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QUANTITATIVE ESTIMATION OF PHYTOCHEMICALS AND ANTIMICROBIAL ACTIVITY STUDY ON CASSIA AURICULATA LINN

Pharmaceutical Science

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ABSTRACT

Background: Phytochemicals are classic secondary metabolites of the plants which have numerous pharmacological benefits. Since most of the plants are used as therapeutic agents, literatures reveals *"Cassia auriculata* Linn" to have high medicinal values and that is used in traditional system of medicines. **Aim:** This study aims to evaluate the leaf and flower parts of "*Cassia auriculata* Linn" in terms of physicochemical, phytochemical study and antibacterial activity. **Material And Methods:** The plant Cassia auriculata Linn, flowers and leaves were collected from Coimbatore District, Tamil Nadu, India. The flowers and leaves were dried, and physiochemical parameters determined. The extracted sample prepared was used for preliminary phytochemical studies and to perform thin-layer chromatography. Further, the sample extract was used to evaluate antimicrobial activity. **Results:** Qualitative phytochemical analysis of Cassia auriculata leaf and flower extracts reported presence of phytoconstituents including alkaloids, carbohydrates, tannins, pyhtosterols, glycosides, and anthraquinone glycosides. The presence of Sennosides in leaf and flower extracts was confirmed by Thin layer chromatography method. The hydro alcoholic extract and the fresh juice of the plant produced a significant decrease of bacterial growth towards Staphylococcus aureus, Bacillus subtilis, Klebsiellaaerogenes, Pseudomonas aeruginosa, Aspergillusniger and Candida albicans. **Conclusion:** In this study the results suggest that different part of plant and extracpossess different antimicrobial activity. The hydro alcohol extract and fresh juice of *Cassia auriculata* Linn. exhibited significant antibacterial activity against both gram +ve and gram –ve bacteria.

KEYWORDS

Antimicrobial activity, Anthraquinone, Cassia auriculata Linn, Phytochemicals

INTRODUCTION

Herbal medicine continues to be the primary source of care for approximately 75–80 percent of the world's population, especially in developing countries.[1] This is due to its better cultural acceptability, better compatibility with the human body and fewer side effects. However, in recent years, there has been a significant increase in the use of herbal medicines, even in the developed world.[1,2]

Cassia species have piqued the interest of phytochemists due to their high medicinal value. All Cassia species are rich in secondary metabolites, particularly anthraquinone derivatives, and have been used in Chinese and Ayurvedic preparations.[3]

Cassia auriculata Linn. (Family: Caesalpiniaceae) commonly known as Tanners Senna, is an annual or biennial shrub found throughout India in open forests. It is mostly found in the dry zones of western, central and southern India, extending up to Rajasthan in the north.[4,5]

Each part of this plant has medicinal value. The leaves are anthelmintic and good for ulcers, skin diseases, and leprosy. It also improves muscle pain, irregular muscle contraction and body pain. The flowers are used in, urinary disorders, throat irritation and as an astringent. The crushed flowers are mixed with goat milk to cure sexually transmitted diseases.[6,7]

Medicinal properties of a plant is mainly due to the presence of phytochemicals like volatile oils, alkaloids, tannins, saponins, glycosides etc. Such plants are a rich source of antimicrobial agents. Thus, it is necessary to carry out a study on medicinal plants for their antimicrobial potential. [8]With this brief introduction, the current study aims to evaluate the leaf and flower parts of "*Cassia auriculata* Linn." in terms of physicochemical, phytochemical study and antibacterial activity.

MATERIALAND METHODS

Collection and Authentication of Plant:

Flowers and leaves *of Cassia auriculata* Linn. were collected in Coimbatore District, Tamil Nadu, India, and authenticated by the director, Botanical Survey of India, Coimbatore. V.No. assigned was BSI/SRC/5/23/2015/Tech/174, and the specimen herbarium was submitted for future reference.

The flowers were collected and cleaned; petals separated except for other floral parts. Also, the leaves were collected and cleaned free of other organic matter before being shade dried, powdered, and passed through a 40 mesh size filter. For future use, powder materials were sealed in an airtight container.

Physiochemical Parameters:

Important physiochemical parameters like ash values, extractive values, total crude fiber content, and moisture content were determined according to standard procedures. [9,10,11]

Procedure

The extract was prepared by a simple maceration process.[11,12]Plant extracts were prepared with various solvents for preliminary phytochemical studies, and the plant extracts were taken for the preliminary phytochemical study according to the standard procedure. [12,13,14]

To isolate sennoside, 250 g of leaf and flower powder were extracted with 750 ml of benzene separately for 2.5 hours in a soxhlet apparatus, then concentrated in vacuum to obtain a dark green viscous mass[15,16]. The isolated compound was orange red in colour, it was confirmed by Borndrager's test [16]and compared with standard drug *Cassia angustifolia* Linn.

Thin Layer Chromatography:

The isolated anthraquinone extract was used for Thin layer chromatography to isolate and identify phytoconstituents in the extract using Silica gel GF254 and a mobile phase (mixture of n-propyl alcohol: Ethyl acetate: Water: Glacial acetic acid in a 40:40:30:1 ratio). The test solution's chromatographic profile matched that of the reference solution (*Cassia angustifolia* extract*)*.[17,18]

Estimation of Sennoside:

The extract was accurately weighed in a 100ml volumetric flask at 0.150 gm, then it was dissolved in water, and diluted to 100 ml. The absorbance was measured at 515nm using methanol as a blank.[17,18]
The Percentage of Hydroxyanthraquinone glycosides present in the extracts of leaf and flower *Cassia auriculata* was calculated as sennoside by using following formula.

% = [Absorbance × 4.167] / Sample taken in gm.

Evaluation of Antimicrobial Activity:

The antimicrobial activity of the test samples (leaf and flower) extracts were carried out by standard disc diffusion method (Kirby Bauer method) [19,20]. The effect produced by the sample was compared with the

effect produced by the positive control (Reference Standard Ciprofloxacin 5µg/disc for bacteria; Nystatin 100µg/disc for fungi).The antimicrobial action was evaluated by measuring the width of inhibition zone.

RESULTS

Physiochemical Evaluation

The physiochemical evaluation of crude drugs, such as ash values, extractive values, moisture content, and total fiber content, was determined and documented for both the leaf and flower of *Cassis auriculata.* The values are tabulated in Table 1.

Table 1: Physiochemical Properties Of *Cassia Auriculata* Leaf And Flower

PARAMETERS	LEAF (% w/w)	FLOWER(% w/w)
Total ash	3.24%	1.84%
Water soluble ash	2.05%	0.95%
Acid insoluble ash	1.28%	0.78%
Sulphated ash	2.67%	1.47%
Benzene Extract	1.72%	1.05%
Chloroform Extract	1.84%	1.02%
Acetone Extract	1.53%	1.20%
Ethyl acetate	2.96%	2.35%
Ethanol Extract	3.25%	2.67%
Methanol Extract	3.51%	2.73%
Water Extract	3.87%	2.89%
Total fiber content	3.16%	1.34%
Moisture content	1.04%	0.64%

*Results are mean values of three sample preparations. (Mean ± standard deviation).

Phytochemical Study

Preliminary Phytochemical Evaluation Of *Cassia Auriculata* Leaf And Flower

The quantitative phytochemical estimation of *Cassia auriculata* leaf and flower reported the presence of alkaloids, carbohydrates, tannins, phytosterols, and glycosides. Quantitative analysis results reported that the total sennosides present in the *Cassia auriculata* leaf and flower were 2.25% and 2.1% respectively.

Table 2. Preliminary Phytochemical Evaluation Of *Cassia Auriculata*

Sl. No	Chemical Test	Water		Ethanol		Methanol		E.Acetate		Chloroform		Acetone		Benzene	
		L	F	L	F	L	F	L	F	L	F	L	F	L	F
1	Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	Proteins	+	+	+	+	+	+	-	-	-	-	-	-	-	-
4	Amino Acids	+	+	+	+	+	+	-	-	-	-	-	-	-	-
5	Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Phytosterols	+	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Saponins	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+

(+): Positive, (-): Negative, (L): Leaf, (F): Flower

Thin layer chromatography

The evaluations of sample extract showed the presence of anthraquinone glycosides. The isolated sennosides reported retention factor (R_f) values same as that of the reference standard. The TLC profiles are depicted in Table3.

Table 3: R_f Value Obtained From Leaf And Flowers Of *Cassia Auriculata*

S.No	LEAF		FLOWER	
	Test sample	Reference standard	Test sample	Reference standard
1	0.33	0.29	0.32	0.29
2	0.51	0.52	0.53	0.52
3	0.76	0.75	0.74	0.75
4	0.79	0.81	0.80	0.81

*Results are mean of three sample preparations. (Mean ± standard deviation).

Anti-bacterial Activity Screening

The anti-bacterial activity of hydroalcoholic extract and fresh juice of leaf and flowers of *Cassia auriculata*s showed good effect towards some micro-organisms.The zone of inhibition was measured and documented in Table 4.

Table 4: Antimicrobial Activity Of Leaf And Flower Of *Cassia Auriculata* Linn

S. No	Name of the Microorganisms	Leaf Extract			Fresh juice	Flower Extract			Fresh juice	Standard
		100 µl	200 µl	400 µl	200 µl	100 µl	200 µl	400 µl	200 µl	
1	*Staphylococcus aureus* (NCIM 2079)	09	14	17	15	11	16	19	17	25
2	*Bacillus subtilis* (NCIM 2063)	10	15	18	16	09	14	18	15	30
3	*Klebsiellaaerogenes* (NCIM 2098)	11	13	17	15	08	11	14	13	32
4	*Pseudomonas aeruginosa* (NCIM 2036)	12	14	18	16	10	16	19	17	30
5	*Aspergillusniger* (NCIM 105)	10	12	15	14	11	16	17	18	25
6	*Candida albicans* (NCIM 3102)	09	15	14	15	10	16	20	18	27

Zone of inhibition: mm. Values are mean triplicate of reading. (Mean ± standard deviation).

Standard- Ciprofloxacin 5µg /disc for bacteria; Nystatin 100 units / disc for fungi. Solvent DMSO.

DISCUSSION

The extensive use of medicinal plants to discover new therapeutics or active pharmacological compounds is an urgent need in order to combat diseases. The current study aimed to evaluate the leaf and flower parts of *Cassia auriculata* Linn. in terms of physicochemical, phytochemical study, and antibacterial activity. Physiochemical evaluation of crude drugs, such as ash values, extractive values, moisture content, and total fibre content, was determined and documented for *Cassis auriculata* leaf and flower. The preliminary phytochemical investigations were qualitative and it was observed that alkaloids, carbohydrates, tannins, phytosterols, and glycosides were present in the extracts. From the evaluations of sample extract by TLC the presence of anthraquinone glycosides was observed. Similar to the observations of the present study, Nille GC, et al. reported that the principle phytochemicals of *Cassia auriculata Linn* are alkaloids, anthraquinone, flavone glycosides, sugar, phenols, terpenoids, flavonoids, tannins, steroids, palmitic acid, linoleic acid, benzoic acid 2-hydroxyl methyl resorcinol, epicatechin, ferulic acid. [7] Quantitative estimation of sennosides in this selected plant also shows it contain considerable quantity 2.25% in leaf and 2.1% flower respectively.

In the current study anti-bacterial activity of hydroalcoholic extract and fresh juice of leaf and flowers of *Cassia auriculata* was reported in *Staphylococcus aureus, Bacillus subtilis, Klebsiellaaerogenes, Pseudomonas aeruginosa, Aspergillusniger and Candida albicans.* Also, for leaf extract maximum inhibition was observed in *Bacillus subtilis* and *Pseudomonas aeruginosa.* These findings were in accordance with a study conducted by Murugan T, et al. who reported that aqueous extracts showed moderate activity against *B. cereus*, *S. aureus* and *P. mirabilis* .[21] Current study findings also corroborate with Prasathkumar M, et al. where the *Cassia auriculata Linn* leaves of methanolic extract exhibited potential antibacterial activities against the tested pathogens in a concentration-dependent manner with maximum zone of inhibition against *B. subtilis*, followed by *MRSA*, *P.aeruginosa* and *E. coli.* [22]

In the present study, the flower extract showed maximum inhibition against *Aspergillus niger* and *Candida albicans*. Whereas, Maneemegalai S et al reported that aqueous extract of dry flower of

Cassia auriculata has minimum activity against *Pseudomonas aeruginosa, Klebsiella pneumoniae*.[23] These inhibitions of growth of bacteria were a result of the presence of the phytoconstituents identified in the crude extracts and the presence of phytochemicals like terpenoids, tannins, flavonoids, saponin, cardiac glycosides are responsible for its antibacterial activity.[8]

The limitation of this study is that only few micro-organisms were considered to assess the antibacterial effect. The extract's inhibitory effect justified the medicinal use of *Cassia auriculata*, and more research is needed to identify the active component of medicinal value. Also there is a need to conduct pharmacological studies to support the use of this herbal plant.

CONCLUSION

In this study the results suggest that different part of plant extracts showed different antimicrobial activity. Our preliminary findings indicate that C. auriculata flowers have a high potential for antimicrobial activity.The hydro alcohol extract and fresh juice of *Cassia auriculata* Linn exhibited significant antibacterial effect against both gram +ve and gram –ve bacteria.

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