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Investigation of HPLC-based phytochemical analysis in mature leaf extract of *Cassia auriculata*

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ABSTRACT :

The present study was carried out to evaluate the phytochemical constituents, biochemical properties, antibacterial activity, and anticancer potential of mature leaf extracts of *Cassia auriculata*. The collected leaf samples were extracted using organic solvents and subjected to qualitative phytochemical screening, biochemical estimation, chromatographic separation, and biological activity assays. Phytochemical analysis revealed the presence of carbohydrates, proteins, tannins, phenolic compounds, flavonoids, steroids, alkaloids, saponins, quinones, phosphate, chloride, and phlobatannins, while vitamin C, amino acids, and terpenoids were absent. The total protein and carbohydrate contents of the mature leaf extract were found to be 70 mg/100 ml and 20 mg/100 ml respectively. Column chromatography yielded five fractions, among which fraction 4 exhibited significant antibacterial activity against *Escherichia coli*. Thin layer chromatography analysis showed an Rf value of 0.78, indicating the possible presence of leucine. HPLC analysis of fractions 2, 3, and 4 revealed the presence of several bioactive compounds with varying retention times and abundance. The findings of the present investigation suggest that *Cassia auriculata* mature leaf extract possesses significant phytochemical and biological activities, supporting its potential for drug development.

Key words: phytochemical, Column chromatography, biological activities, drug development

Introduction

Plants have been a valuable source of natural products for maintaining human health for a long period of time, 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. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Muthukumaran, 2001). 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 sources for new safe, biodegradable and renewable drugs. The use of plants therapeutic agents in addition to being used as food is age long. 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 WHO in several resource poornations (Dutta, 1994). Indian materia medica includes about 2000 drugs of natural origin almost all of which are derived from different traditional systems and folklore practices. Out of these drugs derived from traditional system, 400 are of mineral and animal origin while the rest are of the vegetable origin. India has a rich heritage of traditional medicine and the traditional health care system has been flourishing in many countries. Traditional medicine is an important part of healthcare. Medicinal plants have been widely used in traditional systems of medicine for the treatment of various human ailments because they contain several biologically active phytochemicals with therapeutic properties. Natural products continue to play a major role in modern drug discovery, and more than 50% of currently available drugs are directly or indirectly derived from natural sources (Borris, 1995). Plant-derived secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, phenolic compounds, and glycosides possess significant pharmacological activities including antimicrobial, antioxidant, anti-inflammatory, and anticancer properties (Sofowora, 1993; Harborne, 1998).

Cassia auriculata, commonly known as Tanner's cassia or Avaram, belongs to the family Fabaceae and is widely distributed throughout India and tropical regions. The plant has been extensively used in traditional medicine for the treatment of diabetes, skin diseases, urinary disorders, conjunctivitis, rheumatism, and liver disorders (Kokate et al., 2008). Various parts of the plant including leaves, flowers, roots, and bark are known to contain important phytoconstituents such as flavonoids, tannins, saponins, anthraquinones, and phenolic compounds which contribute to its medicinal significance (Trease and Evans, 2002).

It is the state flower of Telangana. It occurs in the dry regions of India and Srilanka. It is common along the sea coast and the dry zone in Srilanka. Avaram Senna is a much branched shrub with smooth cinnamon brown bark and closely pubescent branchlets. The leaves are alternate, stipulate, paripinnate compound, very numerous, closely placed, rachis 8.8-12.5cm long, narrowly furrowed, slender, pubescent, with an erect linear gland between the leaflets of each pair, leaflets 16-24, very shortly stalked 2-2.5cm long, 1-1.3cm broad, slightly overlapping, oval oblong, obtuse at both ends, mucronate, glabrous or minutely downy, dull green, paler beneath, stipules very large, reinform-rotund , produced at base on side of next petiole into a filliform point and persistent. Its flowers are irregular, bisexual, bright yellow and large (nearly 5cm across) the pedicels glabrous and 2.5cm long. The racemes are few-flowered, short, erect, crowd in axils of upper leaves so as to form a large terminal inflorescence. The 5 sepals are distinct, imbricate, glabrous, concave, membranous and unequal, with the two outer ones much larger than inner ones. The petals also number five are free, imbricate and crisped along the margin, bright yellow veined with orange. The anthers number 10 and are separate, with the three upper stamens barren; the ovary is superior, unilocular, with marginal ovules. The fruit is a short legume, 7.5-11cm long, 1.5cm broad, oblong, obtuse, tipped with long style base, flat, thin, papery, undulately crimpled, pilose, pale brown. 12-20 seeds per fruit are carried each in its separate cavity **(**Rajagopal, 2002).

Phytochemical screening of medicinal plants is essential for identifying bioactive compounds responsible for therapeutic activities. Preliminary

qualitative analysis provides information regarding the presence of different classes of secondary metabolites, while chromatographic techniques such as column chromatography and thin layer chromatography are useful for the separation and purification of active constituents (Harborne, 1998). High Performance Liquid Chromatography (HPLC) is considered one of the most reliable analytical techniques for the identification and quantification of phytochemicals present in plant extracts due to its high sensitivity and accuracy.

The increasing emergence of multidrug-resistant bacterial pathogens has become a serious public health problem worldwide, creating a need for alternative antimicrobial agents from natural sources (Cowan, 1999). Several medicinal plant extracts have demonstrated promising antibacterial activity against pathogenic microorganisms because of the presence of bioactive phytochemicals. Previous studies have reported that leaf extracts of Cassia auriculata exhibit significant antibacterial and antifungal activities against various human pathogens (Duraipandiyan and Ignacimuthu, 2009).

Therefore, the present study was undertaken to investigate the phytochemical constituents, and chromatographic profile of mature leaf extracts of *Cassia auriculata* using qualitative phytochemical analysis, chromatography and HPLC analysis.

2. Materials and Methods:

A)Collection of sample:

Mature leaf sample of *Cassia auriculata* was collected from Aralvaimozhi, Kanyakumari district and was transferred to the laboratory. 2g mature leaf sample materials were

taken and ground using mortar and pestle. The crushed materials were dissolved in 25 ml of methanol.

B)Moisture content and Ash content in leaf:

Dried mature leaves of *Cassia auriculata* sample were weighed into a previously dried and weighed ceramic crucible and dried in a thermostatically controlled oven at 105ºC. The moisture content was then determined by difference and expressed as a percentage of the initial weight of the sample.

Cassia auriculata mature leaf sample was weighed into a previously washed, dried, ignited and weighed ceramic crucible and then the dish with its contents placed in a preheated oven at 400ºC for two hours (2 hrs). The contents were removed and cooled, after which they were weighed. The dry man of the sample was calculated using the below formula,

$$\textbf{\% of Ash} = \frac{Weight\ of\ sample\ remaining}{Weight\ of\ original\ sample}\ X\ 100$$

C) Qualitative analysis of phytochemical constituents in *Cassia auriculata* mature leaf extract :

The mature leaf extract of *Cassia auriculata* was subjected to qualitative phytochemical screening to identify the presence of major bioactive constituents using standard analytical methods. Various phytochemicals including carbohydrates, proteins, amino acids, alkaloids, flavonoids, tannins, phenolic compounds, saponins, steroids, terpenoids, quinones, phytosterols, phlobatannins, vitamin C, phosphate, and chloride were analyzed by specific biochemical tests based on characteristic colour changes and precipitate formation. Total protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as the standard and measuring absorbance at 750 nm. Total carbohydrate content was estimated by the Anthrone method, where the green-coloured complex formed was measured colorimetrically at 620 nm using glucose as the standard.

D)Column chromatography in mature leaf extract of *Cassia auriculata*:

Column chromatography of the mature leaf extract of Cassia auriculata was performed using a glass column packed with silica gel (120–200 mesh) and eluted with ethanol:ethyl acetate solvent system (6:4). The crude extract was loaded onto the column as a slurry and fractions were collected systematically.

E)Thin Layer Chromatography in mature leaf extract of *Cassia auriculata*:

TLC analysis was carried out on silica gel G coated plates using n-butanol:acetic acid:water (16:4:4) as the mobile phase, and separated compounds were visualized by spraying ninhydrin reagent after development. The retention factor (Rf) values were calculated based on the distance travelled by the solute relative to the solvent front.

F)High Pressure Liquid Chromatography in mature leaf extract of *Cassia auriculata*:

High Performance Liquid Chromatography (HPLC) was employed for the identification and separation of bioactive compounds using silica as the stationary phase and a suitable solvent system as the mobile phase under high pressure, where separated compounds were detected and recorded as chromatographic peaks for further analysis.

3. Results and discussion:

A) Collection of sample material:

The collected sample was transported to the laboratory for further analysis and was shown in figure 1. The mature leaves were separated and were used for further analysis protocols.



Figure 1.: Cassia auriculata plant

B. Moisture content and ash content .

The moisture content of the *Cassia auriculata* mature leaf sample extract was found to be 80.63. The ash content of the *Cassia auriculata* mature leaf sample extract was found to be 26.78.

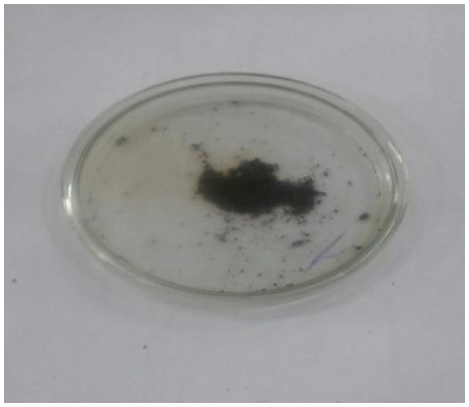


Figure 2: Ash content of *Cassia auriculata* mature leaf extract

C.Qualitative analysis of phytochemical constituents in *Cassia auriculata* mature leaf extract :



Figure 3: Shows mature leaf sample extract in methanol

The mature leaf sample of *Cassia auriculata* was subjected to phytochemical analysis for testing the qualitative analysis of biochemical constituents. The obtained results are given in table (1).

	Phytochemical analysis of mature leaf sample extract	
Sl.no	**Chemical constituents**	**Inference**
1	Carbohydrate	+
2	Protein	+
3	Phosphate	+
4	Chloride	+
5	Tannins	+
6	Phenolic compounds	+
7	Flavonoids	+
8	Steroids	+
9	Vitamin c	_
10	Saponins	+
11	Terpenoids	_
12	Quinones	+
13	Alkaloids	+
14	Amino acids	_
15	Phlobatannins	+

Table 1: Phytochemical analysis of mature leaf sample extract

The qualitative phytochemical analysis revealed the presence of carbohydrates, proteins, tannins, phenolic compounds, flavonoids, steroids, alkaloids, quinones, saponins, phosphate, chloride, and phlobatannins, whereas vitamin C, amino acids, and terpenoids were absent The result indicates that the mature leaf extract of *Cassia auriculata* found to possess significant protein content of about 70mg/100ml. The result indicates that the carbohydrate content was found to be 20 mg/100ml. These findings are in agreement with earlier reports by Murugan *et al.* (2013), who documented the occurrence of carbohydrates, proteins, alkaloids, flavonoids, steroids, and saponins in *Cassia auriculata* extracts. Phenolic compounds and flavonoids are known to contribute significantly to antioxidant, antimicrobial, and anticancer activities because of their ability to scavenge free radicals and inhibit microbial growth (Harborne, 1998; Cowan, 1999). The protein and carbohydrate estimations indicated appreciable biochemical content in the mature leaf extract, suggesting the nutritional and therapeutic value of the plant material.

D. Column chromatography in mature leaf extract of *Cassia auriculata*:

Column chromatography successfully separated the extract into different fractions, among which fraction 4 exhibited maximum antibacterial activity against *Escherichia coli*, indicating the enrichment of active secondary metabolites in this fraction. Similar findings were reported by Raja *et al.,* (2013), who observed enhanced antimicrobial activity in specific chromatographic fractions of medicinal plant extracts due to the concentration of bioactive compounds.

E. Thin Layer Chromatography in mature leaf extract of *Cassia auriculata*:

Thin layer chromatography analysis showed an Rf value of 0.78, suggesting the possible presence of leucine in the extract. Chromatographic techniques such as TLC are considered effective preliminary methods for the separation and identification of phytoconstituents in medicinal plants (Trease and Evans, 2002).



Figure 4. Shows TLC of *Cassia auriculata* mature leaf sample extract

F) High Pressure Liquid Chromatography in mature leaf extract of *Cassia auriculata*:

Fraction 2, 3 and 4 obtained from column chromatography were subjected to HPLC analysis and the results are given in table (2).The control possessed four different compounds named as Cyanocrylate, Sodium triphosphate, Phosphorus pentaoxide and Alkoxide. Among the four compounds, cyanocrylate is an initial as well as peak compound, and its abundance was 287.5% at the RT of 0.09. Moreover, alkoxide is a least abundant compound and its retention time was more 5.09 mts compared with other three compounds.

S.No	Retention time (RT)	Name of the compound	Abundance (%)
Control			
1.	0.09	Cyanoacrylate	287.5
2.	1.09	Sodium tripolyphosphate	170.2
3.	4.1	Phosphorus pentaoxide	145.1
4.	5.09	Alkoxide	115.02
Extract	**Fraction – 2**		
1.	1.7	Hydrazine	380
2.	3.9	Diamino-parachlorotriazine	170
	Fraction – 3		
3.	1.8	Cyanuric acid	160
4.	3.2	Melamine cyanurate	380
5.	3.3	Triacetyl melamine	310
6.	3.4	Diamino-meta-hydroxy 1.5-triazines	300
7.	3.5	N-Dimethylbenzylamine	
	Fraction – 4		
8.	12.5	Cyromasine	400

9.	15.5	Deseethylbenzoatrozolin	340
10.	17	Acylmelamnes	170
11	21	Diethylbenzoatrozolin	210
12.	22	Sulfonylcyanoguanidines	340
13.	31	Trichloro-meta-Isocyanuric acid	310
14.	31.5	Diaminocholorotriazine	280

Table 2: Chemical composition of control and mature leaf sample extract

HPLC analysis further confirmed the presence of multiple chemical constituents in fractions 2, 3, and 4, with fraction 4 containing several abundant compounds such as cyromazine, sulfonylcyanoguanidines, trichloro-meta-isocyanuric acid, and deseethylbenzoatrozolin. The higher abundance and peak intensities observed in fraction 4 may be associated with its enhanced biological activity. Previous studies have suggested that the therapeutic efficacy of *Cassia auriculat*a is attributed to the presence of diverse phytoconstituents with antimicrobial and pharmacological properties (Kheem Singh Dahiya *et al.,* 2016).

4. Conclusion:

The present study demonstrated that the mature leaf extract of *Cassia auriculata* possesses considerable phytochemical and biochemical constituents along with significant chromatographic characteristics, supporting its medicinal importance. Overall, the findings of the present investigation support the medicinal potential of *Cassia auriculata* mature leaf extract and indicate that the identified phytochemicals may contribute to its antibacterial and possible anticancer activities.

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