

Antibacterial activity of *Cassia auriculata* Linn against some pathogens isolated from wound

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ABSTRACT

The in vitro antibacterial activity of *Cassia auriculata* L root extract of (Methanol, Ethanol, Aqueous) has been investigated against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* isolated from wound samples. The fresh and methanol extracts of *Cassia auriculata* L showed high activity against nearly all the test microorganisms. The zone of inhibition of extract is very close and identical in magnitude and is comparable with that of standard antibiotics used.

Key words: *Cassia auriculata* L, Wound, Antibacterial activity, Antibiotics.

1. INTRODUCTION

Cassia auriculata Linn is a common plant in Asia, profoundly used in ayurvedic medicine as a tonic, astringent and as a remedy for diabetes, conjunctivitis and ophthalmia. It is one of the principle constituents as a tonic, astringent and as a remedy for diabetes, conjunctivitis and ophthalmia. It is one of the principle constituents of 'Avaarai panchaga chooranam'- an Indian herbal formulation used in the treatment of diabetes to control the blood sugar value [1]. 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 anthelmintic, seeds used to treat in eye troubles and root employed in skin disease [2].

Cassia auriculata Linn. commonly known as tanner's *cassia*, also known as "avaram" in Tamil language. 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 anthelmintic, seeds used to treat in eye troubles and root employed in skin diseases. The antidiabetic, hypolipidemic and antioxidant in skin disease and hepatoprotective effect [3].

Medicinal plants represent a rich source of antimicrobial agents. The most common bacteria used in the pharmacognostics analyse. Antibacterial potential of methanol, ethanol,

aqueous extract of dry root of *Cassia auriculata* Linn was conducted using agar disc diffusion method. The microorganisms used include *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*. The maximum activity was observed against all microorganisms the minimum inhibitory concentration was determined depending on microorganisms. *Cassia auriculata* Linn was observed to have antibacterial activity and can be used to combat against vast flora of microorganisms.

In an effort to expand the spectrum of antibacterial agents from natural resources, *Cassia auriculata* Linn belongs to leguminosa family has been selected. In the Indian literature, this plant has described to be useful against skin diseases, liver troubles, tuberculosis glands and its use into the treatment of hematemesis, pruritis, leucoderma and diabetes has been suggested. It has been concluded that plant parts could be used as a therapeutic agent in the treatment of hypercholesterolemia partially due to their fiber and mucilage content. Besides its pharmacological uses the plant extract is also recommended as a pest and disease control agents in India. This plant is widely used by tribal people to treat various ailments including ringworm and other fungal skin infections. The leaves are laxative and are useful in skin diseases. This plant organ is known to be an important source of secondary metabolites, notably phenolic compounds.

2. EXPERIMENTAL

2.1. Materials

Ethanol, Methanol, Crystal violet, Gram's iodine, Safranin, Ethyl alcohol, Tetracycline, Ampicillin and Gentamycin were purchased from Madras Scientific Supplies, Trichy.

2.2. Methodology

2.2.1. Collection of samples

The samples were collected by (Government Hospital from Orathanadu(Tk), Thanjavur (district) using sterile cotton swab from the infected patients. Sterile swab gently rotated on the wound region. The collected samples were immediately transferred to the laboratory for isolation of bacterial species.

2.2.2. ISOLATION AND IDENTIFICATION OF ORGANISMS

Nutrient agar medium was poured on to the sterile petriplates. After solidification, the collected wound samples were streaked on the plate. Then the plates were incubated at 37°C for 24 and 48 hours respectively. The isolated colonies were identified by cultural and morphological characteristics.

2.2.3. Identification of bacteria from wound Samples

Gram's staining (Han's Christian Gram, 1884) from the isolated colonies was performed to gain the most valuable cellular and morphological information about the bacteria. Gram's staining can be used to divide most bacterial species effectively into two large groups such as gram positive and gram negative. It can also be used to examine clinical material directly [4].

2.2.4. Motility Test

Hanging drop slide with depression on the upper side was cleaned, flamed and placed on the table. A little vaseline or petroleum jelly was spread around the cavity of the slide. With the help of match stick, petroleum jelly was applied on each of the four corners of the cover slip and placed on a paper. One loopful of culture was transferred in the centre of the cover slip. Depression slide was placed on to the cover slip; with the cavity facing down as a result the depression covers the suspension. The slide was gently pressed to form a seal between the cover slip and the slide. The preparation was lifted and the hanging drop preparation turned quickly so that the culture drop was suspended. The preparation was examined under low-power objective of compound microscope with reduced light. Then switched to the high power objective and preparation was examined again.

2.2.5. Biochemical test

After 24 hours, when growth was observed, the colonies were sub cultured and were later subjected to IMVIC test.

2.2.6. Collection of Plant Materials

The following medicinal plant was selected for the study from the local area based on their basic information available. The medicinal plant *Cassia auriculata* Linn root were collected from Pinnaiyur village, Thanjavur (District) Tamil Nadu, South India, during January (2013).

2.2.7. Preparation of root extract

Cassia auriculata Linn roots were dried in an oven below 60°C for 2 hrs. They were finely powdered and extract with 80% water, ethanol, methanol using Soxhlet apparatus at 55°C. The soluble part was concentrated over water bath maintained at below 60°C and dried in a vacuum metabolic extract of *Cassia auriculata* Linn (MECA).

2.2.8. Antibacterial activity

2.2.9. Disc Diffusion Method

Antibacterial activity was determined by disc diffusion method. Standard suspension of bacteria was inoculated on the surface of Nutrient agar plates. Ethanol, methanol, aqueous (1:1) was used to dissolve the plant extract. Sterilized filter paper disc (5mm) containing 20µL of each extract (100mg/mL) was arranged on the surface of the inoculated plates and incubated at 37°C for 18-24 hours. Along with this 30µg standard Antibiotic discs (Himedia standard -Tetracycline, Gentamycin, Ampicillin) were studied for antimicrobial activity as a positive control whereas the solvent used for preparing extract was used as a negative control. At the end of incubation, inhibition zone formed around the discs were measured with Himedia zone scale. The study was performed in triplicate and the mean values were presented [5].

2.2.10. Comparison of sensitivity of bacteria to plant extract and antibiotics

The comparative study of different antibiotics and plant extract on bacteria were assayed by disc diffusion method.

Antibiotic discs were used to detect antibiotic (Tetracycline, Genta mycin and Ampicillin) sensitivity of the bacterial isolates. The nutrient agar plates were inoculated with 0.1ml of bacterial suspension from nutrient broth. The antibiotic discs were placed on the inoculated plates and incubated at 37°C for 24 hrs. Antibiotic sensitivity was assayed from the diameter of zone of inhibition (Table 2, Figure 1).

3. RESULT AND DISCUSSION

The present study is to prove the antibacterial effect of commonly available medicinal plant root extract against wound causing pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Samples were collected from infected patient by sterile cotton swab. From the collected samples, the pathogens of bacterial species such as *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were recovered. Morphological characteristics of bacterial colonies were confirmed by staining techniques and isolated.

Organisms were also confirmed by the biochemical activities (Table-1). The ethanol, methanol and aqueous extract of *Cassia auriculata* Linn roots were evaluated by disc diffusion method. The ethanolic extract of *C.auriculata* Linn results. were showed the maximum zone of inhibition against *Staphylococcus aureus* 14 mm. The aqueous and methanol extract of plant root extracts were showed the maximum zone of inhibition against *Escherichia coli* 13.5 and 13 mm, (Table-2, and Fig-1).

Among all the extracts, the minimum activity of *C.auriculata* Linn roots showed at *Pseudomonas aeruginosa*(8mm). The use of plant compounds for pharmaceutical purpose has gradually increased. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases. The harmful microorganism can be controlled with drugs and the presence of multiple drug resistant bacteria created alarming clinical situations in the

treatment of infectious diseases. Certain natural products such as plants and different parts of plant leaves, roots, flowers, fruits, barks, seeds and oils are used to cure chronic and wound pathogens. Our study deals with the antibacterial activity of *Cassia auriculata* Linn root extract against some pathogens isolated from wound. The samples were collected from infected patients by sterile cotton swab. From these samples, bacterial pathogens such as *Escherichia.coil*, *Staphylococcus aureus* *Pseudomonas aeruginosa*. The antibacterial potency of *Cassia auriculata* Linn roots were tested with the three extracts (methanol, aqueous and ethanol). From the above three extracts, the ethanol extracts showed the higher zone of inhibition when compared to other by disc diffusion method. Our study correlated with the methanolic extract of flowers showed significant reduction of blood glucose response towards maltose ingestion and concurrently suppress insulin activity in rats [6].

The ethanolic extract of *C. auriculata* Linn showed the maximum zone of inhibition against *Staphylococcus aureus* (14mm). Among all the extracts, the minimum activity of plant root extract showed at *Pseudomonas aeruginosa*. Amsaveni, (2011) evaluate the antibacterial efficacy of *Cassia auriculata* Linn against Extended Spectrum Beta Lactamase (ESBL) produced E. Coli Methanol, ethanol ethyl acetate, hexane and chloroform crude and Soxhlet extracts of ethyl acetate and hexane of leaves, roots and flowers of *Cassia auriculata* Linn were tested for antibacterial activity by well diffusion method against ESBL produced *Escherichia coli*.

Table - 1: Identification of bacterial Isolates by Biochemical test

Name of the test	Name of The Organisms		
	<i>Escherichia Coli</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas earuginosa</i>
Gram staining	+	+	+
Indole test	+	-	-
Methyl red test	+	+	-
Vogasproskauer	-	+	-
Citrate utilization	-	-	+
Catalase test	+	+	+
Triple sugar Iron	+	-	+

Table - 2: Antibacterial activity of Wound isolates

Organisms	<i>Cassia auriculata</i> L	Tetracycline	Gentamycin	Amphicilin
<i>Escherichia coli</i>	14.4±9.6	12.44±4.6	11.9±0.57	10.8±0.08
<i>Staphylococcus aureus</i>	12.0±5.06	10.3±0.05	10.9±0.00	9.9±0.00
<i>Pseudomonas aeruginosa</i>	11.2±0.01	9.7±0.5	9.0±5.88	9.7±2.5

Earlier study of ethanolic and methanolic extracts of the flowers showed antioxidant activity which may be due to presence of flavonoids and tannins. The aqueous and ethanolic extract of flowers at dose 0.25 and 0.5 kg for 30 days exhibited a significant reduction in blood glucose, serum triglycerides, total serum cholesterol level and remarkable increase in plasma insulin level [7]. The combined aqueous extract of *C.auriculata* L. and *Aegle marmelos* L. at doses of 250,350 and 450 mg showed a significant reduction in glucose and lipid in serum on rats in dose dependent manner. It is possessed increment in serum insulin and restoration of beta cells [8]. It is clearly evident through this study that synthetic drugs can be replaced with natural medicine (herbal medicine) which is safer to human systems and also friendly to human body. More research is related to design and pattern this medicine of herbal drugs [9].

4. REFERENCES

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