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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF ROOT EXTRACTS OF *CASSIA AURICULATA* AGAINST *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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ABSTRACT

Cassia auriculata powdered root material was extracted using water. Phytochemical screening of the crude extracts revealed the presence of alkaloids, glycosides, saponins, tannins, carboxylic acid and Flavonoids. This presence of the bioactive constituents have been linked to the antimicrobial activity of the plant material against *Staphylococcus aureus* (gm+ve) and *Escherichia coli* (gm–ve) using agar cup plate method. Minimum inhibitory concentration values ranged from 25-100 mg/ml. The plant can be used as a source of oral drugs to fight infections against susceptible bacteria.

1. INTRODUCTION

This paper discusses about the antimicrobial properties of *Cassia auriculata* against *Staphylococcus aureus* (gm+ve) and *Escherichia coli* (gm-ve) by using agar cup plate method. *Cassia auriculata* belongs to the family of Caesalpiniaceae. *Cassia auriculata* is a glabrous plant which is 8-15 mt in height, cultivated widely throughout South India. It can grow to more than 30 m (98 ft) tall with an average trunk diameter of 1.5 m (4.9 ft). The average height of cultivated specimens, however, is usually between 9 and 15 m (30 and 49 ft) with a trunk diameter not exceeding 50 cm (20 in). The ornamental leaves are medium green and glossy. They are alternate, elliptic to ovate, 7–15 cm long, with an entire margin. The white flowers are inconspicuous and bell-like; with a six-lobed corolla. Tap root system is observed in the *Cassia auriculata*[1].

With the rising prevalence of microorganism showing resistance to antibiotics, there is an urgency to develop new antimicrobial compounds. Since antiquity, plants have been used to treat common infectious diseases. The healing potential of many plants have been utilized by Indian traditional medicines like Siddha, Ayurvedha and Unani. Being nontoxic and easily affordable, there has been resurgence in the consumption and demand for medicinal plants. *Cassia auriculata* L. commonly known as tanners cassia, also known as “avaram” in Tamil language is a shrub belongs to the Caesalpiniaceae family. The shrub is especially famous for its attractive yellow flowers which are used in the treatment of skin disorders and body odour. It is widely used in traditional medicine for rheumatism, conjunctivitis and diabetes. It has many medicinal properties. Its bark is used as an astringent, leaves and fruits anthelminthic, seeds used to treat in eye troubles and root employed in skin diseases. It is also used for the treatment of ulcers, leprosy and liver disease. The antidiabetic, hypolipidemic and antioxidant and hepatoprotective effect of *Cassia auriculata* have been reported[2].

Very few works have been carried out on leaves, stem, bark, fruit, fruit juice for antibacterial activity, antioxidant activity and Seed germination studies. Hence we are taking roots of *Cassia auriculata* for determining antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*[3].

Fig-1- *Cassia auriculata* Plant

2.0 MATERIALS AND METHODS

2.1 Collection of plant material

The roots which were used for the extraction process were primarily collected from local areas of Guntur. Further these roots were subjected to air drying for about two weeks and were used for the extraction[4].

2.2 Preparation of the plant material

The fresh plant was harvested, rinsed with tap water and air dried under shade for about 14 days and reduced to fine powder using blender. The powder was stored in an air tight bottle until needed for use.

2.3 Preparation of the extract

20 gm of the powdered sample was soaked in 100 ml of the solvent contained in a 500 ml of sterile conical flask and covered with a cotton wool. It was then plugged and wrapped with aluminum foil and shaken vigorously. The mixture was left to stand overnight (24 hours) .The mixture was then filtered using a clean muslin cloth and then whatmann no: 1 filter paper. The filterate was then evaporated to dryness at 40°C. The percentage yield was calculated. For the preparation of dilutions of crude extracts for antibacterial activity assay, the extracts were reconstituted by dissolving in the distilled water and further diluted to obtain 100-25 mg/ml[5].

2.4 Microorganisms

Staphylococcus aureus, *Escherichia coli* were obtained from the microbiology laboratory and were stored in a refrigerator.

2.5 Reference and Control

The references were antibiotic in nature and tetracyline was used as the reference for all bacterial species. The Control experiment consists of a plate of solidifying agar onto which was inoculated pure solvent with microorganism mixed in a 1:1 portion.

2.6 Phytochemical screening of the plant material

Phytochemical screening was carried out on the powdered plant material for the presence of different bioactive components such as tannins, carboxylic acids, glycosides, alkaloids, saponins.

2.7 Determination of antimicrobial activity

The antibacterial activity of the aqueous extract of *Cassia auriculata* was determined using agar cup plate method. Cups or wells of 8 mm diameter were punched in the agar medium. Aqueous solutions of different concentrations of the plant extract were dispensed in different wells and incubated at 37º C for 24 hours. The control wells were loaded with saline (negative control) and tetracycline (100 µg/ml) for *Staphylococcus aureus* as positive control. The antibacterial activity was assessed by measuring the zone of inhibition. The relative antibacterial activity of the extract was calculated by comparing its zone of inhibition with the standard drugs.

2.8 Phytochemical Analysis Tests

2.8.1 Tests for Alkaloids

The presence of alkaloids is determined using the mayers and wagners test as described by the Harbone (1988). In this process, two gram of each portion of the powered sample are put into a conical flask and 20 ml of dilute sulphuric acid in ethanol are added into it and then heated in water bath to boil for 5 min. The mixture is filtered and the filtrates are separated treated with 2 drops of Mayers and Wagners reagents in test-tubes. Development of an orange coloration indicated positive result[6].

2.8.2 Test for Saponins

The Froth test and Emulsion test as described by Harbone (1973) are used to determine the presence of saponins. In this process, twenty ml of water is added to 0.25 gm of the powdered sample in 100 ml beaker, boiled and filtered for the test[7].

a) Froth test: 5 ml of the filtrate is diluted with 20 ml of water and shaken vigorously. A stable froth (foam) up on standing indicates the presence of saponins.

b)Emulsion test: 2 drops of olive oil is added to the frothing solution and shaken vigorously the formation of emulsion indicates the presences of saponins.

2.8.3 Test for Tannins

The presence of tannins is carried out using the Harbone (1973) method. 1gm of the powdered sample is boiled with 50 ml of water filtered and the filtrate used to carry out the

ferric chloride test few drops of ferric chloride is added to 3 ml of the filtrate in a test tube. A greenish black precipitate indicates the presence of tannins[6,7].

2.8.4 Test for Flavonoids

The presence of flavonoids in the samples is determined using the Harbone (1973), Sofowora (1993) methods. 10ml Ethyl acetate is added to 0.2gm of the powdered sample and heated in a Water bath for 5 min. The mixture is cooled filtered and the filtrates used for the test[7].

a) Ammonium test- About 4 ml filtrate is shaken with 1 ml of dilute ammonia solution. The layer is allowed to separate and the yellow colour in the ammonia layer indicates the presence of flavonoids.

b) Aluminum chloride solution test: 1 ml of 1% aluminum chloride solution is added to 4 ml of the filtrate and shaken. A yellow coloration indicates the presence of flavonoids.

3. RESULTS AND DISCUSSIONS

Percentage yield of the powdered plant *Cassia auriculata* extract obtained by using water is shown in Table 1. Out of the 20 g of the powdered plant material, the percentage yield obtained was 2.5%.Phytochemical screening of the crude extracts of *Cassia auriculata* revealed the presence of some bioactive components as shown in table 2. It contains tannins, glycosides, alkaloids, saponins, carboxylic acids. These compounds have potentially significant application against human pathogens, including those that cause enteric infections. The presence of alkaloids is interesting, as significant quantities are used as antimalarials, analgesics and stimulants. The presence of glycosides moieties like saponins, are known to inhibit tumor growth and serve also to protect against gastrointestinal infections. Herbs that have tannins as their components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery thus exhibiting antibacterial activity. Tannins are widely used in traditional medicine in treating wounds and to arrest bleeding. Antibacterial activity of the crude extracts of *Cassia auriculata* were evaluated by measuring the diameters of zone of growth inhibition on some members of enterobacteriaceae and the results are presented as shown in table 3. It indicates that the root extract of *Cassia auriculata* showed antimicrobial activity against both gram positive and gram negative bacteria. The MIC values obtained for the test bacteria are high ranging from 25-100 mg/ml when compared to the MIC values of 0.01-10 ug/ml obtained for the test bacteria frequently recorded for conventional antibiotics. It was explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contain some impure substances that may be inert and don't have antibacterial activities.

Although *Cassia auriculata* was found to contain some bioactive compounds with pronounced antibacterial activities, further phytochemical and pharmacological studies will be needed to isolate the active constituents and evaluate the antimicrobial activities against a wide range of microbial pathogens.

Table 1: Percentage yield of the crude extracts of *Cassia auriculata*

Extraction solvent	Raw material powder (gm)	Extracted plant powder (gm)	Percentage yield
Distilled water (aqueous)	20	0.5	2.5%

Table 2: Phytochemical constituents of *Cassia auriculata*

Plant constituents	Water extract
Alkaloids	+
Glycosides	+
Tannins	+
Saponins	+
Flavonoids	+

Key: + = positive, - = negative

Table 3: antibacterial activity of *Cassia auriculata*

Organism	Zone of inhibition	Antibiotic	Zone of inhibition
E.Coli	18 mm	Tetracycline	25 mm
Staphylococcus aureus	11 mm		

Fig-2-Zone of Inhibition of Plant Extract on E-Coli and *Staphylococcus aureus*

4. CONCLUSION

The present study exhibited the antibacterial effect of various extracts of *Cassia auriculata*. The inhibitory effect of the extract justified the medicinal use of *Cassia auriculata* and further study is required to find out the active component of medicinal value. The present study revealed that the ethanol extracts *Cassia auriculata* roots have antimicrobial activities

might be due to the presence of some sorts of bioactive or inhibitory compounds or factors in the extracts or synergism by the existence of some compounds or factors in the extract. Since a variety of constituents are present in the extract studied. So further extensive investigations are necessary to find out the active antimicrobial principles present in this plant parts that may lead to the development of noble antibiotic in future.

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