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PHYTOCHEMICAL SCREENING, QUANTITATIVE ESTIMATION, AND ANTIOXIDANT ACTIVITY OF *CASSIA SPECTABILIS* EXTRACTS

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ABSTRACT

The present study aimed to evaluate the phytochemical composition and antioxidant potential of various extracts of *Cassia spectabilis*. Physicochemical parameters such as ash values and moisture content were determined to assess the purity and quality of the crude drug. The extractive value analysis revealed that the aqueous extract showed the highest yield, indicating the predominance of polar constituents. Preliminary phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, phenols, saponins, tannins, and carbohydrates, especially in the ethanolic and aqueous extracts. Quantitative estimation demonstrated that the ethanolic extract possessed the highest total phenolic (0.75 mg GAE/100 mg) and flavonoid (0.96 mg QE/100 mg) content. The antioxidant potential of the extracts was evaluated using DPPH, hydrogen peroxide, and nitric oxide radical scavenging assays. The ethanolic extract exhibited significant free radical scavenging activity with IC_{50} values of 71.51 µg/mL (DPPH), 278.39 µg/mL (H_2O_2), and 68.25 µg/mL (NO), as compared to the standard ascorbic acid. These findings suggest that *Cassia spectabilis* possesses potent antioxidant activity, which may be attributed to its high content of phenolic and flavonoid compounds, supporting its traditional use as a medicinal plant with therapeutic potential against oxidative stress–related disorders.

Keywords: *Cassia spectabilis*, Phytochemical screening, Antioxidant activity, DPPH, Hydrogen peroxide, Nitric oxide, Phenolic content, Flavonoid content, Ethanol extract.

INTRODUCTION

Medicinal plants are rich sources of bioactive compounds that serve as potential therapeutic agents for the treatment and prevention of various diseases. The growing interest in plant-based medicine is largely due to their pharmacological efficacy, low toxicity, and cost-effectiveness when compared to synthetic drugs (Pandey *et al.*, 2013). Among these, plants belonging to the genus *Cassia* (family Fabaceae) have been extensively studied for their diverse phytochemical constituents and wide spectrum of biological activities, including antimicrobial, anti-inflammatory, antidiabetic, and antioxidant effects (Gupta *et al.*, 2016; Singh *et al.*, 2017). *Cassia spectabilis* DC., commonly known as "Golden Shower Tree," is a tropical ornamental plant distributed widely in Asia, Africa, and South America. Traditionally, various parts of this plant, such as leaves, bark, and flowers, have been used in folk medicine for treating skin diseases, constipation, fever, and infections (Ramesh *et al.*, 2018). Phytochemical studies have revealed that *C. spectabilis* contains a variety

of secondary metabolites, including flavonoids, alkaloids, saponins, tannins, and phenolic compounds, which are known for their antioxidant potential and pharmacological properties (Olayinka *et al.*, 2020; Ahmed *et al.,* 2021).

Antioxidants play essential role in neutralizing free radicals and preventing oxidative stress-related cellular damage, which contributes to the pathogenesis of chronic diseases such as diabetes, cancer, and cardiovascular disorders (Halliwell *et al.*, 2015). Natural antioxidants derived from plants are particularly valued for their safety and efficacy in maintaining redox homeostasis (Birben *et al.,* 2012). Hence, investigating the antioxidant properties of *C. spectabilis* extracts, alongside qualitative and quantitative phytochemical profiling, is vital for understanding its therapeutic potential and justifying its traditional use.

The present study aims to conduct a comprehensive phytochemical screening, quantitative estimation of major phytoconstituents (such as total phenolics and flavonoids), and evaluation of the antioxidant activity of *Cassia spectabilis* extracts using standard in vitro assays. This investigation will provide scientific insight into the bioactive composition and pharmacological relevance of this medicinally important plant.

MATERIALS AND METHODS

Materials

The fresh leaves of *Cassia spectabilis* were collected, shade-dried, and coarsely powdered for extraction. Analytical grade solvents such as chloroform, ethyl acetate, ethanol, and distilled water were used for successive extraction by Soxhlet apparatus. All chemicals and reagents employed, including ferric chloride, Folin–Ciocalteu reagent, aluminum chloride, quercetin, and gallic acid, were of analytical grade and procured from standard suppliers. Ascorbic acid was used as the standard antioxidant for comparative evaluation. All glassware and instruments used were of laboratory grade and properly calibrated before use.

Methods

Procurement of plant materials

Stem of *Cassia spectabilis* were collected from the Bhopal governing region in February 2023. To prevent the phytochemicals from deteriorating, the plants were cleaned after being collected.

Drying

Stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed.

Determination of physio-chemical Parameters

Determination of Ash Values

A. Determination of Total Ash: 2 g of accurately weighed stem powder was incinerated in a tarred platinum or silica dish at a temperature not exceeding 450ºC until free from carbon, cooled and weighed. If a carbon free ash could not be obtained in this way, the charred mass was exhausted with hot water, the residue was collected on an ashless filter paper, incinerated, along with filter paper, evaporated to dryness and ignited at a temperature not exceeding 450ºC. The ash thus obtained was then cooled, weighed and percentage of ash was calculated with reference to the air-dried drug.

B. Determination of Acid Insoluble Ash:

The ash obtained from above procedure was boiled for 5 min. with 25 ml of dilute

hydrochloric acid and the insoluble matter was collected in a Gooch crucible, or on an ashless filter paper. The insoluble matter thus obtained was washed with hot water and filter paper was ignited to a constant weight along with filter paper. The percentage of acid-insoluble ash was calculated with reference to the air-dried drug.

C. Determination of Water Soluble Ash:

The ash was boiled for 5 min. with 25 ml of water, the insoluble matter collected in a Gooch crucible, or on an ashless filter paper, washed with hot water and ignited for 15 min. at a temperature not exceeding 450ºC. The weight of the insoluble matter was subtracted from the weight of the ash. The difference in weight was the water soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried drug.

D. Determination of Moisture Content (loss on drying):

About 10 g of stem (without preliminary drying and cut in parts of about 3 mm in thickness), after accurately weighing (weight to within 0.01g) was placed in a tarred evaporation dish. It was then dried at 105ºC for 5 hours and weighed. Drying was continued and the stem was weighed at 1 h interval until the difference between two successive weighing corresponded to not more than 0.25 percent. Constant weight was reached when two consecutive weighing after drying for 30 min. and cooling for 30 min. in a desiccator, did not show more than 0.01g difference.

Extraction by maceration process

An established and popular extraction method for separating bioactive substances from plant sources is maceration. This technique works especially well for removing phytochemicals from plants that include sensitive components that more aggressive extraction techniques that use heat or solvents might break down. The process described below was used to prepare the extract from the powdered and shade dried herbs (Khandelwal, 2005).

Defatting with petroleum ether

After being coarsely ground, 50 grams of stem of *Cassia spectabilis* were shade-dried at room temperature. Using an electric grinder, the dried plant material was ground into a fine powder, sieved, and stored in plastic bags until it was needed. Shade dried plant material were macerated and extracted using petroleum ether. The extraction process was carried out until the material had been defatted.

Successive extraction with different solvent

Using a 48 hour maceration method, defatted dried powder was extracted using various solvents, including chloroform, ethyl acetate, ethanol and water. By increasing the contact between the plant material and the solvent, the mixture was periodically agitated to improve the extraction efficiency. To separate the liquid extract from the solid plant remains, the mixture is filtered after the soaking period. The dissolved phytochemicals are present in the filtrate. The extract is subsequently concentrated by removing the solvent, typically with a rotary evaporator or by evaporating under lower pressure. To separate particular bioactive components, the concentrated extract can undergo additional processing or analysis.

Determination of extractive value (% yield)

In the phytochemical study of plant materials, determining the extractive value also referred to as the yield percentage is an essential step. By measuring the amount of soluble components extracted from a specific amount

of plant material, it assesses the extraction process effectiveness. This measure is essential for evaluating the extraction method efficacy and comparing the extraction efficiency of various solvents or circumstances. The following formula was used to determine each extracts % yield:

$$\text{Percentage Yield} = \frac{\text{Weight of extract}}{\text{Weight of powdered drug taken}}$$

Qualitative phytochemical analysis

One essential procedure in the fields of pharmacognosy and natural products chemistry is qualitative phytochemical analysis. It entails identifying the different bioactive substances found in plant materials. Alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, and phenolic compounds are some examples of these substances, which are referred to as phytochemicals (Kokate, 1994).

Quantitative studies of phytoconstituents

Estimation of total phenol content

The modified folin-ciocalteu method was used to calculate the extracts total phenol concentration (Gaur Mishra *et al.*, 2017). Various aliquots of 5–25 µg/ml were generated by dissolving 10 mg of gallic acid in 10 ml of methanol. After dissolving 10 mg of dried extract in 10 ml of methanol, the mixture was filtered. This extract was used to estimate the amount of phenol in 2 ml (1 mg/ml). 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/L) of sodium carbonate were combined with 2 ml of extract and each standard. For color development, the mixture was vortexed for 15 seconds and then left to stand for 10 minutes. Using a spectrophotometer, the absorbance at 765 nm was determined.

Estimation of total flavonoids content

The aluminum chloride technique was used to determine the total flavonoid content (Parkhe and Bharti, 2019). Various aliquots of 5–25 µg/ml were produced by dissolving 10 mg of quercetin in 10 ml of methanol. 10 mg of dried extract were dissolved in 10 ml of methanol, and the resulting mixture was then filtered. The flavonoids in 2 ml (1 mg/ml) were estimated using this extract. 2 ml of 2% $AlCl_3$ solution were added to 2 ml of extract of each standard, and the combination was then allowed to stand for 15 minutes at room temperature. The absorbance was then measured at 420 nm.

In-vitro* antioxidant activity of ethanolic extract of *Cassia spectabilis

DPPH method

With a minor modification, the revealed method was applied to assess the extracts overall ability to scavenge free radicals (Parkhe and Jain, 2018). A DPPH solution (6 mg in 100 ml methanol) was made and kept in a dark location. Standard and test concentrations ranging from 10 to 100 µg/ml were generated. 1.5 ml of DPPH and 1.5 ml of each standard and test were prepared in a separate test tube; the absorbance of this mixture was immediately measured at 517 nm. 1.5 ml of DPPH and 1.5 ml of methanol were tested as a control absorbance at 517 nm. Using the following formula, the % inhibition of free radical DPPH was determined:

$$\% \text{ inhibition} = [(\text{absorbance of control} - \text{absorbance of sample})/\text{absorbance of control}] \times 100\%$$

Hydrogen peroxide method

Hydrogen peroxide was used to test the extracts *in-vitro* antioxidant properties (Wasiullah *et al.,* 2023). 2.4 ml of 0.1 M phosphate buffer (pH 7.4), 1.0 ml of ethanolic sample (100-500µg/ml), and 2 ml of hydrogen peroxide (43mol) were added. After ten minutes of maintaining the resultant solution, the absorbance at 230 nm was measured. The blank was ready without hydrogen peroxide, and the control was made without any sample. It was combined with ascorbic acid as a standard compound. The percentage of free radical hydrogen peroxide scavenging activity has been computed.

% inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%

Nitric oxide method

The Griess reagent was measured and sodium nitroprusside was converted to nitric oxide. In aqueous solution at physiological pH, sodium nitroprusside spontaneously generates nitric oxide. It then interacts with oxygen to produce nitric ions, which may be measured using the Griess reagent. Reduced nitric oxide production is the result of nitric oxide scavengers competing with oxygen (Bawane *et al.,* 2020). Different extract concentrations were combined with sodium nitroprusside (10 mmol/L) in phosphate buffer saline (PBS), and the mixture was incubated for 150 minutes at 25°C. Specimens were treated with Griess reagent (1% sulfanilamide, 2% H_3PO_4, and 0.1% napthylethylenediamine dihydrochloride). The absorption of standard ascorbic acid solutions prepared in the same way with Griess reagent as a positive control was measured at 546 nm using the chromophore absorbance produced during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine. The following formula was used to determine the inhibition proportion:

Radical scavenging activity (%) = $(A_{control}-A_{test})/A_{control}\times100$

Where, Atest is the absorption when the extract or standard is present, and Acontrol is the absorption of the control (without extract).

RESULTS AND DISCUSSION

The present study was carried out to evaluate the physicochemical parameters, phytochemical constituents, total phenolic and flavonoid content, and antioxidant potential of various extracts of *Cassia spectabilis*.

The physicochemical evaluation (Table 1) provides preliminary information regarding the purity and quality of the crude drug. The total ash value (4.86%) and acid-insoluble ash (1.52%) were within the acceptable range, indicating minimal contamination with inorganic matter such as silicates and oxides. The water-soluble ash (6.25%) suggested the presence of water-soluble inorganic salts, whereas the low moisture content (1.52%) indicated good stability and reduced risk of microbial contamination during storage.

The extractive values (Table 2) showed that the aqueous extract (11.47 % w/w) yielded the highest extraction, followed by the ethanolic extract (7.23 %). This suggests that the majority of phytoconstituents in *Cassia spectabilis* are polar in nature. The comparatively lower yield from chloroform (1.25 %) and ethyl acetate (2.62 %) extracts indicates a lesser presence of non-polar compounds.

The phytochemical screening results (Table 3) revealed the presence of important bioactive compounds such as flavonoids, phenols, saponins, tannins, and carbohydrates in ethanolic and aqueous extracts, whereas ethyl acetate extract showed the presence of glycosides and flavonoids. Chloroform extract displayed limited chemical diversity, confirming that most of the biologically active constituents are polar. The presence of these phytochemicals is responsible for various pharmacological activities, especially antioxidant and anti-inflammatory properties.

The statistical parameters related to the standard curves of Gallic acid and Quercetin (Table 4) indicate the reliability and accuracy of the calibration data obtained during the quantitative estimation of total phenolic and flavonoid content, respectively. Both standard compounds exhibited a Beer's law range of 5–25 µg/ml, demonstrating linearity within the selected concentration range.

The regression coefficient (R^2) values were found to be 0.999 for Gallic acid and 0.998 for Quercetin, which are very close to 1, confirming an excellent linear correlation between concentration and absorbance.

The regression equations obtained for Gallic acid (*y = 0.034x – 0.000*) and Quercetin (*y = 0.043x + 0.023*) describe the direct proportionality between absorbance and concentration.

Quantitative analysis (Table 5) demonstrated that the ethanolic extract had the highest total phenolic (0.75 mg GAE/100 mg) and flavonoid (0.96 mg QE/100 mg) content, followed by the aqueous extract. This higher concentration of phenolics and flavonoids in ethanolic extract correlates well with its enhanced antioxidant capacity.

The antioxidant activities were evaluated using DPPH (Table 6), hydrogen peroxide (Table 7), and nitric oxide (Table 8) radical scavenging assays. The ethanolic extract exhibited concentration-dependent scavenging activity, with IC_{50} values of 71.51 µg/mL (DPPH), 278.39 µg/mL (H_2O_2), and 68.25 µg/mL (NO), compared to the standard ascorbic acid which showed stronger inhibition at lower concentrations. Although the ethanolic extract was less potent than ascorbic acid, its significant activity indicates the presence of effective antioxidant compounds capable of neutralizing free radicals. The results (Tables 1–8) indicate that *Cassia spectabilis* possesses potent antioxidant potential, which may be attributed to the synergistic effect of its phenolic and flavonoid constituents. These findings support the traditional medicinal use of *Cassia spectabilis* and warrant further pharmacological and chemical investigations to isolate and characterize the active compounds responsible for these effects.

Table 1: Physicochemical test of *Cassia spectabilis*

S. No.	Parameters	Value (% w/w)
1	Total ash	4.86
2	Water soluble ash	6.25
3	Acid insoluble ash	1.52
4	Moisture Content	1.52

Table 2: Extractive values of *Cassia spectabilis*

S. No.	Extracts	% Yield (w/w)
1.	Chloroform	1.25
2.	Ethyl acetate	2.62
3.	Ethanolic	7.23
4.	Aqueous	11.47

Table 3: Result of phytochemical screening of extracts of *Cassia spectabilis*

S. No.	Constituents	Chloroform extract	Ethyl acetate extract	Ethanolic extract	Aqueous extract
1.	**Alkaloids**				
	Wagner's Test:	-ve	-ve	-ve	-ve
	Hager's Test:	-ve	-ve	-ve	-ve
2.	**Glycosides**				
	Conc. H_2SO_4 Test:	-ve	+ve	-ve	-ve
3.	**Flavonoids**				
	Lead acetate Test:	-ve	+ve	+ve	+ve
	Alkaline test:	-ve	-ve	+ve	+ve
4.	**Diterpenes**				
	Copper acetate Test:	-ve	-ve	-ve	+ve
5.	**Phenol**				
	Ferric Chloride Test:	-ve	-ve	-ve	+ve
	Folin Ciocalteu Test:	-ve	-ve	+ve	+ve
6.	**Proteins**				
	Xanthoproteic Test:	+ve	+ve	+ve	+ve
7.	**Carbohydrate**				
	Fehling's Test:	-ve	-ve	+ve	+ve
	Benedict's Test	-ve	-ve	-ve	+ve
8.	**Saponins**				
	Froth Test:	-ve	-ve	+ve	+ve
9.	**Tannins**				
	Gelatin test:	-ve	-ve	-ve	+ve
10.	**Sterols**				
	Salkowski Test:	-ve	-ve	-ve	-ve

+ve= present, -ve=negative

Table 4: Statistical parameters related to standard curve of Quercetin and Gallic acid

Parameters	Gallic acid	Quercetin
Beer's Law Range	5-25 µg/ml	5-25 µg/ml
Regression Coefficient	$R^2 = 0.999$	$R^2 = 0.998$
Regressed line equation (y = mx + c)	y = 0.034x - 0.000	y = 0.043x + 0.023

Table 5: Estimation of total phenol and flavonoids content of *Cassia spectabilis*

S. No.	Extracts	Total phenol content	Total flavonoids content
		Gallic acid equivalent (GAE) mg/ 100 mg	Quercetin equivalent (QE) mg/ 100 mg
1.	Ethyl acetate	-	0.21
2.	Ethanolic	0.75	0.96
3.	Aqueous	0.62	0.83

Table 6: % Inhibition of ascorbic acid and ethanolic extract of using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	10	46.25	29.57
2	20	51.69	32.46
3	40	62.78	40.29
4	60	67.05	43.97
5	80	75.91	54.14
6	100	89.34	61.25
IC_{50} value		**15.91**	**71.51**

Table 7: % Inhibition of ascorbic acid and ethanolic extract of using hydrogen peroxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	100	49.79	31.54
2	200	64.87	40.98
3	300	75.03	52.76
4	400	85.41	62.54
5	500	93.56	73.81
IC_{50} value		**80.55**	**278.39**

Table 8: % Inhibition of ascorbic acid and ethanolic extract of using Nitric oxide method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ethanolic extract
1	20	40.25	27.35
2	40	65.78	37.91
3	60	70.75	49.58
4	80	79.24	56.74
5	100	86.46	60.89
IC_{50} value		**25.08**	**68.25**

CONCLUSION

The present investigation concludes that *Cassia spectabilis* possesses significant phytochemical richness and antioxidant potential**.** The physicochemical evaluation confirmed the quality and purity of the crude drug, while extractive value analysis indicated that polar solvents, particularly water and ethanol, are more efficient in extracting bioactive constituents. Phytochemical screening revealed the presence of important secondary metabolites such as flavonoids, phenols, saponins, tannins, and carbohydrates, which are known for their therapeutic benefits. Among all extracts, the ethanolic extract showed the highest concentration of phenolic and flavonoid compounds and exhibited remarkable free radical scavenging activity in DPPH, hydrogen peroxide, and nitric oxide assays.

These findings suggest that *Cassia spectabilis* is a promising source of natural antioxidants that could play a key role in preventing oxidative stress–related diseases. The study provides a scientific basis for the traditional medicinal use of this plant and encourages further research on isolation, characterization, and pharmacological evaluation of its active constituents for potential therapeutic applications.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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