

## Antimicrobial Activity and Phytochemical Constituents of Leaf Extracts of *Cassia auriculata*

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Murugan, *et al.*: Antimicrobial and Photochemical Evaluation of *Cassia auriculata*

Plants produce a wide variety of phytochemical constituents, which are secondary metabolites and are used either directly or indirectly in the pharmaceutical industry. 'For centuries, man has effectively used various components of plants or their extracts for the treatment of many diseases, including bacterial infections. In the present study methanol, chloroform and aqueous extracts of *Cassia auriculata* leaf were subjected for antimicrobial activity by well-diffusion method against six bacterial strains namely *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. The results revealed that the methanol and chloroform extracts exhibited strong inhibitory activity against all the tested organisms (zone of inhibition of 12-20 mm), except *Pseudomonas aeruginosa* (zone of inhibition 10 mm or nil). The aqueous extracts showed moderate activity by 'Zone of inhibition  $\leq 12$  or nil'. The extracts were screened for their phytochemical constituents by standard protocols' and were shown to contain carbohydrates, proteins, alkaloids, flavonoids, steroids, saponins and tannins. The antibacterial activity of these extracts is possibly linked to the presence of flavonoids, steroid, saponins and/or tannins. Further studies are needed to determine the precise active principles from *Cassia auriculata*.

**Key words:** Antibacterial activity, *Cassia auriculata*, phytochemical constituents

Plants are considered not only as dietary supplement to living organisms but also traditionally used for treating many health problems and the medicinal value of many plants still remains unexplored investigations of plants are carried out to find novel drugs or templates for the development of new therapeutic agents<sup>[1]</sup>. Over 60% of the world human population, 80% in developing countries depends directly on plants for their medicinal purposes<sup>[2]</sup>.

Phytochemicals are nonnutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals in fruits and herbs and each works differently<sup>[3]</sup>. Many plant extracts have been shown to inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in defence reactions of plants against infections by pathogenic microorganisms<sup>[4]</sup>.

*Cassia auriculata* commonly known as Tanner's *Cassia* is an important medicinal shrub used in traditional systems of medicine<sup>[5]</sup>. It holds a very prestigious position in Ayurveda and Siddha systems of medicine<sup>[6]</sup>. It also growing wild in Central Provinces and Western peninsula and cultivated in other parts of India. It is valuable as a tanning material and as a green manure crop<sup>[7]</sup>. The plant has been reported to possess antipyretic, hepatoprotective<sup>[8]</sup>, antidiabetic, antiperoxidative and antihyperglycemic<sup>[9]</sup> and microbicidal activity<sup>[10]</sup>.

Fresh leaves of *C. auriculata* were collected from Thuckalay, Kanyakumari district. The leaves were

cleaned, air-dried for 5 days and ground into a powder. A portion of the powder was subjected to successive extraction with methanol, chloroform and aques for 24-48 h using soxhlet apparatus. The extracts were concentrated by solvent evaporation, each of these extract was weighed and preserved at room temperature until further use.

Six bacterial cultures namely *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Proteus mirabilis* were used in this investigation. The media used for antibacterial test were Nutrient Broth (NB) and Muller-Hinton agar (MHA). The test bacterial strains were inoculated into NB medium and incubated at 37° for 24 h. After the incubation period, the culture tubes were compared with the turbidity standard. Antimicrobial activity was carried out by agar well diffusion method<sup>[11,1]</sup>. Fresh bacterial cultures of 0.1 ml having 10<sup>8</sup> colony forming unit were spread onto MHA plate using sterile cotton swab. The wells were punched off into MHA medium with sterile well puncture. Each well filled with 50  $\mu$ l of each plant extracts by using micro pipette in aseptic condition. The plates were then kept in a refrigerator to allow prediffusion of extract for 30 min and further incubated at 37° for 24 h.

Antibacterial activities of extracts were evaluated by measuring the zone of inhibition (Table 1). The methanol extract of *C. auriculata* leaves exhibit strong antimicrobial activity against all the tested organisms. *B. cereus* (18 mm), *S. aureus* (14 mm), *E. coli* (16 mm), *K. pneumoniae* (14 mm), *P. aeruginosa* (10 mm) and *P. mirabilis* (16 mm). The chloroform extract showed good activity

against *B. cereus* (20 mm), *S. aureus* (12 mm), *E. coli* (14 mm), *K. pneumoniae* (14 mm) and *P. mirabilis* (12 mm). Finally the aqueous extracts showed moderate activity against *B. cereus* (12 mm), *S. aureus* (10 mm) and *P. mirabilis* (8 mm).

The compounds that are responsible for therapeutic effects are usually the secondary metabolites. The extracts were subjected to systematic phytochemical screening for the presence of chemical constituents such as carbohydrates by Benedict's test, proteins by Biuret test, alkaloids by Wagner's reagent test, flavonoids by ferric chloride test, saponins by foam test, steroids by Leibermann-Burchards test and tannins by ferric chloride test<sup>[12,13]</sup>.

The results of the present study revealed that the Gram-positive bacteria are strongly inhibited by all the extracts of *C. auriculata* (L) than the Gram-negative bacteria and it shows minimum activity against *P. aeruginosa*. This study was supported by Mohamed *et al.*, where *C. auriculata* showed significant inhibitory activity against all tested bacteria<sup>[14]</sup>. Samy and Ignachimuthu reported that *C. auriculata* exhibited significant activity against *E. coli* and *S. aureus*<sup>[15]</sup>.

The plant extracts showed the presence of carbohydrate, protein, alkaloids flavonoids, saponin and tannin in methanol and chloroform; carbohydrate, protein, alkaloids, saponin and tannin in aqueous; rest of steroids in chloroform (Table 2). Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defence mechanism against invasion by many microorganisms, insects and other herbivores<sup>[16]</sup>. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection<sup>[17]</sup>. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell<sup>[18]</sup>. Tannins bind to proline rich proteins and interfere with the protein synthesis<sup>[19]</sup>. The medicinal properties and pharmacological actions of *C. auriculata* are well-known to Indian traditional medicine. These plants are known to contain various active principle of therapeutic value and possess biological activity against a number of diseases<sup>[20]</sup>. The antimicrobial activity may be due to the presence of phytochemical constituents like flavonoids and phenolic compounds present in the plant as secondary metabolites<sup>[13,21]</sup>.

**TABLE 1: ANTIMICROBIAL ACTIVITY OF CASSIA AURICULATA LEAVES EXTRACTS ON SELECTED BACTERIA**

Test organisms/plant extracts	Zone of inhibition (mm)		
	Methanol (10 mg/ml)	Chloroform (10 mg/ml)	Aqueous (10 mg/ml)
Gram-positive bacteria			
<i>Bacillus cereus</i>	18	20	12
<i>Staphylococcus aureus</i>	14	12	10
<i>Escherichia coli</i>	16	14	-
Gram-negative bacteria			
<i>Klebsiella pneumonia</i>	14	14	-
<i>Pseudomonas aeruginosa</i>	10	-	-
<i>Proteus mirabilis</i>	16	12	8

Zone of inhibition in 'mm', '-'=No activity

**TABLE 2: PHYTOCHEMICAL INVESTIGATION OF CASSIA AURICULATA LEAVES ON DIFFERENT EXTRACTS**

Phytochemical constituents	Methanol	Chloroform	Aqueous
Carbohydrates	+	+	+
Protein	+	+	+
Alkaloids	+	+	+
Flavonoids	+	+	-
Steroids	-	+	-
Saponin	+	+	+
Tannin	+	+	+

'+'=Presence of compounds, '-'=Absence of compounds

Past some years, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents. Many reports show the effectiveness of traditional herbs against microorganisms as a result, plants are one of the basis for modern medicine to attain new principles. The present study reveals the existence of antimicrobial substances in *C. auriculata* and further studies are required to find out the active components of medicinal properties in this valuable plant.

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