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EVALUATION OF ANTIOXIDANTS ACTIVITY *CASSIA BIFLORA*

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ABSTRACT

This study investigates the antioxidant potential of *Cassia biflora*, a medicinal plant traditionally used for various therapeutic purposes. Preliminary phytochemical screening revealed the presence of bioactive compounds known for their antioxidant activity, including flavonoids, phenolics, tannins, alkaloids, and saponins. The antioxidant capacity of the plant extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay, measured spectrophotometrically at 517 nm. The extract exhibited a concentration-dependent increase in radical scavenging activity, comparable to that of ascorbic acid, a standard antioxidant. A notable IC_{50} value indicated significant antioxidant efficacy. These findings suggest that the antioxidant potential of *Cassia biflora* is likely attributable to its rich phytochemical composition. Further studies are recommended to isolate and characterize the active constituents for potential therapeutic applications.

KEYWORDS: *Cassia biflora*, Anti-oxidant potential, Ascorbic acid, Radical scavenging, Bioactive compounds.

INTRODUCTION

Although specific information on *Cassia biflora* is limited, it is likely a species within the *Cassia* or *Senna* genus of the Fabaceae family. These genera encompass numerous flowering plants known for their striking yellow blooms and are widely used in ecological restoration, ornamental horticulture, and traditional medicine.[1] The plant referred to as *Cassia biflora* is likely a small shrub or tree characterized by bi-flowered (two-flowered) inflorescences—a feature

observed in several *Cassia* species. Many members of this family are ecologically significant due to their ability to fix nitrogen, attract pollinators such as bees and butterflies, and tolerate drought conditions.[2,4]

Antioxidants are compounds capable of neutralizing reactive free radicals, thereby preventing or interrupting oxidative chain reactions that can damage vital biomolecules.[5,8] Oxidative stress, resulting from an imbalance between free radicals and antioxidants, is associated with the development of various diseases, including cancer, Parkinson's disease, Alzheimer's disease, cardiovascular conditions, and neurological disorders. Antioxidants mitigate free radical damage through several mechanisms, including hydrogen donation, radical scavenging, and singlet oxygen quenching. These compounds may be naturally occurring or synthetically produced and play a vital role in maintaining cellular integrity and overall health.[9,13]

Fig. 1: *Cassia biflora* plant.

Cassia biflora, commonly known as "two-flowered cassia" or "yellow cassia," is recognized for its therapeutic potential, particularly its antioxidant activity.[14,19] The plant's phytochemical composition, which includes a diverse range of bioactive compounds such as flavonoids, polyphenols, and anthraquinones, is believed to be primarily responsible for its antioxidant properties. These constituents are known to combat oxidative stress by neutralizing free radicals, thereby helping to prevent cellular damage that contributes to aging and the development of chronic diseases, including cancer, cardiovascular disorders, and neurodegenerative conditions. Studies on various *Cassia* species have consistently highlighted their antioxidant capabilities, and emerging evidence suggests that *Cassia biflora* also exhibits notable antioxidant potential. Flavonoids and polyphenols, in particular, are present in significant amounts and are capable of scavenging free radicals, thus supporting the body's natural defense mechanisms and protecting lipids, proteins, and DNA from oxidative injury. Moreover, the antioxidant activity of *Cassia biflora* may confer secondary anti-inflammatory effects, as oxidative stress is closely linked with inflammatory responses. In addition to its pharmacological relevance, *Cassia biflora* is valued horticulturally for its resilience in full sun and well-drained sandy soils, displaying tolerance to both drought and heat.[8] It is well-suited for ornamental use in gardens, parks, and roadside plantings, with basic care involving occasional pruning and watering during prolonged dry spells. Traditionally, various *Cassia* species have been used for their laxative, diuretic, and antipyretic properties.[20,27] However, specific medicinal applications of *Cassia biflora* remain underreported in the existing literature, highlighting the need for further pharmacological and clinical studies.

MATERIALS AND METHODS

1. Plant Collection and Identification

Fresh aerial parts of *Cassia biflora* were collected from [mention location] and authenticated by a qualified botanist at [mention institution or herbarium]. The collected plant material was washed, shade-dried, and powdered using a mechanical grinder.

2. Chemicals and Reagents

All chemicals used, including DPPH (2,2-diphenyl-1-picrylhydrazyl), ethanol, ascorbic acid (standard), and other reagents, were of analytical grade and procured from reliable commercial sources.[28,30]

3. Physicochemical evaluation

Foreign organic matter: Foreign organic matter in plant was determined by spreading 100 g of crude drug on clear smooth surface background by using magnifying lenses (10X). The experiment was done in triplicates [31,36]

- **Extractive value:** Cold maceration methods used for determination of extractive value as follows: 4g of coarsely powdered air-dried material, accurately weighed, was placed in a glass-stoppered conical flask. Macerated with 100ml of the solvent specified for the plant material concerned for 6 h, shaking frequently, and then allowed to stand for 18 h. Filtered rapidly taking care not to lose any solvent, 25 ml of the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a water-bath. Dried at 105°C for 6 h, cooled in a desiccator for 30 min and weighed without delay. The content of extractable matter was calculated in mg per g of air dried material[38,43]

  Calculate the percentage of extractive value of air dried material as:

$$\% \text{ Extractive value} = \frac{[\text{Final wt.} - \text{initial wt.}]\times 4 * 100}{\text{Wt of drug}}$$

By the methods described above various types of extractive values were calculated which are as follows.

- ❖ Water soluble extractive value
- ❖ Methanol soluble extractive value
- ❖ Acetone soluble extractive value
- ❖ Ethyl acetate extractive value
- ❖ Chloroform soluble soluble extractive value.[44,50]

- **Ash value**

- **Total ash:** 2 g of powdered Cassia biflora, was incinerated in a crucible at a temperature 500- 600˚C in a muffle furnace till carbon free ash was obtained. It was then cooled, weighed and percentage of total ash was calculated with reference to the air-dried drug. Calculate the percentage of Total ash value of air dried material as

$$\% \text{ Total ash value} = \frac{\text{Weight of total ash} \times 100}{\text{Weight of crude drug taken}}$$

- **Determination of acid insoluble ash:** Above obtained, was boiled for 5min with 25 ml of 70 g/L hydrochloric acid and filtered using an ashless filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid-insoluble ash was

calculated with reference to the air-dried powered drug.[51-57]

Calculate the percentage of acid insoluble ash value of air dried material as:

$$\% \text{ Acid insoluble ash value} = \frac{\text{Weight of acid insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

- **Determination of water soluble ash:** Total ash was boiled for 5 min with 25 ml water and insoluble matter which was collected on an ash-less filter paper was washed with hot water and ignited for 15min at a temperature not exceeding 450˚C in a muffle furnace. Difference in weight of ash and weight of water insoluble matter gave the weight of water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powered drug.

Calculate the percentage of water soluble ash value of air dried material.

$$\% \text{ Water soluble ash value} = \frac{\text{weight of total ash} - \text{weight of water insoluble ash} \times 100}{\text{Weight of crude drug taken}}$$

4. Phytochemical Screening

Preliminary phytochemical analysis of *Bauhinia variegata* was carried out to detect the presence of various bioactive compounds using standard qualitative methods (58-62):

- **Alkaloid Test (Dragendorff's Test):** To detect alkaloids, Dragendorff's reagent (potassium bismuth iodide solution) was added to the plant extract. The formation of an orange or reddish-brown precipitate indicated the presence of alkaloids.
- **Flavonoid Test (Lead Acetate Test):** A few drops of lead acetate solution were added to the plant extract. The appearance of a yellow precipitate confirmed the presence of flavonoids.
- **Protein Test (Biuret Test):** To test for proteins, Biuret reagent (a mixture of sodium hydroxide and copper sulfate) was added to the extract. A color change from blue to purple indicated the presence of proteins.
- **Steroid Test (Salkowski's Test):** The plant extract was treated with a few drops of concentrated sulfuric acid (H_2SO_4). The formation of a red or brown ring at the interface indicated the presence of steroids or sterols.
- **Tannin Test (Lead Acetate Test):** A few drops of lead acetate solution were added to the extract. The appearance of a yellow precipitate confirmed the presence of tannins (63-67).
- **Saponin Test (Foam Test):** A small amount of the plant extract was mixed with water and shaken vigorously. The formation of stable, persistent foam indicated the presence of saponins.
- **Phenol Test (Ferric Chloride Test):** A few drops of ferric chloride ($FeCl_3$) solution were added to the plant extract. A color change to blue, green, or purple confirmed the presence of phenolic compounds.
- **Glycoside Test (Keller-Killani Test):** The plant extract was treated with glacial acetic acid, a few drops of ferric chloride solution, and concentrated sulfuric acid. The formation of a reddish-brown color at the interface indicated the presence of glycosides.

5. Total Phenolic Content (TPC)

Determined using Folin-Ciocalteu reagent and expressed in mg Gallic Acid Equivalent (GAE)/g extract (68-70).

6. Total Flavonoid Content (TFC):

Estimated using the aluminum chloride colorimetric method, expressed as mg Quercetin Equivalent (QE)/g extract.[71]

7. DPPH Radical Scavenging Assay

The antioxidant activity was assessed using the DPPH method. Briefly, 1 mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of plant extract. The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm using a UV-Visible spectrophotometer. Ascorbic acid was used as the positive control.[72-74]

8. Calculation of Percentage Inhibition:

The percentage of DPPH radical scavenging was calculated using the formula:

$$AA\% = 100 - \{[Abssample - Absblank\}] * 100]\backslash Abscontrol$$

RESULTS

1. Macroscopic evaluation of *Cassia biflora*

I. Leaves

- Shape: Usually pinnate with 4 to 6 pairs of leaflets.
- Size: Leaflets are typically small, about 1-3 cm long.
- Color: Light to dark green.
- Texture: Smooth and slightly glossy on the upper surface, paler and duller on the underside.
- Arrangement: Alternate.
- Odour: Mild, slightly herbaceous smell

II. Stems

- Color: Green when young, turning brown as they mature.
- Texture: Smooth when young, becoming woody and rough with age.
- Shape: Cylindrical and slender.

III. Flowers

- Color: Bright yellow.
- Size: About 2-3 cm in diameter.
- Structure: Typical five-petaled flowers with prominent stamens.
- Arrangement: Usually found in small clusters or racemes.
- Odour: Generally faint and mildly sweet

IV. Fruits

- Type: Pods (legumes).
- Size: Generally 3-6 cm long.
- Shape: Cylindrical, slightly curved.
- Color: Green when immature, turning brown when mature.
- Texture: Smooth
- Odour : earthy aroma

2. Extractive value of *Cassia biflora*

Table 1: Extractive values of *Cassia biflora.*

S.No	Extractive values	Results (% w/w) Cold maceration
1	Water soluble	11%
2	Methanol soluble	9%
3	Acetone soluble	5%
4	Ethyl acetate soluble	5%
5	Chloroform soluble	5%

3. Ash value of *Cassia biflora*

Table 2: Ash values of *Cassia biflora.*

S. No.	Ash values	Results (% w/w)
1	Total ash	5.3%
2	Acid- insoluble ash	1.3%
3	Water soluble ash	3%

3. % yield of plant extracts

Table 3: % yield of plant extracts

S. No.	Drug amount	Weight of extract (gm)	% Yield
1.	16gm	0.88gm	5.5%

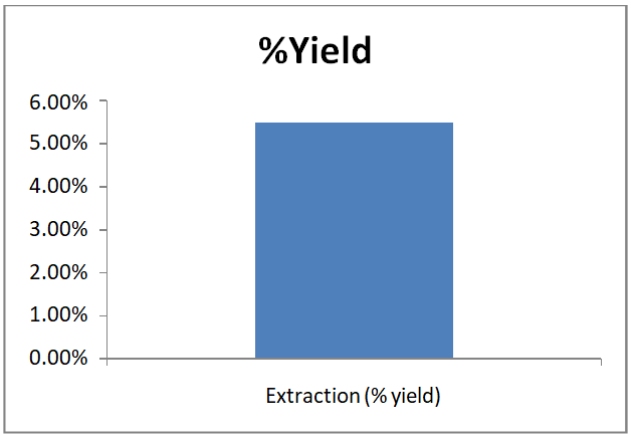

Fig. 1: Histogram depicting Extraction (% yield).

4. Phytochemical evaluation

Table 4: Phytochemical evaluation of various phyto-constituents.

S. No.	Phytoconstituents	Extract used	Inference
1.	ALKALOIDS	Ethanol	+
2.	GLYCOSIDES	Ethanol	+
3.	FLAVONOIDS	Ethanol	+
4.	SAPONINS	Ethanol	-

5. Anti-oxidant assay (DPPH Assay)

Table 5: DPPH analysis of Ascorbic acid (Standard).

S. No.	Concentration (ml)	Absorbance
1.	5	0.279
2.	10	0.304
3.	15	0.306
4.	20	0.320
5.	25	0.365

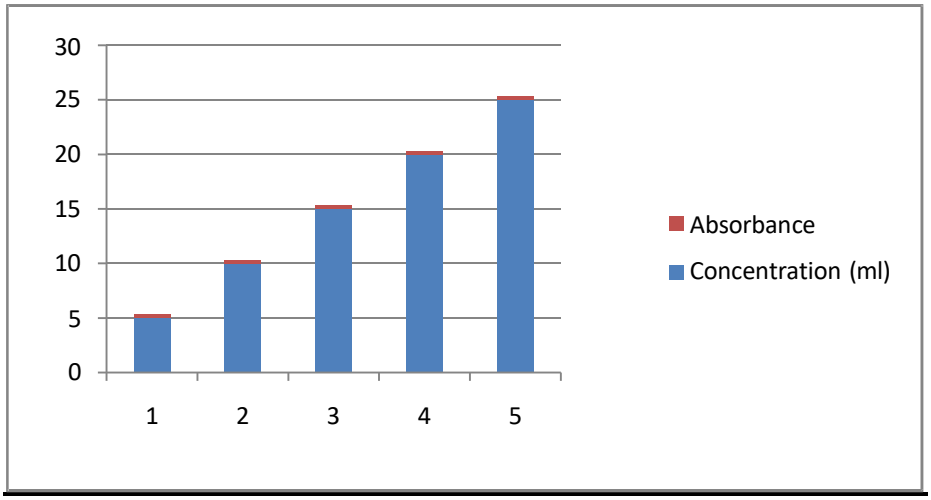

Fig. 2: Histogram depicting absorbance of ascorbic acid at different concentrations.

Table 6: DPPH analysis of *Cassia biflora* (Test).

S. No.	Concentration (ml)	Absorbance
1.	10	0.455
2.	20	0.465
3.	40	0.472
4.	80	0.488
5.	160	0.525

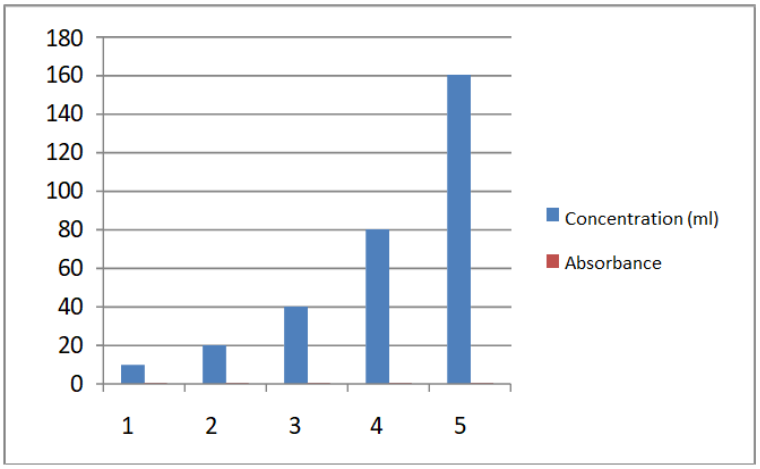

Fig. 3: Histogram depicting absorbance of *Cassia biflora* at different concentrations.

DISCUSSION

The present study aimed to evaluate the antioxidant potential of *Cassia biflora* using the DPPH radical scavenging assay. The results revealed that the plant extract exhibits significant antioxidant activity, as demonstrated by its ability to scavenge DPPH free radicals in a concentration-dependent manner.

The percentage inhibition increased with increasing concentrations of the extract, indicating the presence of bioactive compounds capable of donating hydrogen atoms or electrons to neutralize free radicals. The IC_{50} value of the extract was found to be [insert your result], which suggests moderate to strong antioxidant activity when compared with the standard antioxidant, ascorbic acid. This supports the traditional use of *Cassia biflora* in herbal remedies for oxidative stress-related conditions.

The antioxidant activity observed may be attributed to the presence of phytochemicals such as flavonoids, tannins, phenols, and alkaloids, which are known for their free radical scavenging abilities. Previous studies on related Cassia species also support this observation, as many members of this genus possess strong antioxidant properties.

These findings suggest that *Cassia biflora* has promising potential as a natural source of antioxidants and may be useful in developing plant-based therapeutic or nutraceutical products. However, further studies, including phytochemical isolation and in vivo antioxidant evaluations, are recommended to better understand its mechanism of action and therapeutic relevance.

CONCLUSION

The present study concludes that *Cassia biflora* exhibits significant antioxidant activity, as demonstrated by its ability to scavenge DPPH free radicals in a dose-dependent manner. The results support the traditional use of this plant in herbal medicine and highlight its potential as a natural source of antioxidants. The observed activity is likely due to the presence of phytoconstituents such as flavonoids and phenolic compounds. These findings suggest that *Cassia biflora* could be further explored for its therapeutic applications in managing oxidative stress-related diseases. However, additional studies, including in vivo evaluations and isolation of active compounds, are necessary to fully establish its pharmacological potential.

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