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Research Article

PHYTOCHEMICAL INVESTIGATION OF *CASSIA ALATA* LINN. FLOWERS THROUGH VARIOUS *INVITRO* ANTIOXIDANT ASSAYS

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Abstract:

Antioxidant activities of various crude extracts of *Cassia alata* flowers through *invitro* assays and estimating the flavonoid and phenolic content has been of interest. Qualitative screening tests revealed the presence of numerous therapeutic phytoconstituents. Ethyl acetate fractions recorded higher contents: 56.58 µg for phenolics, 157.71 µg for flavonoid, in contrast to other extracts. Potential antioxidant assays such as DPPH- radical scavenging activity, reducing power, inhibition of lipid peroxidation was carried out and compared with the positive reference compound, butylated hydroxyl anisole. Various concentrations of different extracts (hexane, chloroform, ethyl acetate, methanol, water) presented active SC_{50}/ EC_{50}/ IC_{50} values for these respective assays in a concentration-dependant manner. Being in good agreement with contents, ethyl acetate extract provided better results for the above parameters compared with standard, in contrast with other extracts. Hence these flower extracts could be useful for preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

Keywords: Antioxidant, *Cassia alata*, flavonoid, lipid peroxidation, phenolic, reducing power, scavenging activity

Introduction:

An increasing interest in the search for natural replacements of synthetic antioxidants has led to the antioxidant evaluation of a number of plant sources. The neutraceutical trend towards doubling the impact of natural antioxidants that stabilize food and maximize health impact presents distinct challenges in evaluating

antioxidant activity. Thus, there is a renewing interest in phytomedicine during last decade and nowadays many medicinal plant species are being screened for its pharmacological activities. This has influenced many pharmaceutical companies to produce new formulations extracted from plants or herbs. Natural products have been shown to possess a tremendous and consistent resource for the development of new drugs. India is the major exporter of raw MAP'S (Medicinal Aromatic Plants) and processed plant-based drugs. About 45,000 plant species with medicinal properties have been assigned to several thousands for the turn over of herbal medicines in India as over the counter products, ethical and classical formulations. However, very few plant species have been thoroughly investigated for their medicinal properties. Plant species still serve as a rich source of many novel biologically active compounds. With the development of natural product chemistry, the potential of chemotaxonomy is now becoming increasingly obvious. The application of chemical data to systematics has received serious attention of a large number of biochemists and botanists during the last three decades. Recently, the ability of phenolic substances including flavonoids and phenolic acids to act as antioxidants has been extensively investigated. Several flavonoids are reported to be responsible for the broad therapeutic effects. They are important for human beings due to their antioxidative and radical scavenging effects as well as their potential estrogenic and anticancer activities[1]. The flavonoid family includes flavones, flavonols, flavanols, leucoanthocyanidins, anthocyanidins, aurones, chalcones, and isoflavones.

Cassia alata (Leguminosae) has been reported to have various phytochemical activities. Pharmacological investigations performed so far on *Cassia alata* have shown that this herb has several biological activities, such as antimicrobial[2], antifungal[3], purgative[4], anti-inflammatory[5], analgesic[5], hypoglycemic[5] and antitumor[6] activities. The leaf extracts of this species were abundantly reported for its meaningful biological significance[7]. Several important works have been established on the chemical constituents of its stem part[8]. Many anti-inflammatory reports have also been done on the leaf extracts and flavonoids classes, especially flavonoid glycosides[9] have been isolated. Considering the importance of this area, as a part of our ongoing investigation on natural antioxidants, the useful phytochemical properties of local medicinal plant will be discussed. Therefore, the primary objective of the present

study was to compare the *invitro* antioxidant activity of various solvent extracts of *Cassia alata* flowers which are traditionally used in folkloric remedies for multiple disorders, where free radicals are thought to be involved.

Materials and Methods:

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), gallic acid, (+)-catechin and solvents like hexane, chloroform, ethyl acetate, methanol were purchased from Sigma Chemicals Pvt. Ltd.

Plant selection and Authentification

Our approach involved the consultation with experts, collection (during the specific flowering season), identification, extraction and the phytochemical screening based on their antioxidant activity, derived primarily from a random selection of commonly occurring native plants. All the information regarding their taxonomy and their action were collected from the book "Encyclopedia of Indian Medicinal Plants"[10]**.** At the time of collection, a pressed specimen was prepared and authenticated by a Botanist (Specimen Voucher no. 781), Botanical Survey of India, Southern Circle, TNAU, Coimbatore.

Extraction procedure

The old, insect-damaged, fungus-infected flowers were removed and only the fresh, healthy flowers were selected and washed well with dechlorinated water prior to distilled water, deprived of dusts. It was then chopped, air-dried, and coarsely powdered in a mixture grinder. Soxhlet extraction of the powdered flower samples was carried out using each of the following solvents in increasing polarity: n-Hexane (defatting), ethyl acetate, chloroform, methanol and finally with water which is highly polar comparatively. The soxhelation process was carried out until the solvent was found to be colorless. Then the solvent was filtered and distilled, till the extract turns off into a syrupy consistency. All the crude solvent extracts were stored at 4–5˚C until further use.

Phytochemical screening: The flower extracts of *Cassia alata* were diluted in their respective solvents and subjected for qualitative preliminary phytochemical screening to identify the presence of the secondary metabolites

according to the standard methods[11]. From the intensity of the color inferred for the tests, they will be rated for their presence.

Determination of antioxidant contents

Contents of total phenolics in the extracts were estimated by a colorimetric assay based on standard procedures[12]. Basically, 1ml of sample was mixed with 1ml of Folin and Ciocalteu's phenol reagent. After 3min, 1ml of saturated sodium carbonate solution was added to the mixture and it was adjusted to 10ml with distilled water. The reaction was kept in the dark for 90min, after which the absorbance was read at 725 nm (Analytik Jena 200–2004 spectrophotometer). Gallic acid was used for constructing the standard curve (0.01–0.4mM; y = 2.94848 x -0.09211; R^2 = 0.99914) and the results were expressed as mg of gallic acid equivalents g^{-1} of extract (GAEs).

Flavonoid contents in the extracts were determined by a colorimetric method[12]. The flower extract (250µl) was mixed with 1.25ml of distilled water and 75µl of a 5% $NaNO_2$ solution. After 5min, 150µL of 10% $AlCl_3.H_2O$ solution was added. After 6min, 500µl of 1M NaOH and 275µl of distilled water were added to prepare the mixture. The solution was mixed well and the absorbance was read at 510 nm. (+)-Catechin was used to calculate the standard curve (0.250–2.500mM; Y = 0.2903; R^2 = 1.0000) and the results were expressed as mg of (+)-catechin equivalents (CEs) per g of extract.

DPPH radical-scavenging activity

Various concentrations of *Cassia alata* flower extracts (0.3ml) were mixed with 2.7ml of methanol solution containing DPPH radicals (6 x 10^{-5} mol/l). The mixture was shaken vigorously and left to stand for 60min. in the dark (until stable absorbance values were obtained). The reduction of the DPPH radical was determined by reading the absorbance at 517 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration, using the equation: $\%RSA = [(A_{DPPH} - A_S) / A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract is added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution[12]. BHA and α-tocopherol were used as standards.

Reducing power

Various concentrations of *Cassia alata* extracts (2.5ml) were mixed with 2.5ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was incubated at 50˚C for 20min. After 2.5ml of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 1000 rpm for 8min (Centorion K24OR-2003 refrigerated centrifuge). The upper layer (5ml) was mixed with 5ml of deionised water and 1ml of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically[12] at 700 nm. BHA and α-tocopherol were used as standards.

Inhibition of lipid peroxidation using thiobarbituric acid reactive substances (TBARS)

The procedure[13] using a Fenton reaction-induced lipid peroxidation has been adapted for this assay. The extracts of all species in concentration of 100µg/ml have been mixed with 300µl Tris- HCl buffer, pH=7.5, 500µl of 20mM linoleic acid and 100µl of 4mM $FeSO_4$. The peroxidation was started with the addition of 100µl 5mM ascorbic acid. The reaction mixture was incubated for 60min at 37°C. Thereafter, 2ml of 10% ice cold trichloroacetic acid was added and 1ml aliquot of the samples was added with 1ml of thiobarbituric acid. The TBA/sample mixture was heated in the water bath at 95°C for another 60min. the absorbance was read at 532 nm and the percentage of linoleic acid peroxidation inhibition was calculated using appropriate controls. BHA was used as positive control.

Results:

Phytochemical screening

The preliminary qualitative screening analysis of different extracts of *Cassia alata* flowers revealed the presence of active constituents like alkaloid, phenolics, flavonoids and its classes, saponins, carbohydrates, glycosides, tannins, anthocyanins etc., as listed in table.1. Most of the constituents showed their copious presence in ethyl acetate extract.

Total phenolic content: The total phenolic content for different fractions (hexane, chloroform, ethyl acetate, methanol, water) of the flower extracts revealed the richest source in ethyl acetate extract. Clear differences

between the contents of various crude extracts were recorded. The increasing order obtained for the extracts were crude hexane (12.28µg)> crude chloroform (27.98µg)> crude ethyl acetate (56.58µg)> crude methanol (42.13µg)> crude water (34.64µg) respectively.

Total flavonoid content

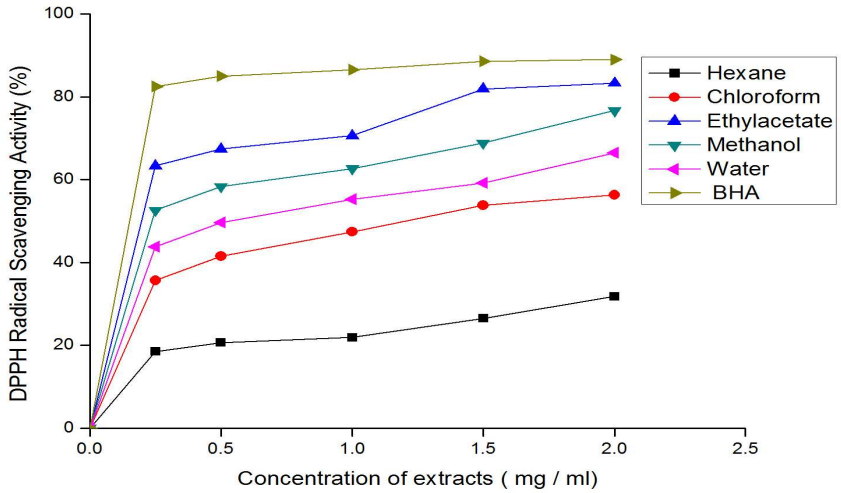
The richest content of flavonoid was exhibited by the ethyl acetate extract. The order of this content was ranked as similar to phenolic content which is as follows: crude hexane (62.5µg)> crude chloroform (98.16µg)> crude ethyl acetate (157.71µg)> crude methanol (146.89µg)> crude water (111.77µg) respectively.

The differences in concentration of these contents are likely to be responsible for their varied scavenging effects.

DPPH- radical scavenging activity

The scavenging effects of various extracts of *Cassia alata* flowers were found to be reliable providing an evidence for their antioxidant effect. Scavenging of DPPH radicals was found to rise with increasing concentration of the extracts (Fig.1), being a concentration dependant manner with effective SC_{50} values. Among the various extract a significant activity was given by ethyl acetate extract (0.1982µg/ml) against the positive control BHA (0.1527µg/ml). The order of the activities of various extracts was in good agreement with their contents.

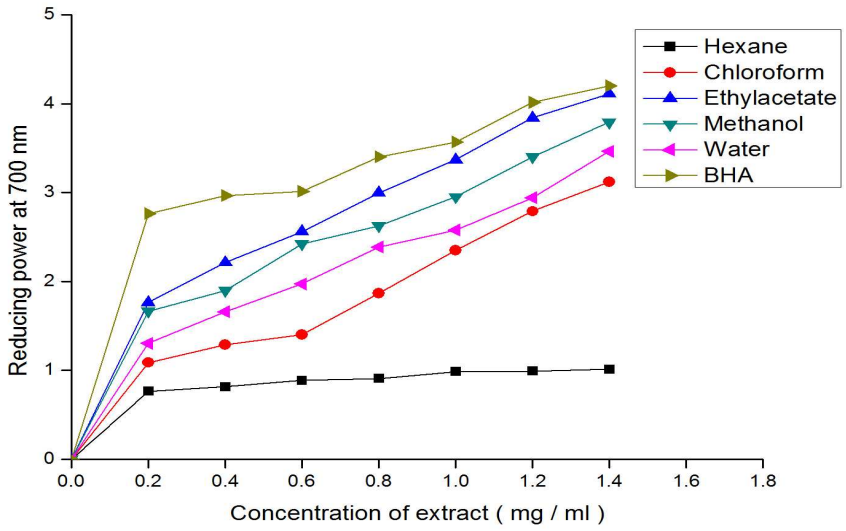
Fig 1: DPPH-radical scavenging activity of *Cassia alata* flower extracts.



Reducing power

Fig.2 represents the ability of various extracts to reduce Fe^{3+} to Fe^{2+} as a function of their concentration. An increase in the reducing power with the increase in concentration was observed which was found to be excellent. The EC_{50} value of all the extracts was calculated showing a significant power for ethyl acetate extract among the other extracts. The standard BHA, showed an EC_{50} value of about 0.0371µg/ml. A least activity was measured in the water extract (0.08914µg/ml).

Fig 2: Reducing power activity of *Cassia alata* flower extracts.



Inhibition of lipid peroxidation induced by thiobarbituric acid reactive substances (TBARS)

The extracts lowered the degree of lipid peroxidation induced by hydroxyl radical generated by an iron/ascorbate system. For comparison, the inhibition was efficient in ethyl acetate extract incontrast to all other extracts, given by their IC_{50} values. A good competence was given by the ethyl acetate extract with the standard (0.2018µg/ml).

The SC_{50}, EC_{50} and IC_{50} values of various extracts are portrayed in table.2, except the hexane extract due to their least activity.

Discussion:

Our results of various free-radical scavenging assays have established the antioxidant potential of *Cassia alata* flower extracts and the major constituents present in it might be responsible for the observed *invitro* scavenging ability of this species. In this context, it emphasizes the fact that each part has its own unique arsenal of flavonoids, polyphenols and other compounds that contribute to this particular activity. The following segment of discussion throws light on the variation in the activities of the solvent extracts that influence the observed antioxidant potential.

The presence of numerous constituents in the qualitative phytochemical screening has provided a support for the assessment of the antioxidant assays like DPPH- radical scavenging activity, reducing power, inhibition of lipid peroxidation (TBARS). Many studies have shown that flavonoids and related polyphenols contribute significantly to the total antioxidant activity, and hence the determination of total phenolic and flavonoid content may be efficient. The order of those contents has recorded their richest amount in ethyl acetate extract. This can be due to compounds got extracted matching to the polarity of the solvent or the chances of those constituents got dissolved in that particular fraction. Methanol, though being more polar compared to ethyl acetate, has not revealed higher contents. The reason behind this may be the presence of compounds of *Cassia alata* flowers may not be as high as compounds matching ethyl acetate polarity. This fact shall be applied for hexane, chloroform and water extracts too. The least content was recorded in hexane extract due to the presence of low polar compounds, mainly lipids and pigments got extracted in the same, which can be referred with their screening tests. Widely the presence of these polar compounds is believed to play the role of antioxidant activity by donating electron to terminate the free-radical chain reaction.

Fig.1 illustrates the antioxidant potential of *Cassia alata* crude extracts through DPPH-radical scavenging activity in various concentrations. Each extract has shown a clear scavenging activity being concentration dependant. Focusing on their absorbance results a systematic decrease in the absorption with increase in

concentration was noted revealing the fading colour change in the test extracts scavenging the stable free radical, DPPH. It is noteworthy that the SC_{50} values are the determining factor of the antioxidant activity through this assay. The amount of each extract needed for 50% of radical scavenging ability (SC_{50}) was considered to be efficient and moreover a significant SC_{50} value was given by the ethyl acetate extract followed by methanol, water and chloroform. Although these extracts were found to be potent scavengers, their SC_{50} values are lesser than the synthetic antioxidant, BHA. At higher concentration (2.0mg/ml) all the extracts except chloroform showed more than 60% scavenging activity. At this particular concentration the ethyl acetate extract exhibited 83.3% which was found to be greater than the %RSA of the standard (82.5%) at its lowest concentration (0.25mg/ml). Thus these extracts that scavenge the free radicals may be of great importance in preventing the onset and propagation of disease causing parameters.

Specially, phenolic substances are known to possess high antioxidative activity based on their ability to donate hydrogen atom to free radicals, thereby exhibiting a wide range of biological effects. The reducing assay is thus considered to be one of the best works with respect to the antioxidant activity. Fig.2 shows the dose-response curve with an increase in concentration. Ethyl acetate fractions showed a remarkable activity to react with its reducing capacity [Fe^{3+} to Fe^{2+}] and hence can reduce free radicals to convert them into more stable non-reactive species and to terminate radical chain reaction. This trend of ferric ion- reducing activities has been followed by all other extracts too, where chloroform and water fractions showed lower reducing activities. The EC_{50} values calculated for the various extracts has presented the efficiency of each extract at the concentration 0.5mg/ml as discussed above a significant EC_{50} value was recorded by ethyl acetate extract. The order of reactivity obtained in this assay was in agreement with the contents and scavenging activity. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process. Ethyl acetate is often used as an extraction solvent with a significant selectivity in the extraction of low molecular weight phenolics and high molecular weight polyphenols[14].

The results obtained for TBARS assay showed a fairly constant high percentage inhibition. IC_{50} values calculated to observe the amount of each extract needed for 50% inhibition. Fig.3 revealed that the inhibition of peroxidation increased by raising the extract concentration. The material extracts had overall a good antioxidant activity (%inhibition) which was about 0.465 mg/ml for ethyl acetate followed by methanol (0.513 mg/ml), whereas 0.66 and 0.692 mg/ml for water and chloroform extracts respectively against the standard BHA (0.2018 mg/ml). These effects are due to the presence of antioxidant compounds which are vital substances that possess the ability to protect body from damage by free radical- induced oxidative stress[15].

Fig 3: Inhibition of lipid peroxidation (TBARS) of *Cassia alata* flower extracts

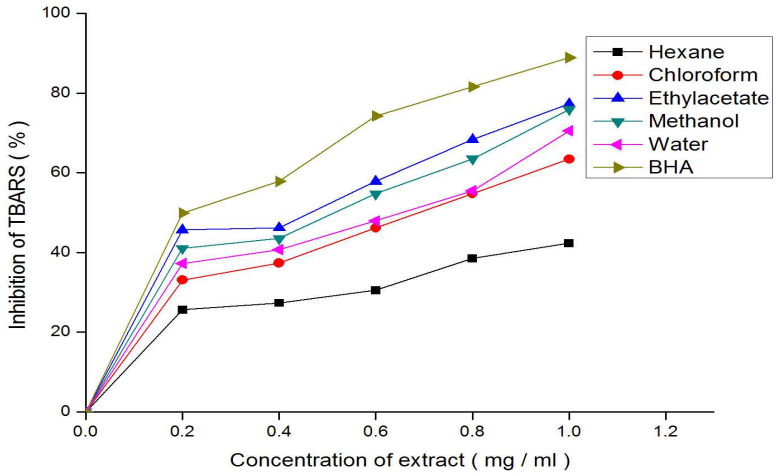


Table 1: Phytochemical screening of various extracts of *Cassia alata* flowers

Constituents	Name of the test	*Cassia alata* flowers in various extract				
		HE	CH	EA	ME	WA
Alkaloids	Wagner's	-	-	+	+	-
	Meyer's	+	-	+	+	+
	Dragendorff's	+	-	-	+	-
Flavonoids	Ferric chloride	-	+	+	+	+
	Shinoda's	+	+	+	+	-
	Fluorescence	-	-	-	-	-

Flavones	Alkaline	-	-	+	+	-
	Mg-Hcl	-	-	+	+	-
	Con. H_2SO_4	-	+	+	+	+
Flavonols	Alkaline	-	+	+	+	-
	Mg-Hcl	-	+	+	+	-
	Con. H_2SO_4	-	-	+	+	+
Isoflavonoids	Alkaline	-	-	+	-	+
	Mg-Hcl	-	-	+	+	+
	Con. H_2SO_4	-	-	+	+	+
Anthocyanins	Alkaline	+	+	+	-	-
	Mg-Hcl	+	-	-	-	-
Phenolics	Ferric Chloride	-	+	+	+	+
	Lead acetate	-	-	+	+	+
	Dichromate	-	-	+	+	+
Tannins	Gelatin	-	-	+	+	-
	KOH	-	-	+	+	-
Saponins	Foam	+	+	-	-	-
Carbohydrates	Molisch's	+	+	+	+	+
	Fehling's	-	-	+	+	-
	Barfoed's	-	-	+	+	+
	Benedict's	+	+	+	+	+
	Borntrager's	-	+	+	-	-
Proteins/ aminoacids	Millon's	+	+	+	+	-
	Ninhydrin	+	-	-	-	-
Steroids	Libermann's & Burchard's	+	-	-	-	-
	Salkowski's	+	-	-	-	-
Terpenoids	Hager's	+	-	-	-	-
	Knollar's	+	-	-	-	-

Glycosides	Keller- killiani	+	+	+	+	-
	Brown ring	+	+	+	+	+
Fats/ Oils	Biuret's	+	+	-	-	-

a) HE- Hexane, b) CH- Chloroform, c) EA- Ethyl acetate, d) ME- Methanol, e) WA-Water

Table 2: SC_{50}/ EC_{50}/ IC_{50} values of various *invitro* antioxidant assays.

***Invitro* assays**	**Extracts**				
	CH	**EA**	**ME**	**WA**	**BHA**
DPPH-radical scavenging activity (SC_{50})	1.1947	0.1982	0.2343	0.1527	0.1527
Reducing activity (EC_{50})	0.08914	0.0531	0.0583	0.0743	0.03715
Inhibition of lipid peroxidation (TBARS) (IC_{50})	0.6921	0.4652	0.5132	0.6606	0.2018

a) HE- Hexane, b) CH- Chloroform, c) EA- Ethyl acetate, d) ME- Methanol, e) WA-Water, f) BHA- Butylated hydroxyanisole

Conclusion:

In conclusion, the present study appraises the antioxidant and free radical propensities present in the flowers of *Cassia alata* crude extracts. *Invitro* biochemical assays of ethyl acetate fraction among the other extracts, merits their applications on food and pharmaceutical industries. Being antioxidative in nature, *Cassia alata* flowers might depend on their total phenolic and flavonoid content to act as a protective shield against numerous free radical mediated diseases.

References

1. Springob K and Saito K. Metabolic engineering of plant secondary metabolism: Promising approach to the production of pharmaceuticals. *Sci. Cul, 68,* 76-85, 2002.
2. Ibrahim D, Osman H. Antimicrobial activity of *Cassia alata* from Malaysia. *J. Ethnopharmacol. 45*, 151-156, 1995.
3. Co LL. Common Medicinal Plants of the Cordillera Region, Bustamante Press, Quezon City, 1989, 11.
4. Rai PP. Alatinone, an anthraquinone from *Cassia alata*. *Curr. Sci. 47*, 271, 1978.
5. Villase˜nor IM, Canlas AP, Pascua MP, Sabando MN, Soliven LAP. Bioactivity studies of *Cassia alata* Linn leaf extracts. *Phytother. Res. 16*, S93, 2002.
6. Belkin M, Fitzgerald Dorothea B, Cogan W. Determination of pharmacologically active compounds in root extracts of *Cassia alata* by use of HPLC. *J. Natl. Cancer Inst. 13*, 139, 1952.
7. Ohsumi K, Ito M, Ohmori Y, Kishi M, Katada T, Riswan S, Kanishi H. Anti-inflammatory activities of some traditional Indonesian crude-drugs. *Natural medicine, 49,* 472-474, 1995.
8. Tiwari RD and Yadava OP. Alatinone, an anthraquinone from *Cassia alata. Planta Medica, 19,* 299, 1971.
9. Hiroyoshi Moriyama, Tohru Iizuka, Masahiro Nagai, Hideki Miyataka, Toshio Satoh. Anti-inflammatory activity of heat-treated *Cassia alata* leaf extract and its flavonoid glycoside. *The Pharmaceutical Society of Japan, 123* (7), 607-611, 2003.
10. Dr. CP. Khare, Encyclopedia of Indian Medicinal Plants. Springer, 1st edition, India, 2007.
11. Trease GE and Evans WCA, Pharmacognosy. 12th Edn., English Language Book Society, Baillire 1985, Tindall, 394.
12. Barreira CM, Ferreira CFR, Beatriz M, Olliveira PP, Jose Alberto Pereira. Antioxidant activities of the extracts from chestnut flower, leaf, skins and fruit. *Food Chem., 107,* 1106-1113, 2008.
13. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MZ. Evaluation of the antioxidant action and flavonoid composition of *Artemisia* species extracts. *Plant Sci. 163*, 1161-1168, 2002.

14. Scholz E, Rimpler H. Proanthocyanidins from *Krameria triandra* root. *Planta medica, 55*, 379-384, 1989.

15. Souri E, Amin G, Sharifabadi AD, Nazifi A, Farsam H. Antioxidant activity of sixty plants from Iran. *Iranian J Pharmaceutical Res., 3*, 55-59, 2004.

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