

**PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF CASIA  
OCCIDENTALIS (L)**

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**ABSTRACT:** The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries. The present study deals with the screening of *Casia occidentalis* leaves for their antimicrobial activity against various strains of bacteria. Plant *Cassia occidentalis* belongs to family Caesalpiniaceae, is a diffuse offensively odorous under shrub. *Casia occidentalis* were shade dried, powered and was extracted using solvents Methanol. The antimicrobial activity test performed by the disc diffusion method. Preliminary phytochemical analysis of the plant extracts fractions of HXF, CTF, CFF and AQF showed the presence of carbohydrates, amino acids, phytosterols, fixed oils and phenolic compounds. The AQF fraction of *C. occidentalis* showed high activity across pseudomonas aeruginosa and staphylococcus aureus bacteria. The present study indicates the potential usefulness of *Casia occidentalis* leaves in the treatment of various diseases caused by micro-organisms.

**Key words:** *Casia occidentalis*, Minimal inhibitory concentration, antibacterial activity, phytochemical.

**INTRODUCTION**

The development of resistance to current antibiotics by disease causing microbes has reinforced research for discovery of new ones. Current trends in drug development process are focused on natural sources, especially sources of plant origin due to some proven correlation between the folkloric medicinal uses of some of these plants to biological activity. Hence the use of plant materials to prevent and treat infectious diseases successfully over the years has continued to attract the attention of scientist worldwide (Osawa *et al*, 1990; Kunle *et al*, 2003; Roopashree *et al* 2008; Kunle and Egharevba 2009; Begum *et al* 2002). *Casia* species have been used as traditional medicine in rain forest and other tropical areas for centuries. The roots, leaves, flowers and seeds have been employed in herbal medicine around the world (Burkill, 1995).

The plant *Casia occidentalis* belongs to family Caesalpiniaceae, is a diffuse offensively odorous under shrub. The plant is sub glabrous, leaflets 3-5 pairs, Flowers are yellow, in short peduncled few flowered racemes; fruits cylindrical containing 20-30 seeds. The plant is distributed throughout India, growing abundantly on wastelands immediately after the rains. In Ayurveda, *Casia occidentalis* leaves has been reported to be used as purgative, expectorant, cures "vata" and "kapha" inflammations, diabetes, and fever. It is good for diseases of the lungs and stomach. It was reported to possess hepatoprotective activity, and anti-inflammatory activity. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries and for the presence of antimicrobial. In the present study, an attempt was made to investigate the antimicrobial activity and preliminary phytochemicals from the leaves of *C. occidentalis* collected from Parvatagiri Village of Torur Mandal, Warangal district, Andhra Pradesh, India.

**MATERIALS AND METHODS****Collection of plant material**

The aerial parts of *Casia occidentalis* were collected from Parvatagiri Village of Torur Mandal, Warangal district, Andhra Pradesh. Voucher specimens were prepared and identified at the Department of Botany, Kakatiya University, Warangal. The *Casia occidentalis* leaves were collected and left at room temperature for two weeks to dry, then ground into powder and extraction with Soxhlet techniques with methanol.

Obtaining methanolic crude extracts of *Casia occidentalis* were then fractionated successively using solvents of increasing polarity, such as, *n*-hexane (HX), carbon tetrachloride (CT), and chloroform (CF) and aqueous fractions (AQ). All the four fractions (HXF, CTF, CFF and AQF) were evaporated to dryness by using rotary evaporator at low temperature (39°C).

### Preliminary phytochemical investigations

The different qualitative chemical tests were performed for establishing profile of given extract for its chemical composition. Phytochemical analyses were done using the procedures of Kokate (1994) and Kokate et al (1995).

### Bacterial species

Bacterial species selected for the study were the four pathogens, namely, two Gram-positive *Staphylococcus aureus* and *Enterococcus faecalis* and two Gram-negative *Psuedomonas aeruginosa* and *Escherichia coli*. All the cultures were maintained on Mueller-Hilton agar at 40°C. The cells were inoculated and incubated at 37°C in broth for 12 hours prior to the screening procedure.

### Minimal inhibitory concentration (MIC)

The serial microplate dilution method developed by Eloff (1998) was used to determine the minimum inhibitory concentration (MIC) for plant extracts using tetrazolium violet reduction as an indicator of growth. Residues of the different extracts were re-dissolved in methanol to a concentration of 1 mg/ml. for each of the four bacteria used, 100µl of each plant extract tested were two-fold serially diluted with 100 µl sterile distilled water in a sterile 96-well microtitre plates. A similar two- fold serial dilution of gentamicine (0.1mg/ml) was used as a positive control against each bacterium. One hundred microlitres of each bacterial culture were added to each well. The plates were covered and incubated overnight at 37°C. To indicate bacterial growth 40 µl of 0.2 mg/ml p-iodonitrotetrazolium violet (INT) were added to each well and the plates incubated at 37°C for 30 minutes. Bacterial growth in the wells was indicated by a red colour, where as clear wells indicated inhibition of the bacterial growth by the plant extracts.

## RESULTS AND DISCUSSION

### Percentage yield

The yield of the methanol crude extract of *Casia occidentalis* was 500gr (7.6%). The percentage yield of these fractions of the methanolic extract of *Casia occidentalis* were showed in the table-1. The HXF and CFF fractions obtained highest yield (2.5%) when compared to other fractions. 1.4% yield obtained in CTF fraction which is lowest.

**Table-1 Percentage of yield**

S.No	Fractions	Yield (%)
1	HXF	2.5
2	CTF	1.4
3	CFF	2.5
4	AQF	1.6

### Phytochemical screening in *C. occidentalis*

Preliminary phytochemical analysis of the plant extracts fractions of HXF, CTF, CFF and AQF showed the presence of carbohydrates, glycosides, amino acids, phytosterols, fixed oils and fats, phenolic compounds, flavanoids, steroids and saponins while alkaloids are absent in all of the tested fractions of methanolic crude extract (Table-2). Carbohydrates and saponins are present in high concentration in AQF fraction of *C. occidentalis*. Glycosides, proteins and amino acids are slightly present in HXF and CTF fractions. Phenolic compounds and flavonoids are moderately present in AQF and CTF.

Table-2. Phytochemical screening for presence of different Phytoconstituents in *Casia occidentalis* leaves extract fractions

Sl. No.	Phytochemicals test	HXF	CTF	CFF	AQF
<b>I</b>	<b>Test for Alkaloids</b>				
a.	Mayer's Test	-	-	-	-
b.	Wagner's Test	-	-	-	-
c.	Hager's Test	-	-	-	-
<b>D</b>	Dragendorff's Test	-	-	-	-
<b>II</b>	<b>Test for Carbohydrates</b>				
A	Molish's Test	-	-	+	+
B	Fehling's Test	-	-	+	+++
C	Barfoed's Test	-	-	+	+
D	Benedict's Test	-	-	+	+
<b>III</b>	<b>Test for Glycosides</b>				
a.	Borntrager's Test	+	+	-	+
B	Legal's Test	+	+	-	+
<b>IV</b>	<b>Test for Saponin</b>				
A	Foam Test	+	+	+++	+++
<b>V</b>	<b>Test for Proteins and Amino acids</b>				
A	Millon's Test	+	+	+	++
B	Biuret's Test	+	+	++	++
C	Ninhydrin Test	+	+	+	++
<b>VI</b>	<b>Test for Phytosteroids</b>				
A	Liebermann – Burchard's Test	-	-	-	++
<b>VII</b>	<b>Test for fixed oils and fats</b>				
A	Spot Test	+	++	-	-
B	Saponification Test	+	++	-	-
<b>VIII</b>	<b>Tests for Phenolic Compounds and Flavanoides</b>				
A	Ferric chloride Test	-	+	-	+
B	Gelatin Test	-	+	-	+
C	Lead acetate Test	-	+	-	++
D	Alkaline Rgt. Test	-	++	+	+
E	Magnesium Test	-	++	+	+

+++ Prominently Present, ++ Moderately Present, + Slightly Present, - Absent

### Antibacterial activity

The MIC values and total activity of the four fractions of methanol crude extract of *Casia occidentalis* plant against all the tested bacteria are presented in Table 3. *Casia occidentalis* AQF had MIC value of 0.08 mg/ml against *S. aureus* and *E. faecalis* respectively.

**Table: 3. MIC values (mg/ml) *Casia occidentalis* plant extracts fractions of the study: *C. occidentalis* against four bacteria: *Escherichia coli* (Ec), *Enterococcus faecalis* (Ef), *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa). Gentamicin was used as a positive control.**

Plant species	Ec	Ef	Pa	Sa
<i>C. occidentalis</i>				
HXF	0.63	0.63	1.25	1.25
CTF	0.63	0.63	0.08	0.08
CFF	0.30	0.63	0.15	0.08
AQF	0.15	0.08	0.15	0.08
Gentamicin (µg/ml)	8.0	1.6	0.2	0.3

HXF=n-hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction and AQF=Aqueous fraction.

In this study the highest total activity was obtained on *C.occidentalis* (295 ml/g) as shown on table-4. *Casia occidentalis* had consistent total activity across *Pseudomonas aeruginosa* and *Staphylococcus aureus* bacteria. AQF fraction of *C. occidentalis* showed high activity across *P. aeruginosa* and *S. aiaureus* bacteria.

**Table-4. Total activity (ml/g) of *C. occidentalis* extracts fractions of this study: *C occidentalis* against four bacteria: *Escherichia coli* (Ec), *Enterococcus faecalis* (Ef), *Pseudomonas aeruginosa* (Pa) and *Staphylococcus aureus* (Sa).**

Plant species	Ec	Ef	Pa	Sa
<i>C. occidentalis</i>				
HXF	37	37	17	17
CTF	24	24	196	196
CFF	78	37	157	295
AQF	104	196	104	196

HXF=n-hexane fraction, CTF=Carbon tetra chloride fraction, CFF=Chloroform fraction and AQF=Aqueous fraction.

Alkaloids are known to possess anti-malaria property; hence the plant may be a good source of anti malaria for which it is traditionally uses in locally (Ronan *et al.*, 2009). Also the use of *C. occidentalis* as genital stimulant may be attributed to the presence of alkaloids. Plant containing saponins are believed to have antioxidant, anti-cancer, anti-inflammatory, and anti-viral properties.

## CONCLUSION

The result of the qualitative phytochemical screening indicates in methanolic crude extracts revealed the presence of alkaloids, carbohydrates, saponins, proteins and Amino acids, Phytosteroids, Fixed oils and Fats, Phenolic compounds and Flavanoids. The presence of these metabolites suggests great potential for the plant as a source of useful phytochemicals.

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