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Original Article

PHARMACOGNOSTIC AND PHYTOCHEMICAL STUDIES OF *CASSIA ABSUS* SEED EXTRACTS

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ABSTRACT

Objective: To carry out the pharmacognostic and phytochemical analysis of different extracts of seeds of *Cassia absus* (CA).

Methods: The CA plant material (seeds) was studied for its organoleptic, microscopic and physicochemical parameters. The seeds of CA were crushed, and their extraction was carried out in succession with 4 solvents of increasing polarities, *viz* hexane, ethyl acetate, alcohol, and hydro alcohol. Preliminary phytochemical screening as per standard methods was carried out on all the extracts. Thin layer chromatography of the extracts revealed their qualitative nature. Quantitative estimation of their phenol, flavonoid and sterol contents was carried out. Their antioxidant capacities were evaluated with the help of DPPH and reducing power assays. Their anti-glycation and anti-inflammatory activities were also measured.

Results: Qualitative phytochemical analysis showed the presence of alkaloids, flavonoids and steroids in the extracts. The hydroalcoholic extract showed higher phenolic content and DPPH activity among other extracts. The flavonoid, sterol and alkaloid content, and Ferric reducing power were found to be higher in ethyl acetate extract. The hexane extract displayed a higher anti-glycation activity, followed by the ethyl acetate extract.

Conclusion: A complete phytochemical screening, along with the evaluation of macroscopic and microscopic characters was carried out for 4 extracts of the seeds of *Cassia absus.* Quantitative estimation of flavonoid, phenol, sterol and alkaloid content was carried out. The antioxidant, anti-inflammatory, and anti-glycation capacities of the extracts were tested. Alkaloids were successfully extracted from the defatted seeds.

Keywords: *Cassia absus*, Seeds, Anti-oxidant, Anti-glycation, Anti-inflammatory, Alkaloids.

INTRODUCTION

Cassia absus Linn. (CA) belongs to family Fabaceae and commonly known as *Chaksu* in the traditional system of Ayurveda. It is distributed throughout India and Sri Lanka and is also seen across the continents of Australia and Africa. It is an erect, sparingly branched annual plant, and has a height of around 1500 m [1]. Its scientific classification is given in table 1.

Table 1: Scientific classification of *Cassia absus*

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Fabales
Family	Fabaceae
Sub-family	Caesalpinioideae
Genus	*Cassia*
Species	*Absus*

The seeds and leaves of the plant are most commonly used for medicinal purposes though the roots have also been studied. The leaves are bitter, acrid and have been used traditionally for a cough, diseases of the nose, and as an astringent to the bowel. The seeds have been used to treat eye diseases since ancient times, and hence, have been rightly called *Chakshu* (eye, in the Sanskrit language) [2].

Since this plant held a lot of importance in the field of ophthalmology, further chemical studies were carried out. The most studied phytochemicals in the seeds of the plant are the alkaloids Chak sine and iso Chak sine [3]. Table 2 lists some of the other phytochemicals isolated from the plant seeds. Studies involving the extracts of the seed have shown antibacterial, antimalarial and blood pressure lowering effects. [1, 4]A recent study has also evaluated the anti-inflammatory and the anti-histaminic activity of an eye drop formulation containing the seeds of the plant [5].

To further highlight the potential of this plant to enter the mainstream medicinal market, a lot of standardization needs to be carried out to assess its purity and its efficacy. This involves a detailed study of the microscopic, macroscopic and physicochemical characteristics of the plant part along with the quantitative determination of the phytochemicals present, which this study has aimed at.

MATERIALS AND METHODS

Plant material

The seeds were collected from a local vendor in Mumbai and were authenticated by Agharkar Research Institute, Pune. A sample was deposited at the herbarium under the voucher number of S-173. The seeds were then crushed till a coarse powder and stored in an airtight and dry container until further use.

Reagents and instruments

All chemicals and reagents used during the experimentation were of analytical grade, purchased from Merck, India. Perkin Elmer Lambda 25 was the spectrophotometer used for all the assays. Motif, B1 Advanced Series photographic microscope was used to study the microscopic details.

Organoleptic characteristics

The organoleptic (macroscopic) observations of the powdered samples, i.e. colour, texture, odour, etc. were made employing the sense organs.

Microscopic studies

For the microscopic evaluation, the ground seed powder was soaked in a solution of 20% chloral hydrate and then mounted on a glass slide with the help of glycerine. The mounted slides were then observed under a photographic microscope with a magnification of 400 X.

Evaluation of physicochemical parameters

After the organoleptic and microscopic examination, physico-chemical parameters, i.e. the determination of ash content, extractable matter and moisture content (or loss on drying) of the powdered samples were carried out.

The ash left after igniting the plant material is measured by different methods to ascertain the amount of total ash, water-soluble ash, sulphated ash and acid-insoluble ash. Determination of extractive values helps predict the amount of active constituents extracted with solvents from a given amount of plant sample. Drying the sample after weighing it and reweighing it after complete drying give a measure of the amount of moisture present in the sample [12].

Table 2: Compounds (and their types) isolated from CA seeds

S. No.	Compound isolated from CA seeds	Type of compound	Ref No
1	Chaksine	Alkaloid	[3]
2	Isochaksine	Alkaloid	[3]
3	Luteolin	Flavone	[6]
4	Allelochemical	Glucoside	[6]
5	Galacto mannan	Carbohydrate	[7]
6	Raffinose	Carbohydrate	[8]
7	CAT1	Protein (Trypsin inhibitor)	[9]
8	Linoleic acid	Fatty acid	[10]
9	Beta-sitosterol	Sterol	[10]
10	Ketooctadec-cis-15-enoic acid	Fatty acid	[11]

Preparation of plant extracts

Powdered seeds were made to undergo successive Soxhlet's extraction (50 g in 300 ml of solvent) with the help of solvents of increasing polarities, i.e. Hexane, ethyl acetate, alcohol and hydroalcoholic. Hence, the extracts were named CA-Hex (hexane extract), CA-EA (ethyl acetate extract), CA-AL (alcoholic extract), and CA-HA (hydroalcoholic extract). The extracts obtained were dried with the help of a rotary evaporator.

Qualitative phytochemical screening

The extracts were subjected to preliminary phytochemical screening as per the standard chemical methods [13]. These methods help the identification of various groups of phytochemicals present in the plant samples.

Thin layer chromatography (TLC)

Thin layer chromatography of all extracts was performed on a 10 × 10 cm Silica gel 60 F_{254} plates (Merck, Darmstadt, Germany). A phytochemical standard beta-sitosterol was also used. Aliquots of each of the extracts were separately applied (samples and standard) to the plate as a 6 mm wide band, 8 mm from the bottom. The 10 ml mobile phase consisted of toluene: ethyl acetate: acetic acid (6:1:1). Development was carried out in a twin glass chamber saturated with the mobile phase, and derivatization was carried out with 1 % vanillin-sulphuric acid, after which the plate was heated at 110 °C for 10 min and viewed under normal light.

Quantitative phytochemical analysis

Total phenol content

The quantitative estimation of the total phenolic content of the extracts was carried out in triplicate by the Folin Ciocalteu's method [14]. Gallic acid was used as the reference standard. Different concentrations (10-50 µg/ml) of gallic acid were prepared in methanol. 0.5 ml of each concentration of gallic acid was mixed with 2.5 ml of (a 10 fold) dilute Folin Ciocalteu reagent and 2 ml of 7.5% sodium carbonate solution. The tubes were allowed to stand for 30 min at RT (room temperature). The absorbance at 765 nm was measured spectrophotometrically after 30 min at RT. Similarly, 0.5 ml of all the extracts (1 mg/ml) was mixed as described above and after 30 min, the absorbance was measured.

Total flavonoid content

The total flavonoid content in extracts was determined with the help of a colorimetric method using aluminum chloride [15]. The standard quercetin was used for the preparation of the calibration curve. From a stock solution of quercetin in ethanol (1 mg/ml) concentrations of 50, 100, 150, 200 and 250 µg/ml were prepared. 0.5 ml of each of these solutions were mixed with 1.5 ml of ethanol, 0.1 ml of 10 % aluminum chloride, 0.1 ml of 1M potassium acetate and 2.8 ml of distilled water. These reaction mixtures were then incubated at room temperature for 30 min, and their absorbance was measured at 415 nm with a spectrophotometer. The amount of 10% aluminum chloride was substituted by the same amount of distilled water in the blank. Similarly, 0.5 ml of all the plant extracts (1 mg/ml) was used for determination of flavonoid content.

Total sterol content

Liebermann-Burchard (LB) reagent was employed for the quantitative estimation of the sterol content in the plant extracts [16]. It was prepared by adding 0.5 ml of concentrated sulphuric acid in 10 ml of acetic anhydride. To 1 ml of each of the extract (1 mg/ml), chloroform was added to make up the volume to 5 ml in a test tube. Two ml of LB reagent was added and mixed well. These tubes were then covered with black paper and kept in the dark for 15 min to avoid any exposure to light. The reaction mixture turned green, which was spectrophotometrically measured at 640 nm. Beta-sitosterol was used as the standard to prepare a calibration curve.

Total alkaloid content

The quantitative estimation of the total alkaloid content was carried out by the technique employed by Sreevidya and Mehrotra [17]. All the 4 plant extracts and the extracted alkaloid were used. Freshly prepared Dragendorff Reagent (DR), standard bismuth nitrate solution (by dissolving 10 mg Bi $(NO_3)_3 \cdot 5H_2O$ in 5 ml concentrated nitric acid and diluting to 100 ml with distilled water), 3 % thiourea, and 1 % disodium sulphide were used.

Calibration curves preparation

Bismuth nitrate pentahydrate stock solution was used to prepare the standard calibration curve. Dilutions of the stock solution were made in series by pipetting out 1, 2, 3, 4, 5, 6, 7, 8, and 9 ml stock solution and diluting them to a precise volume of 10 ml with distilled water. One ml of this solution and 5 ml thiourea solution were mixed. The absorbance of the yellow reaction mixture was measured at 435 nm against a colorless reagent blank.

Procedure for assay of alkaloids from plant extracts

All the extracts were of a fixed concentration. Five ml of the extract was taken, and the pH was adjusted to 2-2.5 with dilute HCl. Two ml of DR was added to it, and the precipitate formed was centrifuged. After centrifugation, the supernatant was decanted completely, and the precipitate was washed with alcohol. The filtrate was discarded and to the residue, 2 ml disodium sulfide solution was added. The brownish black precipitate formed was centrifuged. The precipitate was dissolved in 2 ml concentrated nitric acid. This solution was diluted to 10 ml with distilled water and to 1 ml of this solution; 5 ml thiourea solution was added. The absorbance was measured at 435 nm against the blank containing nitric acid and thiourea.

DPPH radical scavenging activity assay

The free radical scavenging activity of the extracts was measured *in vitro* by 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) assay [14]. A 0.36

mg/ml stock solution of the same was prepared with methanol and stored in the dark at 20 °C. Three ml of this solution was mixed with 100 µl of each of the plant extracts (100 µg/ml). The reaction mixture was shaken and incubated in the dark for 15 min at room temperature. Absorbance was measured at 517 nm. The control was prepared as above with methanol. The scavenging activity of the extracts was calculated using the following equation:

$$\text{Scavenging effect (\%)} = \left[\frac{(\text{Abscontrol} - \text{Abstest})}{\text{Abscontrol}}\right] \text{X } 100$$

Reducing power

The reducing power of a sample tests its ability to transform Fe (III) to Fe (II). Formation of a blue coloured complex confirms the transformation to Fe (II) and can be measured spectrophotometrically at 700 nm. Each of the plant extracts (100 µg/ml, 2 ml) were mixed with 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2 ml of potassium ferricyanide (10 mg/ml). The mixture was incubated at 50 °C for 20 min and 2 ml of trichloroacetic acid (100 mg/l) was later added to it. The mixture was centrifuged at 3000 rpm for 10 min, and the upper layer of the solution was collected. Two ml of this mixture was mixed with 2 ml of distilled water and 0.4 ml of 0.1 % (w/v) fresh ferric chloride. After 10 min, the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicates a higher reducing power [14].

NBT reduction (Anti-glycation assay)

Bovine serum albumin (BSA, 20 mg/ml, 10 ml) was mixed with glucose (500 mM, 5 ml) and 0.02% sodium azide in phosphate buffer (200 mM, pH 7.4). The sample with different concentrations dissolved in phosphate buffer (200 mM, pH 7.4, 5 ml) was added to the reaction mixture, and then the mixture was incubated for 30 d at 37 °C to obtain glycated materials. Amino guanidine was used as a positive control in two concentrations, 300 µg/ml (Pos 300) and 400 µg/ml (Pos 400). A negative was introduced so as to check the amount of glycation occurring in the absence of either a standard inhibitor or any extract. Aliquots of the glycated materials were taken from the system for the procedure ahead in 0, 3rd, 6th, 12th, 18th, 24th and 30thday after incubation. The glycated material (aliquots of 0.5 ml) and NBT reagent (0.3 mM, 2.0 ml) in sodium carbonate buffer (100 mM, pH 10.35) were incubated at room temperature for 15 min, and the absorbance was read at 530 nm against a blank [18].

Anti-inflammatory assay

The reaction mixture consisted of 0.2 mL of 1 % BSA, 2.8 ml of phosphate buffered saline (PBS, pH 6.4) and 2 ml of varying concentrations of extract so that final concentrations become 100, 200, 300, 400, 500 µg/ml. A similar volume of double-distilled water served as control. Then the mixtures were incubated at 15 °C in an incubator for 10 min and then heated at 70 °C for 10 min in a water bath. After cooling, their absorbance was measured at 660 nm by using vehicle (only buffer) as blank. Diclofenac at the final concentration of (100, 200, 300, 400, 500µg/ml) was used as reference drug and treated similarly for determination of absorbance [19]. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ Inhibition} = [(\text{Abscontrol} - \text{Abstest})/\text{Abscontrol}]\text{X } 100$$

Extraction of alkaloids

The ground plant material was first defatted with hexane and petroleum ether. This seed mass was then extracted with methanol, filtered and concentrated. This defatted methanol extract was then suspended in tartaric acid titrated to pH 5, and partitioned with ethyl acetate pre-saturated with water. The ethyl acetate partition phase was discarded. The aqueous acid phase was made alkaline with Na_2CO_3 solution till the pH turned 11, and was partitioned with ethyl acetate again. This partitioning led to two phases: the aqueous phase that contained the quaternary amines and the alkaloids, and the ethyl acetate phase that contained the primary, secondary and tertiary amines [20].

RESULTS

Organoleptic characteristics

An indication of the outward appearance, sense of touch, smell and taste of the plant sample is necessary for its preliminary screening. Such organoleptic studies help determine the nature of the sample and its differentiating characteristics. The results of the organoleptic analysis are given in table 3.

Table 3: Macroscopic characteristics of the plant samples

Plant part	Colour	Odour	Taste	Texture
Seeds	Black(yellow internally)	Odourless	Bitter	Smooth

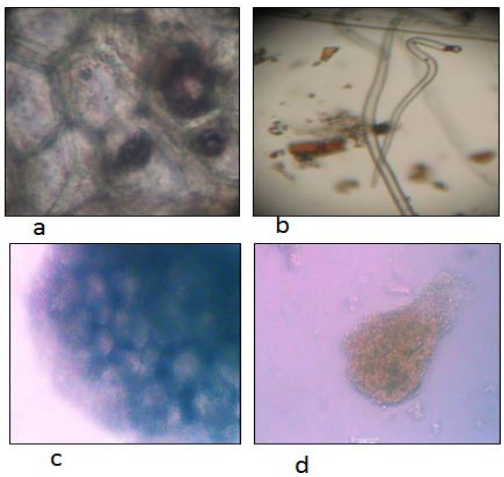


Fig. 1: Microscopy of CA seed powder. a. Fat cells, b. Trichomes, c. Endosperm, d. Epidermis

Microscopic studies

Determination of the internal cellular structure of a plant part helps in the authentication of a plant sample. The powdered seeds, when studied for their microscopic details revealed characteristic features like the presence of trichome, which were un branched and unicellular. Single layer epidermal cells, parts of endosperm and fat cells were also observed. fig. 1 represents the microscopic analysis.

Evaluation of physicochemical parameters

The ash content gives a measure of the quality and purity of the drug. Extractive values state the approximate measure of chemical constituents present in the crude drug. Estimation of loss on drying gives the amount of moisture present in the sample. These parameters were checked, and the results are summarized in table 4.

Table 4: Physicochemical parameters

Parameters (%w/w)	CA
Loss on drying	7.25±0.99
Total ash	5.6±0.66
Sulphated ash	1.61±0.22
Acid insoluble ash	0.20±0.34
Water soluble ash	0.41±0.07
Alcohol soluble extractive value	12.72±1.21
Hydro alcoholic extractive value	27.35±0.40
Hexane extractive value	11.23±1.60
Ethyl acetate extractive value	19.52±0.81

Extraction of the plant material

Solvents are used in plant studies to extract the phytochemical compounds from the plant material into the solvent. Table 5 gives the yield of all the extracts.

Table 5: Percent yields of the plant extracts

Extract	% Yield (w/w)±SD
CA-Hex	7.66±0.97
CA-EA	5.71±0.82
CA-AL	2.41±0.74
CA-HA	2.28±0.91

Qualitative phytochemical screening

The plant extracts were qualitatively screened for their phytochemical constituents using standard procedures. Table 6 represents the results of every extract. Alkaloids, steroids, and flavonoids were majorly found to be present in the extracts.

Thin layer chromatography

A chromatographic examination was carried out for the identification of crude drug based on the use of a major chemical constituent. Here, thin layer chromatography was carried out for all the extracts, (fig. 2) along with the standard beta-sitosterol. The Rf of the standard and that of ethyl acetate extract match, indicating the presence of beta-sitosterol in the ethyl acetate extract of CA. Compared to the other extracts, the hydroalcoholic extract of CA did not yield any separation on the plate.

Total phenolic content

The estimation of the phenolic content was carried out by the Folin Ciocalteu method. Fig. 3a represents the calibration curve constructed using gallic acid, and fig. 3b represents the phenolic contents in the extracts. The phenolic content was shown to be the highest in the hydroalcoholic extract (CA-HA) expressed in µg GAE/mg.

Table 6: Qualitative phytochemical screening of the plant extracts

Main group	Test	CA-Hex	CA-EA	CA-AL	CA-HA
Carbohydrates	Molisch's test	-	-	-	-
	Benedicte's Test	-	-	-	-
	Cobalt Chloride test for hexose sugars	+	-	-	-
	Fehling's test	-	-	-	-
Alkaloids	Wagner's test	-	-	+	+
	Hager's test	-	+	-	-
	Dragendorff's test	-	-	+	-
Steroids	Salkowski's reaction	-	-	-	-
	Liebermann-Burchard reaction	-	+	-	-
Flavonoids	Shinoda test	-	-	-	-
	Sulphuric acid test	-	+	+	+
Cardiac Glycosides	Lead acetate test	-	-	-	-
	Keller–Killiani's test	-	+	-	-

Lanes	Sample	Rf
1	β-sitosterol	0.54
2	CA-Hex	0.52
3	CA-EA	0.54
4	CA-AL	0.52
5	CA-HA	0.50

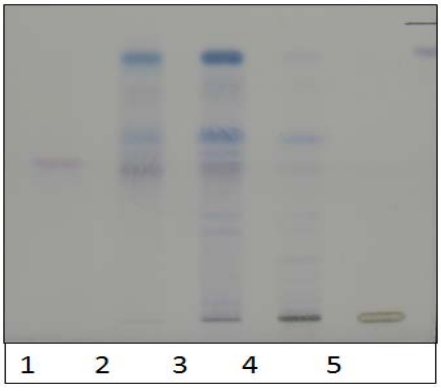


Fig. 2: TLC of all extracts of CA with B-sitosterol as standard (under visible light) Quantitative phytochemical analysis

Total flavonoid content

Fig. 4a represents the calibration curve of quercetin, and fig. 4b represents the flavonoid contents in the extracts. The flavonoid content expressed in µg quercetin equivalents/mg ranged from 87 to 160.54 µg quercetin equivalents/mg showed by the ethyl acetate extract (CA-EA).

Total sterol content

Liebermann-Burchard reagent is one of the standard reagents for the detection of sterols. In this quantitative estimation, the ethyl acetate extract (CA-EA) showed the highest sterol content of 295.9 µg/ml (fig 5).

Total alkaloid content

In the quantitative alkaloid determination by Sreevidya and Mehrotra method, a calibration curve was constructed with the help of bismuth nitrate, and the alkaloidal content was calculated in terms of µg per mg of the extracts. CA-EA showed an alkaloid content of 32.93 µg/mg. (fig. 6).

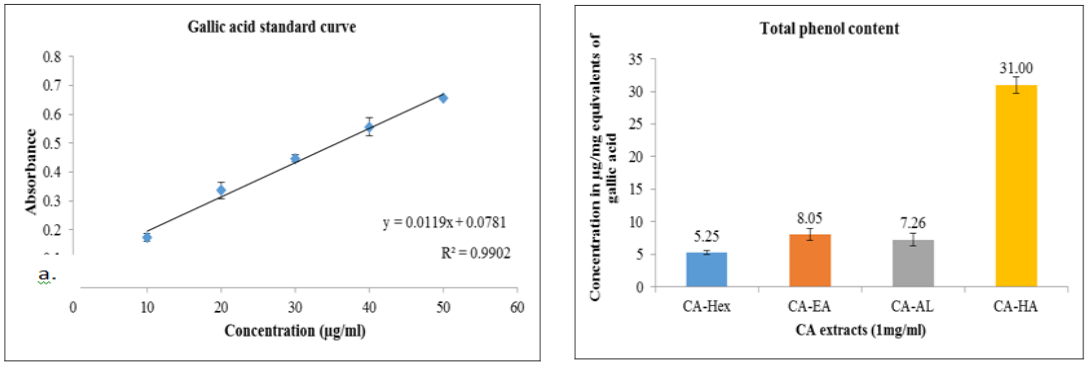
DPPH radical scavenging activity assay

The antioxidant activity of the seed extracts was tested using the DPPH method, and it was found that all the extracts did not exhibit high inhibitory powers. Nonetheless, among all the extracts, CA HA was found to have the highest antioxidant activity, with a % inhibition of 15.41%, and the lowest inhibition was seen in CA-Hex, with a meagre 6.8%. CA-EA and CA-AL displayed intermediate values of 7.77 % and 8.43 % respectively.

Reducing power

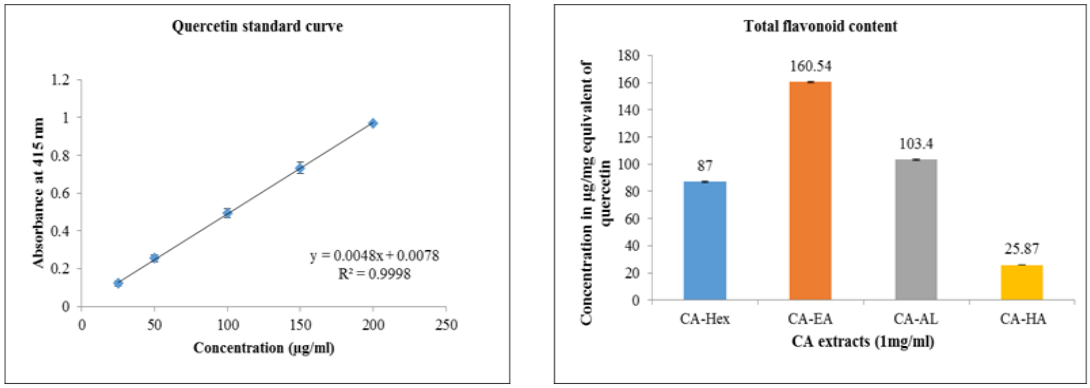
The antioxidant potential in terms of reducing the power of the four extracts, i.e. CA-Hex, CA-EA, CA-AL, and CA-HA was measured in terms of reducing power.

Ascorbic acid, the standard, showed a significantly high reducing power (1.1526) at 700 nm, but among the extracts, CA-EA exhibited the highest reducing power of 0.4108as higher absorbance of the reaction mixture indicates a higher reducing power.(table 7).



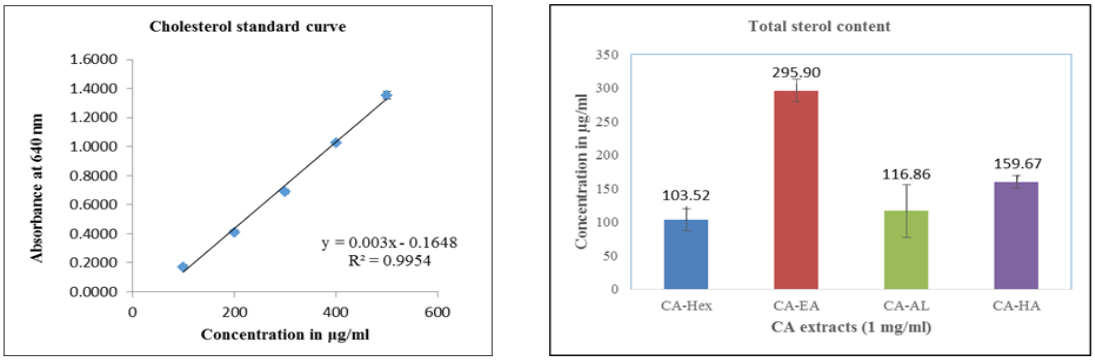
a b

Fig. 3: a. Gallic acid standard calibration curve for the estimation of phenols in the plant extracts, b. Total phenol content of the extracts



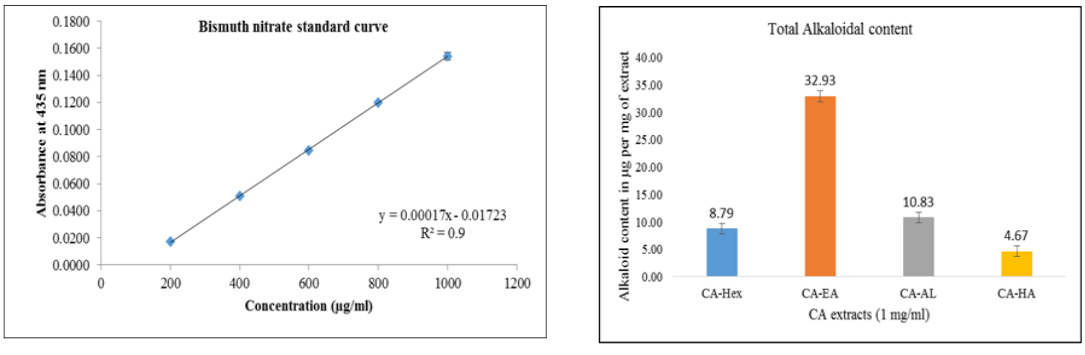
a b

Fig. 4: a. Quercetin standard calibration curve for the estimation of flavonoids in the plant extracts, b. Total flavonoid content of the extracts



a b

Fig. 5a: Cholesterol standard calibration curve for the estimation of sterols in the plant extracts, b. Total sterol content of the extracts.



a b

Fig. 6: a. Bismuth nitrates standard calibration curve for the estimation of sterols in the plant extracts, b. Total alkaloid content of the extracts

Table 7: Reducing power of the CA extracts and standard ascorbic acid

Sample (100 µg/ml)	Reducing power(Absorbance at 700 nm)
Ascorbic acid standard	1.1526±0.12
CA-Hex	0.1247±0.33
CA-EA	0.4108±0.45
CA-AL	0.2251±1.04
CA-HA	0.3356±1.11

NBT reduction (anti-glycation assay)

Glycation of proteins in the body can lead to many complications and degenerative diseases. The measure of an extract to inhibit or restrict the glycation of a standard protein in conditions mimicking *in vivo* ones is given by the anti-glycation assay.

The higher the reduction of NBT by the reaction mixture, the higher is the absorbance. Glycated materials have the ability to reduce NBT. This shows that those extracts that were capable of reducing NBT possess glycated products.

In other words, the lower the absorbance, the lesser is the reduction of NBT and the greater is the efficacy of the extract to inhibit glycation. The Negative (negative control) showed maximum glycation (NBT reduction) over a period of 21 d. As seen in fig. 7, the extent of inhibition of glycation was in the order: Pos 400>Pos 300>CA-Hex>CA-EA>CA-AL>CA-HA>Negative.

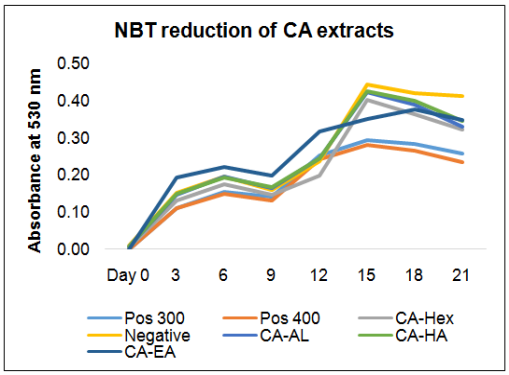


Fig. 7: NBT reduction of the CA extracts and positive standard aminoguanidine

Anti-inflammatory assay

Denaturation of proteins is one of the main causes of inflammation. The ability of the extract to inhibit this denaturation was studied. Table 8 summarises the percent inhibition of albumin denaturation of the drug and the extracts at a concentration of 400 µg/ml. The standard drug exhibited a lesser inhibitory capacity compared to the extracts in general. The percent inhibition of CA-Hex exhibited the highest inhibition at 400 µg/ml compared to the standard drug and other extracts.

Table 8: Percent inhibition of albumin denaturation of standard drug and CA extracts

% Inhibition at 660 nm (400 µg/ml)	
Drug	29.86±0.87
CA-Hex	66.55±1.45
CA-EA	57.11±1.08
CA-AL	19.22±1.63
CA-HA	42.91±1.21

Extraction of alkaloids

The extraction of alkaloids from the defatted seeds gave two fractions: the AQ (aqueous) fraction and the EA (ethyl acetate) fraction. When both the fractions were run on TLC (solvent system of toluene: ethyl acetate: acetic acid, 6:1:1) and derivatized with Dragendorff Reagent, only the aqueous fraction (lane 2, fig. 8) showed a characteristic orange band, which is indicative of the presence of alkaloids. The yield of the aqueous fraction was found to be 12%.

Fig. 8: TLC of the alkaloid fraction (Lane 1-EA fraction, Lane 2-AQ fraction)

DISCUSSION

Though a few studies have been carried out on many species of Cassia, *Cassia absus* is yet to be studied extensively. There are reports of isolation of many components from the seeds of this plant and have been mentioned above [21]. Such studies are very essential to create a chemical profile of the plant. Another set of studies that lays stress on the standardization and authentication of the plant is equally needed.

According to the World Health Organization (WHO), the microscopic and macroscopic studies of the plant are the first steps towards establishing the authenticity and the purity. These studies were carried out and the characteristics that were seen would help in distinguishing not only one species of the genus Cassia from another but also to distinguish different parts of the same plant from each other.

Microscopic studies revealed fat cells, endosperm, trichomes, which are features seen mostly in seeds. Physicochemical parameters like extractive values and ash content were tested in order to measure the amount of adulterants present. The values of these parameters might defer from those in other studies based on the geographical location of the plant, and due the slight variations arisen due to external factors like climatic conditions [10, 22].

The qualitative screening of phytochemicals was carried out in order to understand the basic phytochemical composition of the plant. The hexane extract gave the maximum yield, probably because of higher oil content in it. Alkaloids, steroids and flavonoids were found to be present in the ethyl acetate, alcoholic and hydroalcoholic extracts. Our findings were synonymous with those of another study, where a comparison was made between the phyto-constituents of three different Cassia species [23].

Thin layer chromatography was performed to check for the profiles of the four extracts and the presence of beta-sitosterol in them. CA-Hex and CA-EA showed the presence of beta-sitosterol [10] as their Rfs matched with that of the standard. This result is supported by a finding of a study where beta-sitosterol present in *Cassia absus* was used for molecular docking studies [22]. The hydroalcoholic extract did not show any separation. The TLC profile indicated that further extraction, or alternatively, treatment of the extracts was needed in

order to achieve maximum separation of the extracts into their principal components.

As the qualitative phytochemical screening was carried out to get a glimpse of the phytochemicals present, their quantitation was also done, and the studies revealed that the ethyl acetate extract showed the highest amount of alkaloids, sterols and flavonoids. The phenolic content was the highest in the hydroalcoholic extract, and hence, showed a higher percent inhibition in the DPPH assay, as phenolic compounds are known to be good antioxidants.

Studies on the reducing power, *in vitro* anti-inflammatory and *in vitro* anti-glycation activity of *Cassia absus* have not been reported. The reducing power of a sample evaluates its ability to transform Fe (III) to Fe (II), confirmed by the formation of a blue coloured complex and can be measured spectrophotometrically at 700 nm. The phytochemicals present in the extracts can be antioxidants, either by being able to scavenge free radicals or by being reducing agents. Hence, the highest amount of flavonoid content in the ethyl acetate extract (CA-EA) may have led to the maximum reducing power it displayed.

In the anti-glycation assay, NBT was used as a mediator. Glycated products are formed when the extracts are unable to inhibit the glycation of proteins. Such glycated products possess the ability to reduce NBT. The higher the reduction of NBT by the reaction mixture, the higher is the amount of glycated products present, and the higher is the absorbance. Here, this indicated that CA-Hex possessed the highest anti-glycation activity, followed closely by CA-EA. The negative control which had no extract showed the highest absorbance till day 21. The standard anti-glycating agent amino guanidine showed higher powers of anti-glycation as was expected, compared to the extracts. Polyphenols make up a major group of plant-derived compounds having anti-glycation activity, but some polysaccharides and oligosaccharides were also reported to decrease the AGEs formation [24]. This might be a likely cause of CA-Hex showing a slightly higher anti-glycating activity than the other extracts as its phytochemical screening showed the presence of carbohydrates.

The anti-inflammatory assay employs the principle of deliberate albumin denaturation at high temperatures and checking for agents that inhibit this denaturation. BSA expresses antigens associated with type III hypersensitive reactions and which are related to inflammatory diseases. Hence, agents that inhibit denaturation of serum albumin (or BSA) are likely to have high anti-inflammatory properties. The assay results showed that three out of four extracts, ie CA-Hex, CA-EA and CA-HA i.e. worked better at inhibiting albumin denaturation, compared to the standard drug. CA-Hex exhibited the highest anti-inflammatory activity, which might indicate its extensive use in the traditional system of medicine in the treatment of eye diseases. Though the ethyl acetate extract exhibited the maximum flavonoid, sterol and alkaloidal content, its antioxidant (except its reducing power), anti-inflammatory and anti-glycating effects did little to justify the higher amount of phytochemicals it exhibited.

The alkaloids Chak sine and iso Chak sine were the foremost among the isolated compounds from *Cassia absus.* Keeping this in mind, an attempt, a first of its kind, was made to extract alkaloids from the defatted seeds of CA based on the acid-based shakeout method. Dragendorff reagent is the gold standard for the identification of alkaloids and hence, it was employed for the same. When the end product of the extraction was in the form of two fractions, one aqueous, and the other an ethyl acetate one, only the aqueous fraction displayed a conventional orange band when the fraction was run on TLC. This aqueous fraction can be further treated to obtain the known alkaloids.

CONCLUSION

The present investigation focussed on the pharmacognostic studies and the standardization of the seeds of *Cassia absus.* It also reported the quantitative phytochemical estimation of the seeds in terms of phenols, flavonoids, sterols and alkaloids, and showed that the ethyl acetate fraction exhibited the highest flavonoid, sterol and alkaloid content. *In vitro* anti-inflammatory and anti-glycating activities were higher in the hexane extract, and hence, this extract can be used further in *in Vivo* studies.

Further studies of the seeds need to be carried out to ascertain their role in the prevention of oxidation, as the present study proved to be unsuccessful in giving a conclusive idea. Also, alkaloids that were successfully extracted from the defatted seeds of CA, need to undergo further purification and testing of their potential in *in vitro* as well as *in vivo* studies.

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CONFLICT OF INTERESTS

Declared none

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