European Journal of Medicinal Plants

Volume 35, Issue 6, Page 13-20, 2024; Article no.EJMP.118498
ISSN: 2231-0894, NLM ID: 101583475

Determination of Phytochemical Compositions of Leaves and Flowers of *Cassia auriculate*

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

Article Information

DOI: https://doi.org/10.9734/ejmp/2024/v35i61204

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/118498

Original Research Article

Received: 20/04/2024
Accepted: 22/06/2024
Published: 16/08/2024

ABSTRACT

The present study reveals the morphological characteristics, organoleptic and fluorescence analysis by using various chemicals and reagents to examine the presence of phytochemicals visually in powdered samples of leaves and flowers of *Cassia auriculata*. The qualitative analysis represents the presence of various phytochemicals *viz*., steroids, reducing sugars, sugars, alkaloids, phenols, flavonoids, saponins, tannins, Anthraquinones and amino acids in crude extracts (aqueous, ethanol and petroleum ether) of leaves and flowers of *Cassia auriculata*. The presence of minerals *i.e.,* potassium, phosphorous, sulphur, calcium, were observed in leaves and flowers of *Cassia auriculata* by qualitatively. Quantitative analysis revealed maximum amount of flavonoids in flowers of *Cassia auriculata* and lesser amounts of alkaloids were recorded in leaves and flowers of *Cassia* respectively.

Keywords: Cassia auriculata; fluorescence analysis; phytochemicals.

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Cite as: *Parani, K. 2024. "Determination of Phytochemical Compositions of Leaves and Flowers of Cassia Auriculate". European Journal of Medicinal Plants 35 (6):13-20. https://doi.org/10.9734/ejmp/2024/v35i61204.*

1. INTRODUCTION

"Phytochemicals are a large group of plant-derived compounds that are hypothesized to be responsible for much of the disease reduction conferred by a diet high in fruits vegetables, beans, cereal and plant-based beverages such as tea and wine. Based on their chemical structure phytochemicals can be grouped into such groups as tannins, flavonoids, glycosides, saponins, alkaloids, triterpenoids and sterols" [1].

"For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Sources for new safe, biodegradable and renewable drugs. The use of plants as therapeutic agents in addition to being used as food is age-long" [2].

"Though the therapeutic uses of plants by the primitive people lack scientific explanations there is a great awareness in the use and significance of these medicinal floras by the World Health Organization in several resource-poor nations" [3,4]. "This has led to intensified efforts in the documentation of medicinal plants" [5].

"Therefore, such plants should be investigated to better understand their properties, safety and efficiency" [6]. The cost of drugs in use today is too expensive for the majority of the population in the third world countries and therefore the search for some cheap sources of antimicrobial substances in nature become inevitable plants are good.

Cassia auriculata Linn (Family: Caesalpinaceae) distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha system of medicine. The plant has been reported to possess antipyretic [7]; hepato protective [8]; anti diabetic, antiperoxidative and antihyperglyceamic [9] and microbicidal activity [10]. "The flowers are used to treat urinary discharges, nocturnal emissions, diabetes and throat irritation" [11]. Hence, the objectives of the present study is focused on evaluating the phytochemicals qualitatively and quantitatively of the *Cassia auriculata* using various extracts.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

Leaves and flowers of *Cassia auriculata* (*Caesalpinaceae*)were collected from Karuppanathi Dam near Chokampatti, Tenkasi (TK.) Tamil Nadu.

2.2 Organoleptic Study

The plant powder characteristics such as color, odor, taste and nature were evaluated.

2.3 Preparation of Crude extracts

The collected plant samples were thoroughly washed under running tap water and shade dried. The samples were pulverised with the help of a blender / mixer and soaked in aqueous, ethanol and petroleum ether were prepared by macerating one gram of powder with 10ml of solvents taken in flasks wrapped separately in Erlenmeyer flasks. The preparation were allowed to stand for 4 hrs. at room temperature. Then the extracts were filtered using Whatmann filter No. 1 and stored for further use. The crude extracts were analysed qualitatively and quantitatively and percentage yield of the extract was determined by using the equation [12].

$$\text{Yield (\%)} = W_2 - W_1 / W_0 \times 100$$

Where W_2 - weight of the extract and container
W_1 – weight of the empty container and W_0 is the weight of the initial dried sample.

2.4 Fluorescence Analysis of the Powder

The fluorescence analysis of powdered samples *i.e.*, leaves and flower mixed with different solvents and reagents were carried out using long ultraviolet (UV) lamps (365nm) and visible wavelengths [13 – 15].

2.5 Preliminary Phytochemical Analysis

The qualitative tests for extracts to detect the presence of phytochemicals such as alkaloid, tannin, saponin, flavonoid and phenol were carried out using standard procedures [16].

2.6 Quantitative analysis of Phytochemicals

2.6.1 Phenol determination

100 mg of the extract of the sample was weighed accurately and dissolved in 100 ml of triple distilled water (TDW). 1 ml of this solution was

transferred to a test tube, then 0.5 ml 2N of the Folin Ciocalteu reagent and 1.5ml 20% of Na_2CO_3 solution was added and ultimately the volume was made up to 8 ml with TDW followed by vigorous shaking and finally allowed to stand for 2 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of gallic acid.

2.6.2 Alkaloid determination

"Five gram of the powdered plant samples were weighed into 200 ml of 20% acetic acid in ethanol was added and covered to stand for 4 h. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed" [17].

2.6.3 Flavonoid determination

"To estimate flavonoids quantitatively, 10 g of powdered sample of each plant material was extracted twice with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatmann filter paper No.1, the filtrate was later transferred into crucibles, and evaporated to dryness on a water bath to a constant weight" [17].

2.6.4 Tannin determination

"Distilled water (50 ml) was added to 500 mg of the sample taken in a 500 ml flask and kept in a shaker for 1 h. It was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pippetted out into a test tube and mixed with 2 ml (10 fold diluted) of 0.1 M $FeCl_3$ in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 605 nm within 10min" [18].

3. RESULTS AND DISCUSSION

"Plant based antibacterial preparations are known to have enormous therapeutic potential due to the presence of several antibacterial substances" [19]. "In order to identity the antibacterial active compounds of the herbs or medicinal plants, such factor should be taken into consideration including the extraction and bio assay techniques employed. Generally, the type of solvent used for the extraction plays a significant role in the solubility of the active principles of plant material that not only affected the amount of representative compounds where consequently will influence the antibacterial activity of the extract" [20]. Flowers of *Cassia auriculata* are showy with dense bunches, brightly yellow color corolla, leaf obovate with tapering apex, dark or prominent vein, waxy coated and coriaceous (Table 1).

3.1 Organoleptic Studies

"It is an important parameter of powder analysis which is technique for the qualitative detection of morphological and sensory profile of drugs" [[21]. The study revealed the characteristic color, odor, taste and nature of powdered medicinal species (Table 2). "The results of pharmacognostical and phytochemical studies conducted in the bark and leaves of *Terminalia travancorensis*Wight & Arn. (Combretaceae), a tree, endemic to the Western Ghats and their pharmacognostical studies included the organoleptic, physico-chemical and fluorescence analysis of the bark and leaf powder" [22].

3.2 Percentage Yield of Crude Extracts

Table 3 represents the percentage yield of crude extracts in medicinal plants in which high yield occurred in ethanolic extract of leaves (24%) and low yield was observed in flowers of *Cassia auriculata* (6%). "Highest yield percentage accounted for 1.70% was obtained in maceration with methanol followed by ethyl acetate at 1.28% and n-hexane at 0.93% in a study done on *Psidium guajava* leaves extract, suggested that methanol was the best solvent for solubility of several compounds" [23]. "Nevertheless, the preferred extraction method should be simple, fast, economical and importantly able to retain the important phytoconstituents" [24].

3.3 Fluorescence Analysis

In fluorescence analysis study shows specific colour appeared with specific reagents (Table 4). The powdered leaves of *Cassia* recorded as light green to brown colour under ordinary white light and fluorescent green to dark brown appeared in long UV light (365 nm). The *Cassia* flower powder appeared as light yellow to dark black in ordinary light, while under long UV light (365 nm) exposed showed fluorescence green to dark red in colour.

Table 1. Morphological features *of Cassia auriculata*

S. No	Parameters	Leaf	Flower
1.	Habit	Shrub	Shrub
2.	Root system	Tap root	Tap root
3.	Stem	Branches	Branches
4.	Leaf	Lanceolate	Lanceolate
5.	Flower	Large and showy	Large and showy
6.	Colour	Green	Yellow
7.	Inflorescence	Racemose	Racemose

Table 2. Oraganoleptic character *of Cassia auriculata*

Characters	Leaf	Flower
Odour	No smell	No smell
Taste	Bitter	Bitter
Colour	Normal green	Yellow
Texture	Fine	Fine

Table 3. Percentage yield of *Cassia auriculata*

Yield percentage				
Ethanolic Extract	**Wt. of initial dried sample (W_0)**	**Wt. of empty container (W_1)**	**Wt. of extract & container (W_2)**	**Yield (%)**
Leaves	1.0	65.40	65.64	24
Flowers	1.0	61.37	61.43	6

Table 4. Fluorescence analysis of *Cassiaauriculata*

S. No	Reagent Used	*Cassia* leaf		*Cassia* flower	
		Long UV light (365 nm)	**Visible light**	**Long UV light (365 nm)**	**Visible light**
1.	Concentrated HNO_3	Dark brown	Light brown	Dark red	Dark red
2.	Concentrated HCL	Greenish brown	Pale green	Dark green	Light green
3.	Acetone	Dark brown	Brown	Light yellow	Light yellow
4.	NH_3 + Ammonia	Brownish green	Brown	Flourescence green	Light yellow
5.	Chloroform	Dark green	Light green	Dark yellow	Light yellow
6.	Benzene	Dark green	Light green	Greenish yellow	Light yellow
7.	Ethanol	Dark brown	Light brown	Greenish yellow	Light yellow
8.	Petroleum ether	Dark green	Light green	Dark yellow	Light yellow
9.	Glacial acidic acid	Dark brown	Dark brown	Greenish yellow	Light yellow
10.	$HNO_3 + NH_3$	Greenish brown	Brown	Dark black	Dark red
11.	H_2SO_4	Dark brown	Dark brown	Dark black	Dark black
12.	50% HNO_3	Greenish brown	Brown	Dark black	Dark red
13.	50% HCL	Dark green	Light green	Greenish yellow	Light yellow
14.	1N Aqueous NaOH	Dark green	Light green	Dark red	Dark black
15.	1N Alcoholic NaOH	Dark brown	Dark brown	Dark black	Light black
16.	50% H_2SO_4	Dark brown	Light brown	Brownish green	Light yellow
17.	Ferric chloride	Flourescent green	Brown	Dark red	Light red
18.	40% of NaOH + 10% Lead acetate	Dark green	Brown	Greenish yellow	Light red

Table 5. Phytochemical analysis of *Cassiaauriculata* in various extracts

S.No	Phytoconsitituents	***Aqueous***		Ethanolic		**Petroleum ether**	
		Leaf	Flowers	Leaf	Flowers	Leaf	Flower
1	Steriods	-	-	+	-	+	-
2	Reducing sugar	-	+	-	+	-	-
3	Sugar	+	+	-	+	+	+
4	Alkaloids	+	-	-	-	+	-
5	Phenol	+	+	-	+	+	+
6	Flavonoids	+	-	+	+	-	-
7	Saponin	+	-	+	+	-	+
8	Tannin	+	+	-	+	+	+
9	Anthroquenine	-	-	-	-	-	-
10	Amino acids	+	-	-	+	-	-

(+) : Present (-): Absent

Table 6. Quantitative analysis of phytochemicals

S.NO	Phytoconsituents	***Cassia* leaf**	***Cassia* flower**
1.	Flavonoids (%)	14	28
2.	Phenols (mg / g)	0.16	0.20
3.	Tannins (mg / g)	0.65	0.47
4.	Alkaloids (%)	6.0	5.0

“Herbal drug which are used in various traditional medicine needs detailed investigation with an ethano pharmacological approach. The present study provides information in respect of the identification, standardization of herbal drug of *Cassia auriculata* of Ayurvedic compendia. Correct identification and quality assurance of the starting materials is an essential pre requiste to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy” [25].

“The fluorescence analysis is adequately sensitive and enables the precise and accurate determination over a satisfactory concentration range without several time consuming dilution steps prior to analysis of pharmaceutical samples” [26]. The fluroscencecolour is specific for each compound. A non fluorescent compound may fluorescence it mixed with impurities that are fluorescent.

3.4 Qualitative Analysis

As shown in the Table 5. maximum number of phytochemicals were observed in water extract of *Cassia* leaf followed by ethanol extracts of *Cassia* flower. Lesser number of phytochemicals was seen in ethanol extract of *Cassia*leafs.

“Our results are positively correlated with ethanolic extracts revealing the presence of a high concentration of tannins, reducing sugars and steroids in the stem, bark and roots” [27]. Flavonoids, phenolics and protein were prevented in high concentration in the stem, bark while anthraquinone, glycosides and alkaloids were present in the leaves and roots of *Cassia abbreviate* respectively.

“Bioactivity properties of herbs are where closely related to their phytochemicals consitutents which are classified into various major groups” [28]. Ethanolic extract of *Cassia* flower possess reducing sugars, phenol, tannins, and steroids can summarized in Table 3. However, it is important to highlight that the type of diluent used was the main factor that could influence in variation of phytoconstituents being extracted.

“While in aqueous extract of *Cassia* flower showed the absence of phytoconstituents namely alkaloids, saponins, and antheroquinone other study that evaluated the existence of phytochemicals of petroleum ether, ethanol and aqeous extracts also revealed the difference in the solubility of active compouds” [29].

“Phenolic compounds were the most common secondary metabolites implicated with microbial growth inhibitory action in herbs” [30,31]. Plants are rich in wide variety of secondary metabolites such as tannin, terpenoids, alkaloids and flavonoids etc., which have been *in vitro* to have antibacterial and antifungal properties.

3.5 Quantitative Estimation of Major Phyto Components

Table 6 reveals the amount of phytochemicals quantitatively, in which more amount of flavonoids was recorded in flowers of *Cassia auriculata* (28%) followed by leaves of *Cassia* (14%). Lowest amount of alkaloids was observed in leaves and flowers of *Cassia* (6% and 5% of alkaloids) respectively, while tannins were more in leaves of *Cassia* (0.65 mg/g) followed by *Cassia* flowers (0.47 mg/g).

"Plant based compounds have several biological applications. An alkaloid compound has been reported to exhibit lethal effects against colon and breast cancer cells and has been used for antimicrobial, antiviral, antiprotozoal and anti tumor applications" [32]. "Flavonoids have been used for anti diabetic, anti microbial activities, anti -inflammatory and anti aging preparations" [33]. "Previous researchers have shown that the plant phenolic compounds offer the role of potential natural antioxidants" [34,35,36,37].

"Flavonoids and phenols have raised particular interest because of their potential biological characteristics as antioxidant, antiestrogenic, anti inflammatory, immune modulatory, cardio protective and anti carcinogenic compounds" [38,39]. "Tannins play an essential role in many biological applications because of their anti inflammatory, cardio protective and anti microbial properties" [40]. "Presence of alkaloids, tannins, total phenols, carbohydrates, total tannins, saponins, terpenoids and total glycosides in varying content using various solvents *viz.*, ethanol, methanol, acetone, choloroform, petroleum ether and also in water, clearly shown that the more number of photochemical compounds are maximum soluble in ethanol solvent" [41].

3.6 Qualitative Analysis of Minerals

The presence of minerals *i.e.,* potassium, phosphorous, sulphur, calcium, were observed in leaves and flowers of *Cassia auriculata* by qualitatively. Similarly reports that different plant species, elemental accumulation depends on various factors such as the type of soil, fertilization method, plant species and environmental conditions [42].

Proximate and mineral nutrient analysis validates the significance of the extracts with a high amount of carbohydrates and proteins along with significantly high amount of zinc, iron, manganese, calcium, magnesium and potassium involved in various metabolic reactions of *Calligomumcrinitum* [43].

4. CONCLUSION

The present study reveals the morphological characteristics, organoleptic and fluorescence analysis by using various chemicals and reagents to examine the presence of phytochemicals visually in powdered samples of leaves and flowers of *Cassia auriculata.* The presence of minerals i.e., potassium, phosphorous, sulphur, calcium, were observed in leaves and flowers of *Cassia auriculata* by qualitatively. The maximum amount of flavonoids was found in flowers of *Cassia auriculata* and lesser amounts of alkaloids were found in leaves and flowers of Cassia respectively.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/118498