cassia occidentalis* seed and flower extracts in different concentrations. Each value represents a mean \pm SD.



MES=Seed Methanol extract; CHS=Seed chloroform extract; PES=Seed Petroleum ether; MEF=Flower Methanol extract; CHF=Flower chloroform extract; PEF=Flower Petroleum ether; AA=Ascorbic acid; TP= α -Tocopherol.

Figure 2. SC₅₀ Values of DPPH radical scavenging activity compared with various extracts with standards like α -tocopherol, Ascorbic acid.

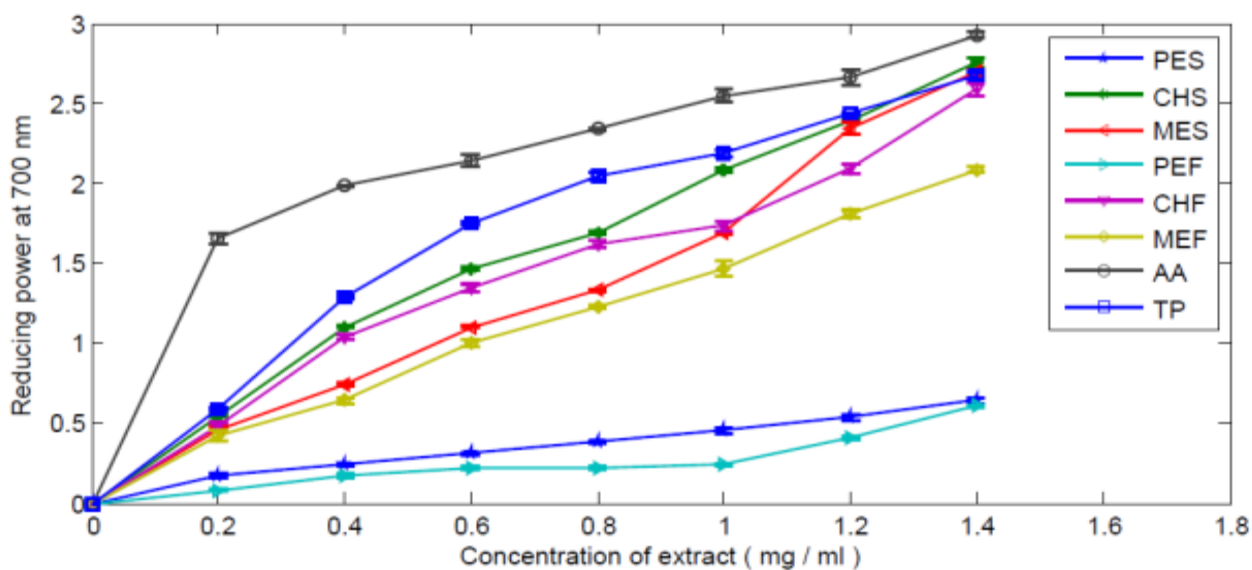


Campus, Salem. The plant materials were identified and confirmed by Botanical Survey of India (BSI / SRC / 5 / 23 / 10-11 / Tech.423). The plant material washed with tap water, prior to distilled water were shade dried and powdered. The powdered plant materials were subjected to successive extraction with petroleum ether, chloroform and methanol using Soxhlet Extractor. The extracts were dried in vacuum pump at 40°C. The dried extract was stored in freezer at 0°C for future use. Phytochemical Screening: The preliminary phytochemical screening tests

were carried out to screen the useful constituents by standard methods¹².

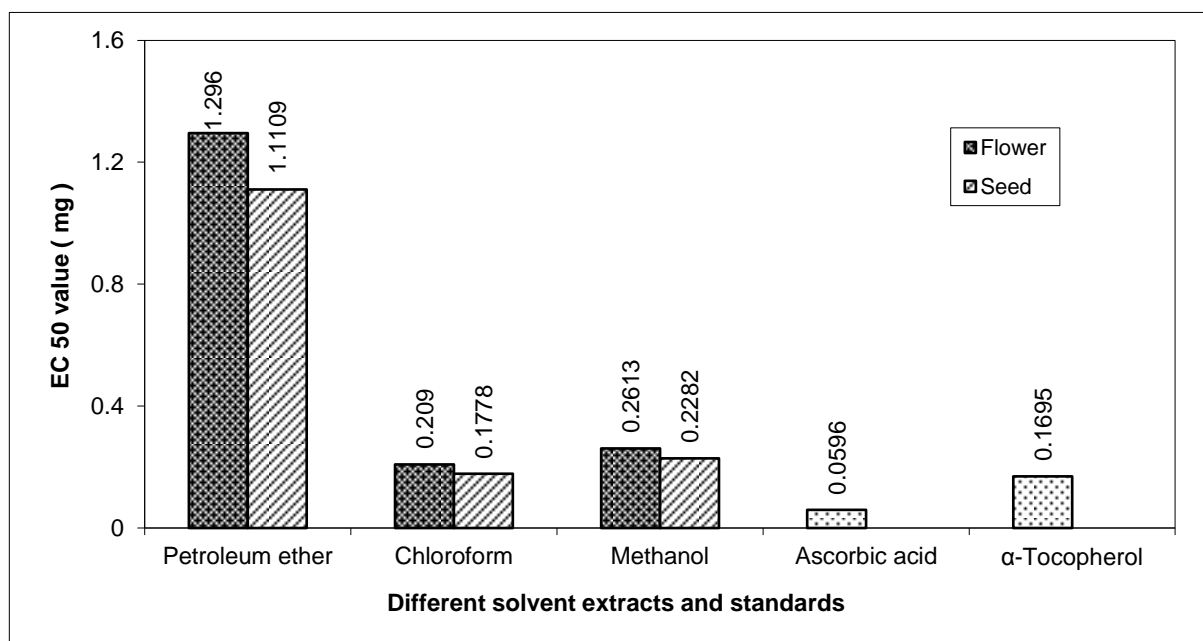
Determination of Total Phenolic Contents: The total phenolics in the extracts were estimated by Spectrophotometric assay¹³. One mL of sample (concentration 1 mg/mL) was mixed with 1 mL of Folin and Ciocalteu's phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which

Figure 3. Reducing power of *Cassia occidentalis* seed and flower extracts in different concentrations. Each value represents a mean \pm SD.



MES=Seed Methanol extract; CHS=Seed chloroform extract; PES=Seed Petroleum ether; MEF=Flower Methanol extract; CHF=Flower chloroform extract; PEF=Flower Petroleum ether; AA=Ascorbic acid; TP= α -Tocopherol.

Figure 4. EC₅₀ Values of Reducing power activity compared with flower and seed extracts with standards like α -tocopherol, Ascorbic acid.



the absorbance was read at 725 nm. Gallic acid was used for constructing the standard curve (20-100 μ g/mL, $Y=0.094x+0.0117$, $R^2=0.9673$) and the results were expressed as μ g of gallic acid equivalents/mg of extract (GAEs).

Determination of Total Flavonoid Contents: Flavonoid contents in the extracts were determined by Spectrophotometric method¹³. The (250 μ L) extract (concentration 1 mg/mL) was mixed with 1.25 mL of

distilled water and 75 μ L of a 5% NaNO₂ solution. After 5 min, 150 μ L of 10% AlCl₃ solution was added. After 6 min, 500 μ L of 1 M NaOH and 275 μ L of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (\pm)-Catechin was used to calculate the standard curve (20-120 μ g/mL, $Y=0.0085x+0.0375$, $R^2=0.999$) and the results were expressed as μ g of (\pm)-catechin equivalents (CEs) per mg of extract.

Table 1. Phytochemical screening of *Cassia occidentalis* seed and flower extracts using various solvents.

Phytochemical constituents	Tests	Flower			Seed		
		PE	CH	ME	PE	CH	ME
Alkaloids	Mayers test	-	+++	++	-	+++	+++
	Wagners test	-	++	+	-	++	++
	Hagers test	-	++	++	-	++	+++
Phenolics / Tannins	FeCl ₃ test	+	+++	+++	+	+++	+++
	Lead acetate test	-	++	+	-	+++	+++
	K ₂ Cr ₂ O ₇ test	-	++	+	-	+++	++
Flavonoids	Shinoda test	+	+++	+++	+	+++	+++
Proteins / Amino acids	Ninhydrin test	-	-	-	-	+	+
	Biuret test	-	+	+	-	-	-
Carbohydrates	Molisch's test	-	++	++	-	++	+++
	Fehling's test	-	+	+	-	+++	+++
	Barfoed's test	-	-	-	-	++	+++
Fats / Oils	Sudan IV test	+++	+	+	+++	+	-
Steroids	Salkowski test	-	++	+	-	+	++
	Liebermann's test	-	+	+	-	+	++
Saponins	Foam test	+++	-	-	+++	-	-
Terpenoids	Knollar's test	-	++	++	-	++	++
Cardiac glycoside	Keller-Killiani test	-	++	+	-	++	+
Anthraquinones	Borntrager's test	-	-	-	-	++	+

PE=Petroleum ether extract; CH=Chloroform extract; ME=Methanol extract:

+++ = Copiously present, ++ = Moderately present, + = Slightly present, - = Absent

Estimation of Total Flavonol Contents: One mL of extract (concentration 1 mg/mL) was mixed with 1 mL aluminium trichloride (5 mg/mL) and 3 mL sodium acetate (25 mg/mL). The absorbance read at 440 nm was read after 2.5 h. The absorption of standard rutin solution (0.5 mg/mL) in methanol was measured under the same conditions¹⁴. All determinations were carried out in triplicates. The amount of flavonols in plant extracts in rutin equivalents (RE) were calculated by the following formula. $X = (A.m0)/(A0.m)$, where X is the flavonol content, mg/mg plant extract in RE, A is the absorption of plant extract solution, A0 is the absorption of standard rutin solution, m is the weight of plant extract (mg), m0 is the weight of rutin in the solution (mg).

Estimation of Tannins Content: Tannin content of the extracts were measured by Folin-Denis method¹⁵. 50µL of extract was made upto 7.5mL by adding double

distilled water. Then 0.5mL Folin- Denis reagent and 1mL of Na₂CO₃ were mixed with it. Again volume was made upto 10mL by double distilled water. Absorption was recorded at 700nm.

DPPH Radical Scavenging Activity: Various concentrations of *Cassia occidentalis* extracts (0.3 mL) were mixed with 2.7 mL of methanol solution containing DPPH radicals (6×10⁻⁵ mol/L). The mixture was shaken vigorously and allowed to stand for 60 min in the dark. The reduction of the DPPH radical was determined by reading the absorbance at 517 nm¹⁶. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: % RSA = [(ADPPH - AS)/ADPPH] x 100, where AS is the absorbance of the solution when the sample extract is added at a particular level and ADPPH is the absorbance

Table 2. Total phenolic, tannin, flavonoid and flavonol content of *Cassia occidentalis* seeds and flowers by various solvents.

Solvents Used	Plant parts	Total phenolic content (μg gallic acid / mg of plant extract)*	Total Tannin content (μg Tannic acid / mg of plant extract)*	Total flavonoid content (μg (\pm)-catechin / mg of plant extract)*	Total flavonol content (mg rutin / mg of plant extract)*
Chloroform	Flower	41.31 \pm 1.759	107.99 \pm 1.145	123.09 \pm 4.002	156.807 \pm 3.858
	Seed	48.98 \pm 2.272	113.60 \pm 2.961	132.87 \pm 4.002	159.76 \pm 4.174
Methanol	Flower	21.28 \pm 3.091	80.862 \pm 2.356	47.88 \pm 3.493	60.193 \pm 0.779
	Seed	26.41 \pm 1.161	103.38 \pm 0.905	55.13 \pm 1.185	104.211 \pm 1.705
Petroleum ether	Flower	7.06 \pm 1.713	9.533 \pm 1.717	9.32 \pm 0.884	25.021 \pm 1.102
	Seed	7.34 \pm 1.185	20.082 \pm 2.719	11.92 \pm 0.377	27.413 \pm 1.92

*Each value represents a mean \pm SD (n=3).

of the DPPH solution. The extract concentration providing 50% of radical-scavenging activity (SC50) was calculated from the graph of RSA percentage against extract concentration. Ascorbic acid and α -tocopherol were used as standards.

Reducing Power: The reducing power of *Cassia occidentalis* extracts were determined according to the method¹⁵. Various concentration of different solvent extract (1mL), phosphate buffer (1mL, 0.2M, pH=6.6) and potassium ferricyanide (1 mL, 10 mg/mL) were mixed together and incubated at 50°C for 20 min. Trichloroacetic acid (1 mL, 100 mg/mL) was added to mixture and centrifuged at 8,000 rpm for 5 min. The supernatant (1 mL) was mixed with distilled water (1mL) and ferric chloride (0.1 mL, 1 mg/mL) and then the absorbance was measured at 700 nm.

Statistical Analysis: All the assays were carried out in triplicate. The results are expressed as mean values and standard error (SE) or standard deviation (SD) of the mean. Results are given as mean arithmetic values \pm SE.

RESULTS

Phytochemical Screening: The preliminary phytochemical screening of the chemical constituents present in the parts of *Cassia occidentalis* are listed in Table.1.

Phenolic Content: Satisfactory phenolic contents were observed for all the extracts. A significant content of about 41.31 μg was obtained for flower and 48.98 μg of seed in chloroform extract.

Tannin Content: Chloroform extract of both flower and seed has recorded higher contents. Very closely the methanolic extract of seed has presented a good content of about 103.38 μg .

Flavonoid Content: About 123.09 μg and 132.87 μg were recorded for the flavonoid content of flower and seed in chloroform extract. These contents have shown a greater difference from the methanol extracts thereby revealing its potency.

Flavonol Content: Excellent flavonol contents of about 156.81 μg and 159.76 μg was given by the plant parts in chloroform extract comparatively. For a note, the methanolic seed extract recorded 104.211 μg .

DPPH Radical Scavenging Activity: An increase of % RSA value with increase in concentration has been displayed in Fig. 1 for all the solvent extracts of flower and seed against the standard ascorbic acid and α -tocopherol. SC50 values for the parts are presented in Fig.2. The chloroform extract of seed has recorded 0.2318 mg as SC50 value, proving its potency with reference to the standard.

Reducing power: Reducing power of flower and seeds in various extracts were found to increase with increase in concentration (Fig.3). An efficient EC50 value of 0.1778 mg was observed for seed in chloroform extract and 0.209 mg was observed as EC50 for flower in chloroform extract (Fig.4). For all the estimations, the petroleum ether extracts of both flower and seed showed lesser contents comparatively (Table 2).

DISCUSSION

The qualitative screening of the subjected plant parts has revealed the presence of many useful chemical constituents. They have been confirmed for their presence by one or two tests and displayed from copious, moderate to slightly present. Many researchers have shown that vegetables prevent cancer and cardiovascular diseases due to their high amount of antioxidants including anthocyanin, total phenolic contents³. Hence, it is worth determining the important contents like flavonoid, flavonol, tannin and phenolics. They have shown their enormous presence in the screening tests too. Many of phenolics have been shown to contain high levels of antioxidant activity. This activity of phenolics is mainly due to their redox properties that would allow the extracts to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelating potential¹⁷.

As per general way, the contents of these constituents estimated were often found to be high in highly polar solvent extracts. It was surprising to record significant contents in chloroform extract for all the estimations.

A closely related constituents (i.e) phenolics and tannins have shown their contents to be high in chloroform extracts of both the parts. In particular, the seed extracts have produced good contents in contrast to that of flower extracts. It can be noted that the tannin content were

found to be greater than phenolic groups. Hence the polyphenolic compounds might be present in large amounts in seeds than free phenolic groups. Tannins were greatly considered to be responsible for the antioxidant activity, due to the presence of number of hydroxyl groups. Similar to chloroform extract, seeds have produced higher contents in methanol and petroleum ether extracts for almost all the contents, compared to flower.

In the classes of flavonoids, excellent contents were observed for both flavonol and flavonoid. For instance, a seed in chloroform extract has recorded greater contents than any other extract, in case of the flavonol content. Chemical structure activities of the flavonols by various methods have consistently shown that flavonols have superior antioxidant activity to flavone-3-ol, which may be due to the presence of an *o*-dihydroxy group and 2, 3-double bond in conjugation with 4-oxo functions. This increases the stabilization of the generated radical¹⁸. In all the extract, seeds have been observed to produce good contents compared to that of flower, which was same in the case of phenolic and tannin contents. Thus the higher contents in chloroform extract for both flower and seed may be due to the fact that the equally polar compounds present in the plant might have got extracted in chloroform. Also it can be assumed that the chloroform extract can exhibit good antioxidant and radical scavenging activity when compared with methanol and petroleum ether extract.

The two invitro antioxidant assays viz. DPPH radical scavenging activity and reducing power activity has given a support to the potency of both seed and flower, especially their chloroform extracts. DPPH-scavenging activity is a method based on the reduction of DPPH• solution by hydrogen-donating process of the extracts. It can be inferred that this activity increased with increasing concentration of the extract which is due to the ability of that concentration of the extract to scavenge the free radical. The highest scavenging capacity of seeds was observed in the chloroform extract. The seed extract recorded the lowest 50% inhibition activity (0.2318mg) proving the strongest scavenging activity of the chloroform extract. It can be checked out that there is an inverse relationship between the SC50 value and percentage of DPPH scavenging potential.

Reducing power activity works on the principle of reducing Fe³⁺ to Fe²⁺ by donating electron. As per this assay, an increase in absorption with increase in concentration was observed for all the extracts. The chloroform extract of seed showed a considerable ability of reducing Fe³⁺ to Fe²⁺ ions compared to the flower chloroform extracts varying with concentration. Also, a very low EC50 value (0.1778 mg) for this extract showing that, this extract reduces the ions with such a low concentration. Hence even a low concentration of the extract shall reduce the ions. Methanol extracts of flower and seed exhibited satisfactory activities not much better than chloroform. Whereas, the petroleum ether extract was found to have poor radical scavenging activity value and reducing activity for both the parts, due to the

presence of constituents like oils / fat, lipids, saponins etc. It does not contain any active compounds like phenolic, flavonoid, alkaloids, tannins etc., thus its lower activity. From this work it can be deeply observed that the seeds of *Cassia occidentalis* can be recognized for its immense antioxidant activity and can be used for free radical mediated diseases. Future work is concentrated on the identification and isolation of active compounds present in these parts.

CONCLUSION

In the present study, the chloroform seed extract had higher antioxidant activity when compare to flower extracts. Phenolic, flavonoids, flavonols and tannin compounds contribute a major part to the antioxidant properties of *Cassia occidentalis*. EC50 values for radical scavenging activity and reducing power activities were significant in seed extract in contrast to flower extract. This leads a support to traditional knowledge and can serve as a basis for selecting the most active medicinal plant to use in traditional medicinal practice in future. *Cassia occidentalis* seeds being safe, non-toxic, economic and potent can be used as a natural antioxidant. The future work will be focussed on isolation of active compounds.

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