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A COMPREHENSIVE REVIEW ON THE EVALUATION TECHNIQUES AND STANDARDIZATION PARAMETERS OF CRUDE DRUG – CASSIA ANGUSTIFOLIA

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ABSTRACT

Cassia angustifolia, a member of the Fabaceae family, is known for its traditional medicinal value in treating various illnesses caused by pathogens. This study aims to evaluate the phytochemical properties and in vitro antimicrobial activity of Cassia angustifolia extracts, ranging from non-polar to polar solvents. Results indicate that the extracts contain significant amounts of secondary metabolites, with methanol proving to be the most effective solvent for extracting these compounds. All extracts contain detectable levels of phenols and flavonoids, with methanol extracts showing the highest concentrations, followed by water, acetone, and hexane extracts. The extracts also exhibit antimicrobial activity against various pathogenic bacteria and fungi, with methanol extract displaying the strongest effect, followed by water, acetone, and hexane extracts. Further research is recommended to enhance the development of plant-based drugs from Cassia angustifolia.

KEYWORDS: *Cassia angustifolia, secondary metabolites,HPTLC, antioxidant activity.*

INTRODUCTION

Cassia angustifolia, commonly known as senna, belongs to the Leguminosae family and is widely used for treating constipation in both Eastern and Western countries[1,2].The laxative properties of senna are attributed to the presence of two anthraquinone glycosides sennoside A and sennoside B. Additionally, C. angustifolia contains various other compounds, including rhein-8-diglucoside, sennosides C and D, rhein, rhein-8-glucoside, aloe-emodin, anthrone diglucoside, and naphthalene glycosides such as tinnevellin glycoside and 6-hydroxy musizin glycoside. It also includes kaempferol (a flavonoid), phytosterols, resin, and calcium oxalate [3,4].

Historically, the first variety of senna was discovered along the Nile River in Egypt and Sudan. Today, it is commercially cultivated in regions like Kutch (Gujarat) and Jodhpur (Rajasthan) in India. Senna can be grown as a perennial crop with a cultivation duration of approximately 2-3 years [2].

Taxonomical Classification [5]

Kingdom	Plantae
Sub division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Sub class	Rosidae
Order	Fabales
Family	Fabaceae/Leguminoceae
Genus	Cassia
Species	Angustifolia

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Medicinal Uses

Cassia angustifolia, commonly known as senna, is used medicinally for various treatments. It is effective in treating splenic enlargements, anemia, typhoid, and cholera as a febrifuge. Additionally, it serves as a blood purifier, an anthelmintic, and a remedy for constipation [5].Senna has also been included in the Indian Pharmacopoeia (I.P.) as a purgative due to the presence of active compounds such as rhein, aloe-emodin, kaempferol, and isorhamnetin [6].

MATERIALS & METHODS

Collection of Plant Material

Leaves of Cassia angustifolia were collected from the Seshachalam forest and authenticated by Dr. K. Madhava Chetty, Assistant Professor in the Department of Botany at Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh. The collected plant material was thoroughly washed first with running water, followed by distilled water. The leaves were then separated from the stems, chopped, and left to dry under shade. The dried material was stored in sterilized polythene bags for further study [7].

Extraction Technique

The dried leaf material was powdered and subjected to sequential extraction using a Soxhlet apparatus with solvents of varying polarities, including hexane, acetone, methanol, and water [8].

The extracts were concentrated using a rotary evaporator, then dried and weighed to calculate the extractive yield. Finally, the dried crude extracts were stored in airtight bottles for further analysis [9].

Powdered drug was used for moisture content, ash values, swelling index, and fluorescence studies were carried out by treating 0.5 g of powdered drug with different reagents and observation in color was made in visible light, UV light of short (254 nm), and long wavelength (365 nm) under UV chamber. Photomicrography was performed using Olympus C7070 camera [10].

For Antioxidant Study: Preparation of plane material extraction: Cassia angustifolia leaves were grinded to fine powder by using electrical blender. The fine powder of 20 g was weighed separately and transferred to 250 ml of different solvent (petroleum ether, methanol and distilled water) were subjected to orbital rotary shaker for 24 h at 25 ºC at a speed of 150 rpm. Then the samples were centrifuged for 15 min at 2000 rpm at room temperature and are filtered through what man no 1 filter paper. The crude extracts of petroleum ether and methanol was evaporated through rotary evaporator at 60 ºC and 70 ºC respectively under constant pressure. While, aqueous extract was evaporated on hot plate at 100 ºC for 2 hours. The Crude plant extracts were stored at 4 ºC until further usage [11].

For HPTLC, 0.5 g of powdered seed samples from four different regions (DehraDun, Hyderabad, Jodhpur, and Mumbai) were separately refluxed with 5 ml of 50% methanol for 5 minutes on a water bath, then filtered. The filtrate was concentrated, and the extract was prepared in a known volume [12].

For analysis, 25 µl of each test solution was applied on a 10 x 10 cm precoated silica gel 60-F254 Merck TLC glass plate using a Camag Linomat IV applicator. Alongside the seed samples, Sennoside A (0.1 mg) and Sennoside B (0.55 mg) standards in isopropyl alcohol were also applied. The plate was developed to a distance of 8.7 cm at room temperature (33°C) using a solvent system of toluene: ethyl, acetate: methanol in the ratio of 85:15:0.5.

After development, the plate was sprayed with anisaldehyde–sulfuric acid reagent and heated at 120°C for 10 minutes, following the protocol by Wagner et al. (1984).

A fingerprint profile was then obtained using a Desaga Video Documentation Unit (Providoc II). Quantitative estimation of Sennosides A and B was performed with a Camag densitometric scanner.

Phytochemical Analysis

Preliminary Qualitative Screening of Cassia angustifolia

Standard screening tests were conducted on extracts of Cassia angustifolia to determine the presence or absence of various secondary metabolites.

The analysis included testing for alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins, and anthraquinones, following established procedures [13].

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1)Detection of Alkaloid
Extract was dissolved individually in dilute Hydrochloric acid and the resultant solution was clarified by filtration

Mayer's Test: Filtrate was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow colour precipitate indicates the presence of alkaloids

Wagner's Test: Filtrate was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown / reddish precipitate indicates the presence of alkaloids.

Dragendroff's Test: Filtrate was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids.

Hager's Test: Filtrate was treated with Hager's reagent (saturated Picric Acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2) Detection of Phenols
Ferric Chloride Test: The filtered solution of extract was treated with three drops of freshly prepared 1% Ferric Chloride and Potassium Ferro cyanide. Formation of bluish- green colour is taken as positive.

3) Detection of Flavonoids
Alkaline Reagent Test: The Extract was treated with few drops of Sodium Hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute HCl, indicates the presence of flavonoids.

Lead Acetate Test: The Extract was treated with few drops of Lead Acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

4) Detection of Anthraquinones
Free Anthraquinones Test (Borntrager's test): The extract of the plant material (equivalent to 100 mg) was shaken vigorously with 10 ml of Benzene, filtered and 5 ml of 10% Ammonia solution was added to the filtrate. The mixture was shaken and the presence of a pink, red, or violet colour in the ammonia (lower) phase indicates the presence of free anthraquinones.

5) Detection of Phytosterols
Salkowski's Test: The extract was dissolved in 2 ml Chloroform in a test tube. Concentrated Sulfuric Acid was carefully added unto the wall of the test tube to form a lower layer. A reddish brown colour at the interface indicates the presence of a steroid ring.

6) Detection of Terpenoids
The extract was added to 2 ml of Acetic Anhydride and Concentrated H_2SO_4. Formation of blue, green rings indicate the presence of terpenoids.

7) Detection of Tannins
Ferric Chloride Test: The extract was dissolved in water and the resultant solution was clarified by filtration to which 10 % Ferric Chloride solution was added to the clear filtrate. This was observed for a change in colour to bluish black.

Lead Acetate Test: The extract was dissolved in water and to that 10 % Lead Acetate solution was added. Appearance of yellow precipitate confirms presence of tannins.

Potassium Dichromate Test: The extract was dissolved in water and to it a strong potassium dichromate solution was added. Yellow colour precipitate indicates presence of tannins and phenolic compounds.
8)Detection of Reducing Sugars
Extract was dissolved individually in 5 ml distilled water and filtered. The filtrate was used to test for presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dilute HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

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Keller Kiliani test (for deoxy sugars in cardiac glycosides): Fifty (50) mg of each extract was dissolved in 2 ml chloroform. H2SO4 was added to form a layer and presence of colour at interphase was noted. Brown ring at interphase is characteristic of deoxysugars in cardenolides.

Pharmacognostic evaluation of the plant:
The plant material was used for quantitative determination of physicochemical values. ash values, loss on drying, and extractive values of Cassia angustifolia [14].

Antioxidant Study
The antioxidant activities of aqueous and organic extracts of C. angustifolia and gallic acid were evaluated using the DPPH free radical scavenging test, based on IC50 values. IC50 represents the concentration required to inhibit 50% of DPPH free radicals. A lower IC50 value indicates higher antioxidant potential in the plant extracts [15].

To conduct the test, different extracts of C. angustifolia and standards were measured for absorbance at 517 nm, and the percentage of DPPH scavenging was calculated.

The extracts displayed dose-dependent antioxidant activities; as concentrations increased, so did their scavenging effectiveness.

The crude medicinal plant extract was diluted in methanol at concentrations of 5%, 10%, and 20%. For each concentration, 2 ml of the plant extract was added to 0.5 ml of 0.2 mmol/L DPPH ethanolic solution, with ascorbic acid used as a control. Each sample was tested in triplicate.
The reaction mixtures were incubated in the dark for 30 minutes, after which antiradical activity was measured with a UV spectrophotometer at 517 nm.

The DPPH scavenging effect was calculated as follows:

DPPH scavenging effect = ((A0 – A1)×100% /A0))
Where the AO of the control at 30 minutes, A1 the absorbance of the sample at 30 minutes [16].

RESULTS & DISCUSSION

Macroscopic Characteristics
Senna leaves are delicate and grayish-green with a specific odor and a mucilage-like, slightly bitter taste. The compound leaves consist of 5-8 pairs of oval-lanceolate leaflets, each measuring about 1.5-6.0 cm in length and 0.5-1.5 cm in width.. Senna produces the leaflets possess short and stout petioles, which may rarely break [4,17].The flowers of the Senna plant are large and yellow medium-sized, oblong-shaped pods containing flat, yellowish seeds [2].



Fig.1. Flower & Leaf of Cassia Angustifolia

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Fig.2. Leaves of Senna

Miroscopical Evaluation

The transverse section (T.S.) of the leaf shows an isobilateral structure, featuring paracytic stomata, nonlignified unicellular trichomes with warty walls, a fibrovascular bundle lined with abundant prisms of calcium oxalate, a 4-5 tiered palisade layer, and sclerenchyma. These structures are observed in the T.S. of the Cassia angustifolia leaf, as represented in the figure 3 above [17].

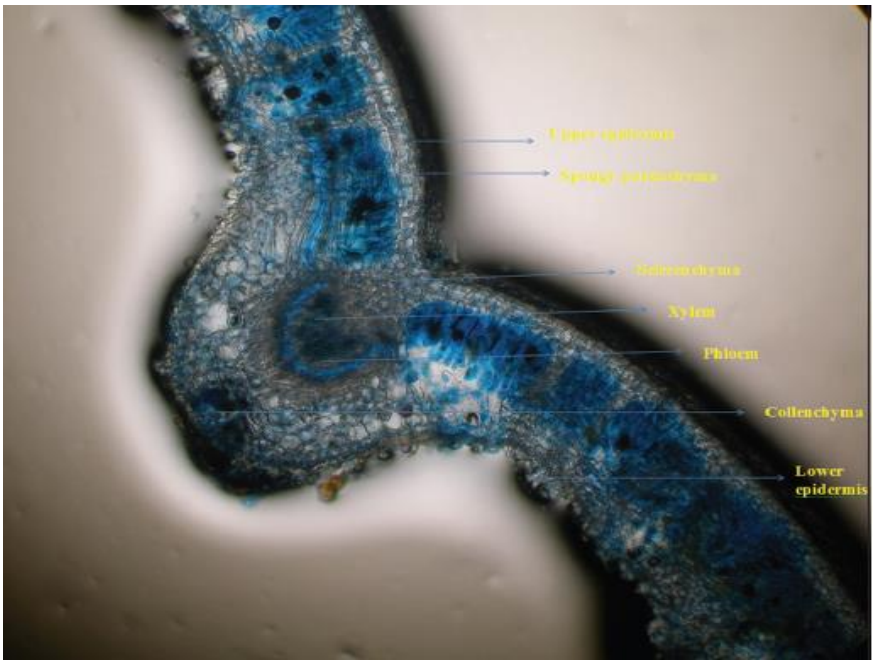


Fig.3.The transvers section of leaf of cassia angustifolia

Preliminary Screening

A preliminary qualitative analysis of the extracts provided initial information about the presence or absence of various metabolites in the plant extracts in table1 below.

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S.r. No.	Tests	Hexane Extract	Acetone Extract	Methanol. Extract	Water Extract
01.	***Alkaloids*:**				
	1. Mayer's Test	-	+	+	+
	2. Wagner's Test	-	+	-	+
	3. Dragendrof'fs Test	-	+	+	+
	4. Hager's Test	+	+	-	+
02.	***Phenolics*:**				
	1. Fecl2 Test	-	+	+	+
03.	***Flavonoids*:**				
	1. Leadacetate Test	-	+	+	+
	2. NaoH Test	+	+	+	-
	Anthraquinones:				
	1. Borntrager's Test	-	-	-	-
04.	***Phytosterols:***				
	1. Salkowski Test	+	+	+	+
05.	***Tannins:***				
	1. Fecl2 Test	-	+	+	+
	2. Lead acetate Test	-	+	+	+
	3. Pot. Dichromate Test	-	+	+	+
	4. Saponin Froth Test	-	-	+	-
06.	***Coumarin:***				
	1. NaOH Test	-	-	-	-
07.	***Reducing Sugars:***				
	1. Fehling' s Test	+	+	+	+
	2. Keller-Killani Test	+	+	+	+

(-): Negative (+): Positive

Table 1: Comparative Analysis of Phytochemical Analysis of Whole Arial Part Extracts of Cassia angustifolia .

The phytochemical screening results for hexane, acetone, methanol, and water extracts of Cassia angustifolia were summarized in the table above.These results revealed the presence of phenolics, steroids, alkaloids, flavonoids, and reducing sugars in all extracts of Cassia angustifolia. Polar solvent extracts (acetone, methanol, and water) showed the presence of tannins. Other metabolites, such as anthraquinones, saponins, anthocyanins, leucoanthocyanins, and coumarins, were completely absent in both nonpolar and polar extracts of Cassia angustifolia [13].

Physicochemical Parameters

The Cassia angustifolia (Linn.) leaf's physicochemical parameters are summarized below in table 2.

Analysis revealed a total ash content of 11.2%, indicating a significant presence of mineral and earthy components.

Further breakdown showed that 1.5% was acid-insoluble ash, while 4.7% was water-soluble ash, suggesting the presence of acidic compounds, sugars, and inorganic substances.

Moreover, drying the leaf at 105°C resulted in a 1.90% weight loss, indicating its moisture content [18].

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Table 2. Phisiochemical parameters of Cassia Angustifollia

Sr.No	Parameters	Values (%w/w)	Values (%w/w)	Values (w/w)	Mean±SD (% w/w)
01.	Total ash value	11.2	11.5	11.0	11.23±0.25
02.	Water-insoluble ash value	6.5	6.75	6.1	6.45±0.32
03	Water-soluble ash value	4.7	4.5	4.2	4.46±0.25
04.	Acid-insoluble ash value	1.5	1.3	1.4	1.4±0.1
05.	Loss on drying	1.90	1.8	1.9	1.86±0.05

Table 3. Extractive values of cassia angustifolia

Sr.No.	Solvent	Value (% w/w)	Value (% w/w)	Value (% w/w)	Mean±SD (% w/w)
01.	Water	16.5	16.9	16.4	16.6±0.26
02.	Ethanol	3.8	3.9	3.6	3.7±1.75
03.	Chloroform	0.8	0.9	0.8	0.83±0.05
04.	Petroleum ether	1.5	1.6	1.7	1.6±0.1
05.	Methanol	3.0	3.2	3.5	3.2±0.25

Successive extractive values of the Cassia Angustifolia using solvents Water, ethanol, chloroform, petroleum-ether and water, were determined and showed in table 3 found to be 16.6±0.26 %(w/w), 3.7±1.75 %(w/w), 0.83±0.05 %(w/w), 1.6±0.1 %(w/w) and 3.2±0.25 %(w/w) respectively [18].

Estimation of cassia Angustifolia By TLC (Thin Layer Chromatography):

Chromatographic parameter

Chromatography was performed by on glass packed silica gel 60 Gf254 HPTLC layers (20 cm ×20cm : 03mm layer thickness) prepared using a camag The plate Sample and standard compounds 1 and 2 of known concentration were applied at 8nm wide bands using Camag linomat automated TLC applicator with nitrogen flow from syringe [19].

Detection and Quantification

After completion of sample application the plate was developed in a camag twin through glass tonk presatu
rated with mobile phase of 2-propanol:ethyl acetate : water: formic aid (17:19:12:2) for one hour

The TLC runs were performed under laboratory Conditions of 25-27°C and 60% of relative humidity. After that played were taken off and dried by drier [20].

Sennosides A and B were quantified using a c TLC scanner model 3 equipped with camag loincasis software applying the following conditions:Slit width 6×0.5nm,wavelength 3500 nm absorption reflection scan mode.The identification of sennoside A and Sennoside B in formulations was confirmed bysuperimposing the UV spectra of samples and standards within Rf window [19,20].
The Rf value of sennoside A and Sennoside B is 0.991 and 0.997 respectively

Chemical Standardization through HPTLC

HPTLC studies were conducted to develop a characteristic gross HPTLC fingerprint profile that could serve as a marker for the quality evaluation and standardization of the drug.

In this study, eight chemical marker components were identified, with Rf values of 0.10, 0.21, 0.27, 0.32, 0.52, 0.58, 0.62, and 0.64, each displaying a characteristic color in all seed samples collected from different geographical zones across the country. (showed in table 3 and figure 4.)

Sennosides, the primary active components in Cassia angustifolia, have been previously studied in the leaves and pericarp of the fruit, though no data exists on their concentration in seeds. Because this study focused on the medicinal and commercial value of seeds, the

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percentage of sennosides was also measured. It was observed that sennoside B concentration was notably high in most of the samples [21,22].

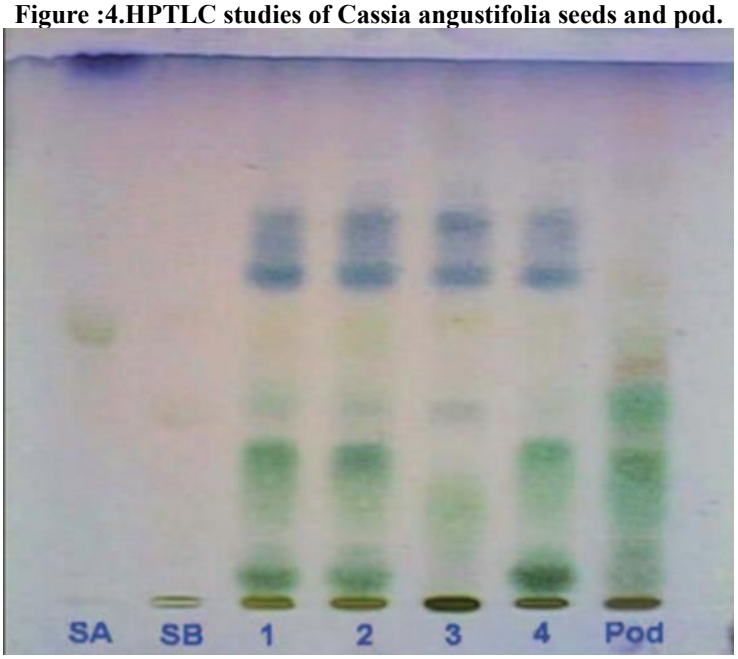
The plate in UV 254 and visible light after spraying with detecting. Reagent showed the presence of sennoside A and sennoside B at Rf of 0.52 and 0.32 respectively, in all of the seed samples [23].

Sennoside A and B, when qualitatively estimated,were found to be present in all the seed samples though their quantity varied from one region to another, possibly due to geographical variations.

The effect of different geographical zones on the concentration of sennosides (A and B) was also studied and showed in table below.

The concentration of sennoside A varied from 0.21% to 2.13% in seeds of different regions, while sennoside B showed a wide range of variation from 1.34% to 8.52% [21].

Figure :4.HPTLC studies of Cassia angustifolia seeds and pod.



(SA; Sennoside-A; SB; Sennoside-B; 1-Bombay; 2-Dehradun; 3-Hyderabad; 4-Jodhpur; Pod- Pod sample from Mumbai) Solvent system: Butanol: Acetic acid: Water (6:1:2)

Hyderabad seeds possessed a maximum of sennoside A (2.13%), which was least in the Bombay seeds,limited to just 0.219%. On the contrary, sennoside B was found to be maximum in the case of Jodhpur seeds(8.52%), which was about 7-times more than the seeds of Bombay, (i.e., 1.34%). Hyderabad seeds also possessed an appreciable concentration of sennoside B, 7.4%, while in Dehradun seeds the concentration was 3.95% [21,22].

Table 4: Quantitative HPTLC estimation of sennosides in C. angustifolia seeds of different zones in visible light after spraying

REFERENCE STANDARDS	MUMBAI		HYDERABAD		DEHRADUN		JODHPUR	
	Rf value	Colour	Rf value	Colour	Rf value	Colour	Rf value	Colour
	0.1	Green	0.1	Green	0.1		0.1	Green
	0.21	Yellowish green	0.21	Yellowish green	0.21	Yellowish green	0.21	Yellowish green
	0.27	Green	0.27	Green	0.27	Green	0.27	Green
Sennoside A	0.32	Greyish blue	0.32	Greyish blue	0.32	Greyish blue	0.32	Greyish blue

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Sennoside B	0.52	Light green	0.52	Light green	0.52	Light green	0.52	Light green
	0.58	Blue	0.58	Blue	0.58	Blue	0.58	Blue
	0.62	Blue	0.62	Blue	0.62	Blue	0.62	Blue
	0.64	Blue	0.64	Blue	0.64	Blue	0.64	Blue

TABLE 5 : Percentage concentration of sennoside A and B in seed samples of different geographical regions.

	DEHRADUN	HYDERABAD	JODHPUR	MUMBAI	PODS MUMBAI
Sennoside A	1.000%	2.134%	1.856%	0.219%	1.247%
Sennoside B	3.951%	7.446%	8.526%	1.348%	3.793%

Antioxidant Study

Radical scavengers present as antioxidants in products may directly react and quench with peroxide radicals and terminate the peroxidation chain reaction and improve the quality and stability of food product [24].

Cassia angustifolia has only high antioxidant potential at 5% concentration with petroleum ether and aqueous extracts, and the remaining concentration showed low antioxidant activity listed in table 6 [25].

The stable DPPH radical has been used to evaluate antioxidants for their radical quenching capacity.
The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity [26].

Table 6 : Antioxidant activity of plant extracts:

Sr.no	Solvent Extract	Conc. (µg/ml)	A. b (nm)	IC50%	Std. Ascorbic acid A. b (nm)
01.	Petroleum ether	5 10 15	0.405 0.376 0.360	24.015 74.439 86.327	0.533
02.	Methanol	5 10 15	0.117 0.252 0.291	78.048 82.868 88.947	1.471
03.	Distilled water	5 10 15	0.328 0.600 1.095	38.416 59.211 58.412	2.633

CONCLUSION

The present study conducted preliminary phytochemical and physicochemical investigations on Cassia angustifolia (C. angustifolia), focusing on its significant secondary metabolites, which are responsible for various pharmacological activities. These investigations are essential for drug identification and exploration of bioactive constituents in medicinal herbs. The phytochemical analysis of the ethanolic extract revealed the presence of flavonoids, glycosides, carbohydrates, and tannins, which contribute to multiple pharmacological effects, including anti-inflammatory, chemoprotective, antioxidant, antidiabetic, antianxiety, and antidepressant activities.

The identification and authentication of Cassia angustifolia seeds were carried out through macroscopic and microscopic studies, further confirmed by similar HPTLC profiles. Quantification of sennosides A and B revealed variations based on geographic location, with sennoside A ranging from 1.00% to 2.14% and sennoside B from 1.34% to 3.95%.

The antioxidant potential of Cassia angustifolia extracts was assessed using the DPPH free radical scavenging assay at three different concentrations (5, 10, and 20 µg/ml), demonstrating that this medicinal plant is a promising candidate as an antioxidant. This study supports the need for further in-depth assays of traditional herbal medicinal plants for potential use in pharmacological preparations

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