PHYTOCHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF LEAF AND FLOWER EXTRACTS OF CASSIA AURICULATA

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ABSTRACT:

The present study aims to investigate the phytochemical constituents in the leaf and flower extracts of Cassia auriculata and to evaluate the antibacterial efficacy of the flower extracts against three bacterial species: Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes. The evaluation was conducted using five solvents: acetone, ethanol, methanol, chloroform, and water. Phytochemical analysis indicated the presence of various bioactive compounds, including alkaloids, saponins, terpenoids, phenols, tannins, flavonoids, carbohydrates, and proteins. Specifically, detailed carbohydrate analysis of the flower extract revealed the presence of ketose groups. Among the extracts, the ethanol flower extract exhibited the highest total phenolic content in qualitative analysis.

The antibacterial activity was assessed using the agar well diffusion method with flower extract volumes of 10, 20, 30, and 40 µl. The antibacterial efficacy of the extracts followed the order: ethanol > methanol > acetone > water > chloroform. This order was confirmed through the disc diffusion method. Ethanol and methanol extracts demonstrated significant antibacterial activity against E. coli, P. aeruginosa, and S. pyogenes. Moderate antibacterial potential was observed in the acetone and water extracts, while no antibacterial activity was recorded for the chloroform extract.

Overall, this study highlights the potent phytochemical profile and antibacterial properties of Cassia auriculata flower extracts, with ethanol and methanol proving to be the most effective solvents for antibacterial activity.

Keywords: *Cassia auriculata, flower extract, Leaf extract, phytochemical analysis, antibacterial property.*

INTRODUCTION:

Medicinal plants are the foundation of primary health care for the majority of the global population. They are essential sources for the development of potent antimicrobial drugs and have played a significant role in maintaining human health since ancient times. Particularly in developing countries, medicinal plants are highly valued for their therapeutic properties, believed to be safe and non-toxic. They provide a renewable and biodegradable source of new drugs. Among these, Cassia species are of significant interest due to their exceptional medicinal properties (Guruprasad *et al.,* 2015). All Cassia species are rich sources of secondary metabolites, which contribute to their medicinal value. Cassia auriculata Linn, commonly known as Tanner's Senna or Avaram tree, is renowned for its medicinal benefits. This plant is found throughout the hot deciduous forests of India, particularly in the dry regions of Madhya Pradesh, Tamil Nadu, Rajasthan, and other parts of the country. Cassia auriculata possesses numerous medicinal properties, including antipyretic, hepatoprotective, antidiabetic, antiperoxidative, antihyperglycemic, and microbicidal activities. It has demonstrated antiviral and antispasmodic activities. Traditionally, it has been used to treat female infertility, leprosy, worm infestations, diarrhea, and diseases related to the pitta dosha. It is also known for its benefits in treating rheumatism and conjunctivitis and for alleviating diabetes symptoms (Anushia *et al.,* 2009).

The leaves are alternate, stipulate, and paripinnate compound, with numerous, closely placed leaflets. The rachis measures 8.8 – 12.5 cm long, slender, and pubescent, with an erect linear gland between each pair of leaflets. The leaflets are 16-24 in number, very shortly stalked, 2 – 2.5 cm long, and 1 – 1.3 cm broad. They are oval-oblong, obtuse at both ends, glabrous or minutely downy, and dull green, paler beneath. The stipules are large, reniform-round, and persistent. The leaf extract has protective effects against alcohol-induced oxidative stress in cells, evidenced by lower tissue lipid peroxidation and elevated levels of enzymatic and non-enzymatic antioxidants. It also shows emollient effects (Kavimani *et al.,* 2015).

The flowers of Cassia auriculata are irregular, bisexual, bright yellow, and large (nearly 5 cm across). The pedicels are glabrous and 2.5 cm long. The racemes are few-flowered, short, erect, and crowded in the axils of upper leaves to form a large terminal inflorescence. The 5 sepals are distinct, imbricate, glabrous, concave, membranous, and unequal, with the two outer ones much larger. The petals, numbering 5, are free, imbricate, and crisped along the margin, bright yellow veined with orange. There are 10 anthers, with

the three upper stamens barren. The ovary is superior, unilocular, with marginal ovules (Anandan *et al.,* 2011). The flowers are used to treat urinary discharges, nocturnal emissions, diabetes, and throat irritation. Both the leaves and petals are mildly astringent and help regulate urine flow and aid in the absorption of necessary fluids in the kidneys and intestines.

METHODOLOGY:

Collection of sample and preparation of solvent extract: The plant materials, specifically the flowers and leaves, were collected from Thoodugadu, near Sriperumbudur, Tamil Nadu. It was ensured that the collected flowers and leaves of Cassia auriculata were healthy and free from infections. Upon collection, the fresh plant materials were thoroughly washed with tap water, followed by rinsing with sterile distilled water. They were then shade-dried for two weeks. After drying, the flowers and leaves were crushed into small pieces using a pestle and mortar. The resulting powdered samples were stored in sealed containers for the extraction process (Deshpande *et al.,* 2013). For the extraction, twenty-five grams of the powdered flowers and leaves were individually soaked in 50 ml of acetone, chloroform, ethanol, methanol, and water for one week. After the soaking period, the mixtures were filtered using Whatman No. 1 filter paper. The liquid extracts obtained were stored in airtight containers for further analysis (Mali *et al.,* 2012).

Phytochemical analysis: The acetone, chloroform, ethanol, methanol, and water extracts of the flowers and leaves of Cassia auriculata were analyzed to determine the presence of various phytochemical constituents. The compounds tested for included alkaloids, carbohydrates, proteins, amino acids, glycosides, phytosterols, steroids, terpenoids, phenols, tannins, flavonoids, phlobatannins, cardiac glycosides, resins, saponins, starch, phenolic compounds, coumarins, triterpenoids, and anthraquinones (Deshpande supriya S *et al.,* 2013).

Antimicrobial susceptibility test: For the present study, the microorganisms Escherichia coli, Pseudomonas aeruginosa, and Streptococcus pyogenes were selected. The susceptibility of these microbes to the different solvent extracts of Cassia auriculata was analyzed. Chloramphenicol was used as the standard for comparison in these susceptibility tests (Manimegalai S *et al.,* 2010).

RESULTS AND DISCUSSION:

The phytochemical characteristics of five different extracts of Cassia auriculata flowers are summarized in Table 1. This preliminary screening revealed the presence of medicinally active constituents in the five extracts studied. The study indicated that proteins

and alkaloids were present in all extracts, while glycosides, triterpenes, phlobatannins, coumarin, and anthraquinone were absent in all five extracts. Carbohydrates, terpenoids, and steroids were absent only in the chloroform extract. Saponins were absent in both chloroform and methanol extracts. Phenols, flavonoids, cardiac glycosides, and resins were absent in both chloroform and water extracts. Amino acids were absent in both acetone and ethanol extracts. Tannins were present only in the water extract. During the osazone test, yellow needle-shaped crystals were observed under the microscope, indicating the presence of glucose, fructose, or mannose. Detailed analysis of carbohydrates using alkaline potassium ferricyanide revealed the presence of fructose in the flower extract. Further sodium nitroprusside testing confirmed the presence of a keto group (fructose) in the flower extracts across the five different solvents.

The phytochemical characteristics of five different extracts of Cassia auriculata leaves are summarized in Table 2. The study concluded that alkaloids, carbohydrates, proteins, phytosterols, steroids, terpenoids, triterpenoids, and anthraquinone were present in all extracts. In contrast, amino acids, glycosides, starch, and coumarin were completely absent in all five extracts. Phenols, tannins, cardiac glycosides, and resins were absent only in the chloroform and water extracts. Flavonoids were absent only in the chloroform extract, while phlobatannins were present only in the methanol extract. Saponins were found in the acetone and ethanol extracts. The phenolic compounds were most abundant in the ethanol extract, followed by methanol, chloroform, acetone, and finally water. The total phenolic content was highest in the methanol extract of the leaves (Fig. 1).

Antibacterial Assay: The antibacterial assay revealed that among the five different extracts, the ethanol and methanol extracts of Cassia auriculata flowers demonstrated significant antibacterial activity against Escherichia coli and Streptococcus pyogenes. Ethanol and methanol extracts exhibited the highest inhibitory activity compared to the other solvents. Following these, the acetone extract showed good inhibitory activity, while the water extract had moderate activity. The chloroform extract exhibited the lowest inhibitory activity among the tested solvents. The acetone extract was effective against Escherichia coli, Streptococcus pyogenes, and Pseudomonas aeruginosa. The chloroform extract showed limited activity, being slightly effective against Pseudomonas aeruginosa only. The water extract was active against Escherichia coli and Streptococcus pyogenes.

The antibacterial efficacy of the various extracts at different concentrations against the test pathogens is presented in Table 3. The ethanolic extract showed the largest zone of inhibition against E. coli, measuring 16 mm in diameter at a concentration of 40 µl. The methanolic extract demonstrated a maximum zone of inhibition of 12 mm in diameter at 40 µl concentration against both E. coli and Streptococcus pyogenes. The acetone extract had a maximum inhibition zone of 12 mm in diameter at 40 µl concentration against Streptococcus pyogenes. The aqueous extract exhibited a maximum inhibition zone of 12 mm in diameter at 40 µl concentration against E. coli.

In the present study, flower extracts of Cassia auriculata were subjected to preliminary screening to identify their phytochemical constituents and evaluate their potential as antibacterial agents, given the increasing resistance to widely marketed antibiotic formulations. The qualitative phytochemical analysis revealed the presence of alkaloids, saponins, terpenoids, phenols, tannins, flavonoids, carbohydrates, proteins, and amino acids in the extracts. Among these, the ethanol and methanol extracts exhibited the highest inhibitory effects, suggesting that these solvents are more effective in extracting the active ingredients from the plant parts compared to acetone, chloroform, and water.

Cassia auriculata flowers are known to contain various therapeutically valuable active principles and exhibit biological activity against numerous diseases (Muthukumaran *et al.,* 2001). The antimicrobial activity of Cassia auriculata extracts is likely attributed to the presence of phenolic constituents. The methanol extract, in particular, contains saponins, flavonoids, alkaloids, tannins, and phenolic compounds, which are the phytochemicals responsible for its antibacterial properties (Shihabudeen *et al.,* 2010) (Ayyanar *et al.,* 2008).

CONCLUSION:

Among the five extracts tested, methanol and ethanol demonstrated high activity against E. coli, Pseudomonas aeruginosa, and Streptococcus pyogenes. Acetone and aqueous extracts exhibited moderate antibacterial potential. However, the chloroform extract showed no antibacterial activity against the tested pathogens.

REFERENCES:

Anandan A, R.Eswaran, A.Doss, G.Sangeetha, and S.P.Anand (2011). Chemical compounds investigation of cassia auriculata Leaves – A Potential folklore medicinal plant. Bulletin of Environment, Pharmacology and Life Sciences, Volume 1, Issue 1, Pages 20-23.

Anushia C, P.Sampath Kumar, and L.Ramkumar (2009). Antibacterial and antioxidant activities in Cassia auriculata. Global Journal of Pharmacology, Volume 3, Issue 3, Pages 127-130.

Ayyanar M, S.Ignacimuthu (2008). Pharmacological action of Cassia auriculata Linn, A short review. Journal of Pharmacology and Toxicology, Pages 213-221.

Deshpande H, Bhalsing S.R (2013). Recent advances in the phytochemistry of some medicinally important Cassia species. International Journal of Pharmacology, Medicinal and Bioscience, Volume 2, Pages 2278-5221.

Deshpande Supriya S, Kewatkar Shailesh, Paithankar Vivek V (2013). Anticlastogenic activity of flavonoids rich extracts of Cassia auriculata Linn on experimental animals. Indian Journal of Pharmacology, Volume 45, Pages 184-186.

Guruprasad C, Nille, K.R.C. Reddy (2015). A phytopharmacological review of Plant–Cassia auriculata. International Journal of Pharmaceutical & Biological Archives, Volume 6, Issue 6, Pages 1-9.

Kavimani V, A.Ramadevi, K.Kannan, S.Gnanavel, G.Sivaperumal (2015). Antibacterial activity of Cassia auriculata Linn. Journal of Chemical and Pharmaceutical Research, Volume 7, Issue 9, Pages 479-485.

Mali A.A, Hivrale M.G, Bandawane D.D, Chaudhari P.D (2012). Study of anti-inflammatory activity of Cassia auriculata Linn. leaves in Wistar rats. Indian Drugs, Volume 49, Pages 182-192.

Manimegalai S, T.Naveen (2010). Evaluation of antibacterial activity of flower extracts of Cassia auriculata Linn. Ethnobotanical Leaflets, Volume 14, Pages 182-192.

Muthukumaran P, M.Elayarani, P.Shanmuganathan, A.Cholarajan (2001). Antimicrobial activity of Cassia auriculata Linn. International Journal of Research in Pure and Applied Microbiology, Volume 1, Issue 2, Pages 9-12.

Shihabudeen M.H, H.D.Priscilla, K.Thirumurugan (2010). Antimicrobial activity and phytochemical analysis of selected Indian folk medicinal plants. International Journal of Pharmacological Sciences Research, Pages 430-434.

Table 1: Qualitative Phytochemical Evaluation of Flower extracts

Test	Acetone	Ethanol	Methanol	Chloroform	Water
Test for alkaloids					
a) Hager's Test	+	+	+	+	+
b) Wager's Test	+	+	+	+	+
C)Mayer's Test	+	+	+	+	+
Test for carbohydrates					
a)Molisch's Test	+	+	+	+	+
b)Fehling's Test	+	+	+	+	+
c)Benedict's Test	+	+	+	+	+
d)Barfoed'Test	+	+	+	-	-
e)Seliwanoff's Test	+	+	+	+	-
f)Bial's Test	-	-	-	-	-
g)Osazone Test	+	+	+	+	+
h)Iodine Test	-	-	-	-	-
i)Anthrone Test	+	+	+	+	+
j)potassium ferricynaide test	+	+	+	+	+
k)Sodium nitroprusside Test	+	+	+	+	+
Test for Protein					
a)Xanthoprotein Test	+	+	+	+	+
b) Biuret Test	+	+	+	+	+
c) Millon's Test	+	+	+	+	+
Test for Amino acids					
a) Ninhydrin Test	-	-	+	+	+
Test for Glycosides					
a)Modified Borntrager's Test	-	-	-	-	-
b) Picrate Paper Test	-	-	-	-	-
Test for Phytosterols					
Extract + CHCL3 + Con.H2SO4	+	+	+	+	+
Test for Steroids					
a)Libermann-Burchard's Test	+	+	+	-	+
b) Salkowski Reaction	+	+	+	-	+
Test for Terpenoids					
Extract + CHCL3 & Filter + Con.H2SO4	+	+	+	-	+
Test for Phenols					
Extract + Ferric chloride	+	+	+	-	-

Table 2: Qualitative Phytochemical Evaluation of leaf extracts

Test	Acetone	Ethanol	Methanol	Chloroform	Wat er
Test for alkaloids					
a) Hager's Test	+	+	+	+	+
b) Wager's Test	-	-	+	+	+
C)Mayer's Test	+	+	+	+	+
Test for carbohydrates					
a)Molisch's Test	-	-	+	+	-
b)Fehling's Test	+	+	-	+	+
c)Benedict's Test	+	+	+	-	-
d)Barfoed'Test	-	+	-	-	-
e)Seliwanoff's Test	-	-	-	-	-
f)Bial's Test	-	-	-	-	-
h)Iodine Test	-	-	-	-	-
i)Anthrone Test	+	+	+	+	+
j) potassium ferricynaide test	-	-	-	-	-
k)Sodium nitroprusside Test	-	-	-	-	-
Test for Protein					
a)Xanthoprotein Test	+	+	+	+	+
b) Biuret Test	-	-	-	-	-
C) Millon's Test	+	+	+	+	+
Test for Amino acids					
a) Ninhydrin Test	-	-	-	-	-
Test for Glycosides					
a)Modified Borntrager's Test	-	-	-	-	-
b) Picrate Paper Test	-	-	-	-	-
Test for Phytosterols					
Extract + CHCL3 + Con.H2SO4	+	+	+	+	+
Test for Steroids					
a) Libermann-Burchard's Test	+	+	+	+	+
b) Salkowski Reaction	+	+	+	+	+
Test for Terpenoids					
Extract + CHCL3 & Filter + Con.H2SO4	+	+	+	+	+
Test for Phenols					
Extract + Ferric chloride	-	-	-	-	-
Test for Tannins					
Extract + 10% alcoholic Fecl3	+	+	+	-	-
Test for Flavonoids					
a) Extract + NaoH	+	+	+	-	-
b)shinod's Test	+	+	+	-	+
c) Zn-Hcl Reduction Test	-	-	-	-	-
Test for Phlobatannin					

Extract + Hcl	-	-	+	-	-
Test for Cardiac Glycosides					
a) Legal Test	+	+	+	+	+
b) Keller Killiani Test	+	+	+	-	-
Test for Resin					
Extract + acetone +D/W	+	+	+	-	-
Test for Saponins					
a) Froth Test-1	+	+	+	-	-
b) Froth Test–2	+	+	+	-	-
Test for Starch					
starch-Iodine Test	-	-	-	-	-
Test for Phenolic Compounds					
Extract + 0.5%Fecl3	+	+	-+	+	-
Test for Coumarin					
Extract + alcoholic NaOH	-	-	-	-	-
Test for Triterpenoids					
Libermann-Burchard's Test	+	+	+	+	+
Test for Anthraquinone					
Extract + Con.H2so4+CHCL3+Ammonia	+	+	+	+	+

Table 3. Antibacterial effects of various extracts of Cassia auriculata at different concentration against test bacterial pathogen.

Extract	**Bacteria**	**10µl**	**20µl**	**30µl**	**40µl**
Ethanol extract	*Escherichia coli*	12	14	15	16
	Pseudomonas aeruginosa	-	-	-	-
	Streptococcus pyogenes	11	11	12	13
Methanol extract	*Escherichia coli*	9	10	11	12
	Pseudomonas aeruginosa	-	-	8	8
	Streptococcus pyogenes	10	12	12	12
Acetone extract	*Escherichia coli*	8	9	9	10
	Pseudomonas aeruginosa	-	-	-	-
	Streptococcus pyogenes	-	11	11	12
Aqueous extract	*Escherichia coli*	7	9	11	12
	Pseudomonas aeruginosa	-	-	-	-
	Streptococcus pyogenes	-	8	9	10

Fig. 1. Estimation of Phenolic acid in flower and leaf extract