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Antioxidant and antibacterial activities of *Cassia auriculata* in urinary tract infection patients

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Abstract
This work aimed to evaluate the *in-vitro* antioxidant activity and antibacterial activity of *Cassia auriculata* Linn. This belongs to the family Fabaceae/Caesalpiniaceous. The potential antioxidant activity of the extract was screened using tests such as DPPH assay, $ABTS^{\cdot}$ + Free radical scavenging assay and hydrogen peroxide-scavenging assay. The in-vitro antioxidant assays showed that the plant extract also possessed antimicrobial and antioxidant activity and exhibits vigorous defensive activity against the tested microorganisms. Therefore, *C.auriculata* Linn. Could be considered beneficial for the production of nutraceuticals as effective antioxidants to treat numerous human ailments.

Keywords: DPPH, $ABTS^{\cdot}$ +, hydrogen peroxide- scavenging, nutraceuticals, *Cassia auriculata*

Introduction
As a health-protecting factor, antioxidant compounds play an important role in food. Fruits, whole grains and vegetables are primary sources of naturally occurring antioxidants. The trapping of free radicals in the living system is the main characteristic feature of an antioxidant. The oxygen species and reactive free radicals can induce degenerative diseases. Antioxidant compounds like polyphenols, phenolic acid and flavonoids are commonly found in plants and have numerous biological effects, including antioxidative properties (Brown and Rice-Evans, 1998) [2].

Phytoconstituents are non-nutritive plant substances that have defensive or disease-preventing properties (Argal and Pathak, 2006) [1]. Plants yield these substances to safeguard themselves, but current research shows several phytoconstituents can protect humans against various infections. There are various phytochemicals in fruits and herbs and each works differently. Many plant extracts inhibit the growth of microorganisms. These extracts consist of chemicals and are usually considered to play a role in the plant's defense reactions against infections by pathogenic microorganisms (Fawcett and Spencer, 1970) [5]. *Senna auriculata* (L.) Roxb. Is a native plant found in India and belongs to the family Fabaceae. Its synonym is *Cassia auriculata* L. and is commonly known by other names such as Avaram, Matara tea, styptic weed or Tanner's Cassia (Lim, 2003). In Tamil, it is known as the Avaram tree. It is distributed throughout the hot deciduous forests of India and is found in the dry regions of Tamil Nadu, Madhya Pradesh, Rajasthan and other parts of India (Guruprasad *et al*., 2016) [6]. This plant exhibits antipyretic (Rao and Vedavathy, 1991) [11], hepatoprotective, anti-peroxidative, anti-hyperglycemic (Pari and Latha, 2003) [9] and microbicidal activity (Prakash, 2006) [10]. It is used in the traditional medicine system for female UTI (Urinary Tract Infections), antifertility, leprosy, worm infestation, diarrhea and pitta disease (Daisy and Kani, 2013) [3]. The flowers are used as a treatment for urinary discharges, nocturnal emissions, diabetes and throat irritation (Sharada *et al*., 2012) [12]. The seeds of *Cassia auriculata* find their application in purulent ophthalmia, *i.e*., eye inflammation or conjunctiva. The seeds should be finely powdered and blown into the affected eyes (Mali *et al*., 2012) [8]. The present study focuses on determining the antioxidant potential and antibacterial effects of the plant, i.e., flavonoids rich extract of *C. auriculata* Linn. Which has traditional claims for several diseases.

Materials and Methods
Antibacterial Assay
The potential antibacterial activity of *Cassia auriculata was* studied against gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and gram-negative (*Klebsiella pneumonia, Escherichia coli*) bacteria by agar well diffusion method. 100 mL of a fresh culture containing 1x108 CFU/mL of bacteria was spread onto the Mueller Hinton Agar (MHA) plates using sterile swabs. The antibacterial activity was tested at concentrations of 25, 50, 75, and 100 µL of *Cassia auriculata* extract (methanol and hexane as solvents). The size of the inhibition zone in the agar plates was measured after 24 hours of incubation at 37 °C, under visible light. The standard antibiotic amoxicillin (30 µg) was used as a positive control. Assays were carried out in triplicate.

Determination of MIC and MBC
The minimal quantity of *Cassia auriculata* required for the inhibitory and bactericidal activity was determined by adding different concentrations (25-100 µL) of *Cassia auriculata* (methanol and hexane) extract into the bacterial cultures. After 24 hours of incubation, each culture was plated, and the growth was determined by the colony formation method. The minimal concentration, at which there was no visual growth of cultures on test plates, was designated as the Minimum Inhibitory Concentration (MIC). The Minimum Bactericidal Concentration (MBC) is the lowest concentration at which there is no bacterial regrowth when transferred from the test plate into a new medium.

Antioxidant Assay
DPPH Radical Scavenging Assay
The DPPH free radical scavenging activity was carried out by (Sridhar and Charles, 2019) [13]. For the DPPH radical dot

assay, 0.70 ml sample extract or standard (ascorbic acid) with varying *Cassia auriculata* (methanol and hexane) extract concentrations (10, 20, 30, and 40 µg/mL) were added to the same volume of DPPH radical methanolic solution (100 µM). Mixtures were shaken vigorously and left to incubate for 20 mins in the dark at room temperature. A decrease in absorbance was measured at 515 nm against a blank of methanol without DPPH radical using a UV/Vis spectrophotometer. The inhibition percentage of DPPH radical discoloration was calculated using the following Equation (1):

Inhibition percentage = {A Control OD – A Treated OD/A Control OD} x 100 (1)

Where A control is the absorbance of control and A treated is the absorbance of the extract.

ABTS· + Radical scavenging assay

ABTS· + Radical scavenging activity was followed by (Sridhar and Charles, 2019) [13]. The stock solution ABTS· + radical was prepared by mixing ABTS aqueous solution (7 mM) and 2.45 mM aqueous potassium persulfate solution in equal quantities and were then allowed to react for 12– 16 hrs at room temperature in the dark. Then, 1 mL of ABTS·+ radical solution was mixed with 0.5mlof the aqueous extract or standard (ascorbic acid) at different *Cassia auriculata* (methanol and hexane) extract concentrations (10, 20, 30, and 40 µg/mL). The mixture was then kept for incubation at room temperature for 10 minutes in the dark. The control was prepared by mixing 1 ml of ABTS· + Radical solution with 0.5 ml of double-distilled water. The percentage results of scavenging activity were calculated as inhibition percentage using Equation (1).

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging activity was determined by (Fafal *et al*., 2017). Hydrogen peroxide (40 mM) solution was prepared in phosphate-buffered saline (0.1 M, pH 7.4). 1 ml of the ASP and *Cassia auriculata* (methanol and hexane) extract were rapidly mixed with 600 µL of hydrogen peroxide solution. The absorbance at 230nm was measured in UV/Vis spectrophotometer after 10 minutes of incubation at room temperature against a blank (without hydrogen peroxide). The percentage results of scavenging activity were calculated as the percentage of inhibition using the above Equation (1).

Results and Discussion

Medicinal plants produce these photochemical compounds to safeguard them self from the attack of pathogens. In recent decades, many photochemical compounds are used by humans to protect against various antibacterial, antifungal, anti-inflammatory diseases. Alcohol (methanol and hexane) based plant extracts inhibit the growth of microorganisms. *Cassia auriculata* extracts consist of enormous amounts of photochemical that are usually considered to play a role in the plant's defense reactions against infections by pathogenic microorganisms.

Antibacterial Assay

The antibacterial activity of *Cassia auriculata* extracts (methanol and hexane) were tested against gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and gram-negative (*Klebsiella pneumonia, Escherichia coli*) bacterial strains using agar well diffusion method as shown in Figure 1A and 1B. The antibacterial activity was tested at concentrations of 25, 50, 75 and 100 µL of *Cassia auriculata* extract (methanol and hexane). The diameters of inhibition zones and MIC values ranged from 8 to 9 mm and 2.2 and 7.5 mg/mL for *Cassia auriculata* methanol and hexane extracts studied against gram-positive and gram-negative bacteria, respectively in Table 1 and Table 2.

Table 1: Zone of inhibition of the *Cassia auriculata* extract (methanol and hexane solvent) samples tested against Gram-positive and Gram-negative bacteria.

Test organism	Zone of inhibition (mm) Methanol				Zone of inhibition (mm) Hexane			
	25 µL	50 µL	75 µL	100 µL	25 µL	50 µL	75 µL	100 µL
S. aureus	-	-	14	17	-	-	-	-
S. pyogenes	-	-	-	-	-	-	-	10
E. coli	-	-	-	19	-	-	8	12
K. pneumonia	-	-	16	18	-	-	-	11

Table 2: Determination of MIC of the *Cassia auriculata* extract (methanol and hexane solvent).

S. No	Test Organism	Methanol in mg/mL	Hexane mg/mL
1	*S. aureus*	2.2	-
2	*S. pyogenes*	-	7.5
3	*E. coli*	2.2	7.5
4	*K. pneumonia*	2.2	7.5

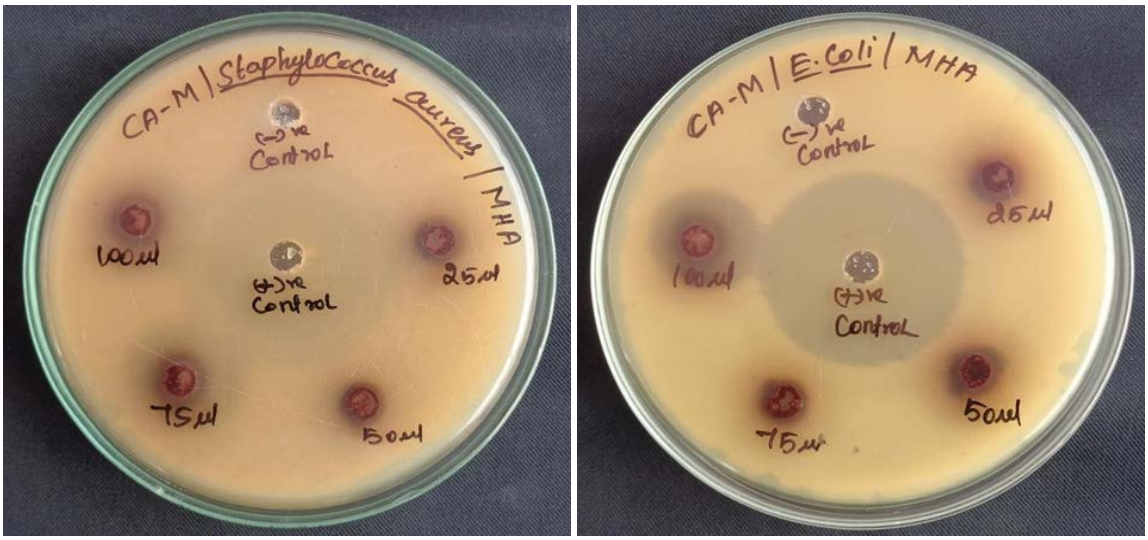

Fig 1A: Antibacterial activity of the *Cassia auriculata* extract (Methanol solvent) samples tested against Gram-positive and Gram-negative bacteria.

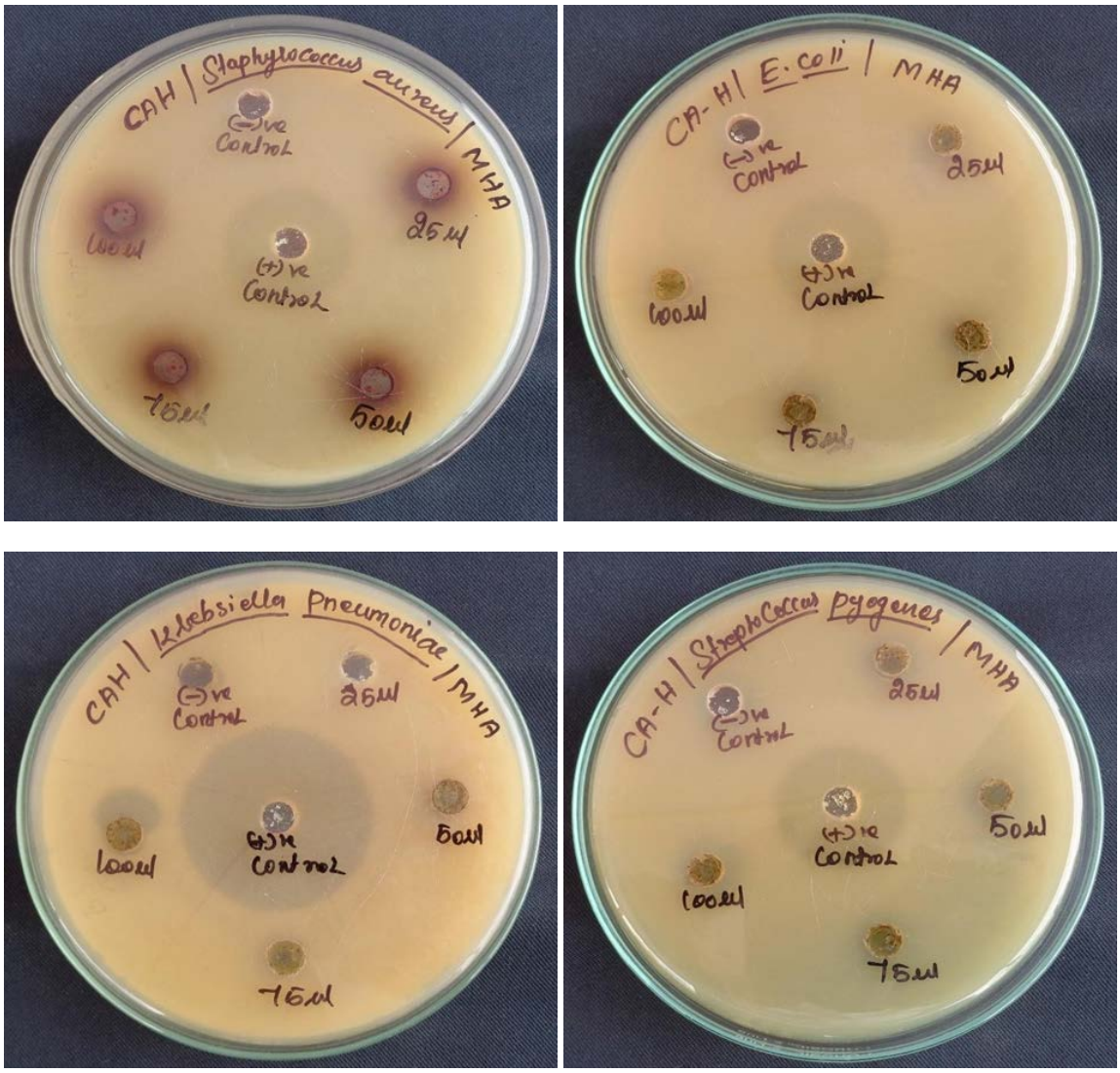

Fig 1B: Antibacterial activity of the *Cassia auriculata* extract (Hexane solvent) samples tested against Gram-positive and Gram-negative bacteria.

Antioxidant Assay

DPPH Radical Scavenging Assay

Free radical scavenging activity of the *Cassia auriculata* extracts (methanol and hexane) was determined by DPPH assay. Free radical scavenging capacity was increased with increasing extract concentrations. In Table 3, *Cassia auriculata* extract from different solvents exhibited good radical scavenging activity. The highest radical scavenging

activity was recorded by hexane solvent with IC50 value 92.91% and methanol solvent with IC50 value 89.37%.

ABTS. + Radical scavenging assay

The ABTS· + Radical scavenging assay of *Cassia auriculata* extract (methanol and hexane solvent) exhibits similar activity. The prepared plant extract has a potential antioxidant activity with IC50 values of 49.06 and 46.79 µg/mL (Table 4) for *Cassia auriculata* extract in methanol and hexane solvent, respectively.

Hydrogen Peroxide Scavenging Assay

The H2O2 scavenging activity of *Cassia auriculata* extract (methanol and hexane solvent) is shown in Table 5. The H2O2 scavenging activity of methanol extract is more significant than hexane solvent. However, the IC50 value of the methanol and hexane extracts are 49.06 and 46.79 µg/mL, respectively. Therefore, the observed antioxidant activity dependent on the inhibition percentage of different concentrations of DPPH radical scavenging assay, ABTS· + radical scavenging assay and hydrogen peroxide radical scavenging assay of *Cassia auriculata* in methanol and hexane solvents is Shown in Figure 2(A) and 2(B).

Table 3: DPPH radical scavenging assay of *Cassia auriculata* extract (methanol and hexane solvent).

Methanol				Hexane		
Concentration µg/mL	OD value	Inhibition %	IC50 value	OD value	Inhibition %	IC50 value
10	0.797	5.79	89.37	0.791	6.5	92.91
20	0.756	10.63		0.786	7.09	
30	0.699	17.37		0.676	20.09	
40	0.596	29.55		0.587	30.61	

Table 4: ABTS.+ radical scavenging assay of Cassia auriculata extract (methanol and hexane solvent).

Methanol				Hexane		
Concentration µg/mL	OD value	Inhibition %	IC50 value	OD value	Inhibition %	IC50 value
10	0.426	35.35	50.15	0.43	35.74	50.91
20	0.319	51.59		0.302	54.17	
30	0.255	65.85		0.23	65.09	
40	0.112	98.33		0.135	79.51	

Table 5: Hydrogen peroxide radical scavenging assay of *Cassia auriculata* extract (methanol and hexane solvent).

Concentration µg/mL	Methanol			Hexane		
	OD value	Inhibition %	IC50 value	OD value	Inhibition %	IC50 value
10	0.412	45.06	49.06	0.405	46	46.79
20	0.368	50.93		0.352	53.2	
30	0.283	62.26		0.29	61.33	
40	0.235	68.66		0.24	68	

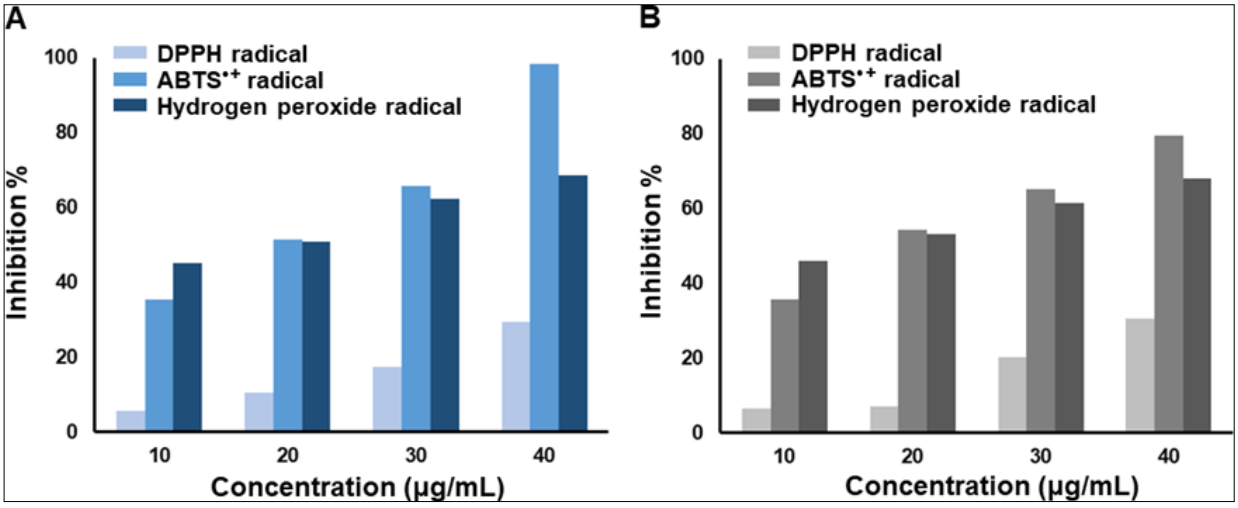

Fig 2: Antioxidant activity observed by DPPH, ABTS.+ and Hydrogen peroxide radical scavenging assay of *Cassia auriculata* in (A) methanol and (B) hexane solution at concentration level of 10, 20, 30 and 40 (µg/mL).

Conclusion

Researching plant sources may lead to new natural products in pharmacological, cosmetics and food production. An *in-vitro* antioxidant study offers scientific confirmation to the traditional claims on the medicinal value of the member of *Cassia* species. On the basis on the results of the present study, it was concluded that the *Cassia auriculata* Linn. Possesses a substantial level of antioxidant activity and antimicrobial activity. The presence of a sufficient amount of phenol, flavonoids and saponin composites may account for this fact. Hence, the findings of the current study recommend that this plant is a potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds, and *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

Abbreviations

UTI: Urinary Tract Infection
MIC: Minimum Inhibitory Concentration.
MBC: Minimum Bactericidal Concentration.
DPPH: 2, 2-Diphenyl-1-picrylhydrazyl.
ABTS· +: 2, 2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid.

Conflict of Interest

There is no conflict of interest among authors or from the concerned institution.

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