

Phytochemical Investigation and Antibacterial Activity of Pod Extracts of *Cassia obtusifolia* Linn.

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Department of Chemistry,
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Phytochemical have played a vital role in the past and will continue to do so in the future. The source of many compounds used in modern medicine today can be traced down to plant origin. Various fractions of *Cassia obtusifolia* pods have been studied for in vitro antibacterial activity against urinary pathogens. All fractions showed inhibitory activity against all test pathogens but maximum inhibition was seen with ethanol fraction

Keywords: Antibacterial Activity, *Cassia Obtusifolia*, Fabaceae, Phytochemical, Urinary Pathogens, Cup Plate Methods.

Introduction

Cassia obtusifolia Linn (Family Fabaceae) is commonly known as Chakawar in Hindi. It is widely distributed along road side and other tropical and temperate regions of Asia, Africa and America [1]. Due to persence of several chemical constituents in the different components of the plant were used for its antiplasmodial activity [2], anti-inflammatory activity [3], laxative activity [4], purgative [5], psoriasis [6], anthelminatic [7] and in the treatment of half headache with ring worm [8]. In previous communication we have reported the presence of anthraquinone glycosides with free amino acids from the fermented leaves, seeds, roots and pods of the test plant *Cassia obtusifolia* [9].

Urinary tract infection is a common disease, which occurs asymptotically in both sexes at any ages and is mainly treated by use of antibiotics such as ciprofloxacin, cephaloridine, gentamycine and streptomycin but prolong used of these antibiotics may result in adverse effects such as degenrative lesions of the renal perenchyma tubular dysfunction, acute tubular necrosis and damage to proximal tubule etc. [10]

In view of this, present study was undertaken to assess the antibacterial activity of the partially purified fractions *Cassia obtusifolia* pod extract made in different solvents against pure cultures of clinical strains the common urinary tract pathogens like *Escherichia coli*, *Proteus mirabills*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* which were procured from Department of Pathology, I.M.S., B.H.U. Varanasi (U.P.) and identified by standard methods [11].

Material and methods**Plant material**

The fresh mature pods of *Cassia obtusifolia* is collected from the rural area of Jaunpur district and identified by Toxonomist of Botany Department, S.G.R.P.G. College Dobhi, Jaunpur (U.P.) and voucher specimen was deposited at herbarium and assigned that voucher specimen no.127.

Preparation of extract

Cassia obtusifolia pods was manually scraped from the plant and dried in oven at 48°C. Dried coarse powder (20 gm) was successively extracted with 140 mL each of petroleum ether, benzene, chloroform, ethanol and water respectively using soxhlet extractor. After extraction the solvent was evaporated under vaccum and the extract was dried at room temperature. Presence of secondary metabolities in these fractions was confirmed by performing standard rapid qualitative chemical test [12].

Antibacterial activity

Antibacterial activity was assayed by cup plate method [13]. The respective fractions were reconstituted in DMF to make 10 mg/mL stock solutions. A well of 8 mm diameter in the agar plate seeded earlier with test organism was filled with 250 mL of different fractions respectively. Plates were incubated at 38°C for 24 h and the diameter of the zone of inhibition

surrounding each of the wells (250 µL) was used as standard. was noted. Ciprofloxacin (250 µg/mL) was used as standard.

Minimum inhibitory concentration and bactericidal concentration of alcohol fraction was assayed by macrobroth dilution method [11]. The test solution (pod extract) was added in 8 mL nutrient broth in order to attain final concentrations such as 500 mcg/mL, 250 mcg/mL—3.95 mcg/mL. 0.1 mL cell suspension (1×10⁴ cells/mL) of test organisms was added to each tube and the tubes were incubated at 37° for 24 h. Tube containing only medium and

inoculum was used as control. Simultaneously DMF control was also maintained. MIC was determined as the concentration at which there was absence of visible growth. For estimation of MBC bacteria from tubes containing MIC and subsequent extract concentrations respectively were inoculated on agar medium and once again incubated at 37° for 24 h. Concentration at which there was complete absence of growth was taken as MBC [13].

Table 1 : Phytochemical composition of pod extract of *Cassia obtusifolia* and percentage extractive value of each formation

Constituents and Colour test	Petroleum ether	Benzene	Chloroform	Alcohol	Aqueous
Fixed oil	P	A	A	A	A
Flavanoids (Shinoda's test)	A	P	P	P	A
Phenolic compounds (Ferric chloride test)	A	P	P	P	P
Tannis (Gelatine ppt. Test)	A	A	A	P	P
Glycosides (Molich reagent)	A	P	P	P	P
Sterols (Lieberman Burchard's test)	A	A	A	A	A
Alkaloids (Mayer's reagent)	A	A	A	A	A
% w/w of extract	0.35%	0.25%	0.1%	46.1%	10.7%
Abr.	P = Present		A = Absent		

Results and discussion

Percentage extractive value and chemical constituents present in petroleum ether, benzene, chloroform, ethanol and aqueous fractions are shown in table 1. Maximum percentage extractive value i.e. 46.1% was found in alcohol fraction. Alkaloids and sterols were absent in all fractions. Fixed oil was

present only in petroleum ether fraction while benzene and chloroform fraction contained flavnoids and phenolic compounds. Alcohol fraction showed presence of phenols, flavonoids, tannis as well as glycosides, where as aqueous fraction contained phenols, tannis and glycosides.

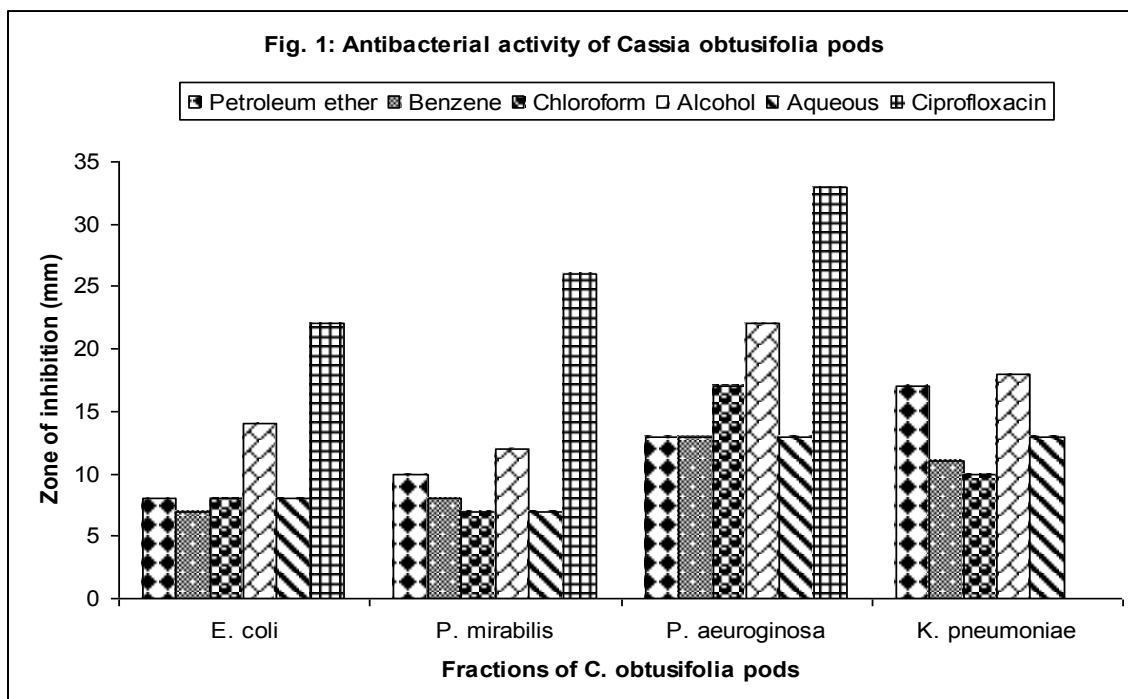


Fig. 1 suggests that all bacteria were most susceptible to alcohol fraction of pod extract. All fraction showed significant inhibition of *P. aeruginosa* followed by *K. pneumoniae*, *P. mirabilis* and *E. coli* respectively. DMF did not show antibacterial activity. *K. pneumoniae* was found to be resistant to the standard drug whereas all fractions showed significant inhibition of this organism. Best inhibitory activity was also observed against *K. pneumoniae* as its growth was inhibited at 31.25 mcg/mL followed by *E. coli* and *P. aeruginosa* (MIC; 250 mcg/mL). *P. mirabilis* was inhibited at 500 mcg/mL. Effect of alcohol fraction was bacteriostatic, except for *K. pneumoniae* where, 250 mcg/mL concentration of alcohol fraction was found to be the MBC. This differential inhibition of test organisms by different fractions implies that organism susceptibility is dependent on chemical nature and mode of action of the fraction.

Results of the present study therefore suggest that *C. obtusifolia* pod extract is antibacterial in nature. The antimicrobial activity may be due to the presence of secondary metabolites especially flavanoids, phenols, tannins, anthraquinone, glycosides etc. which are known to exhibit antimicrobial activity against both human and plant pathogens [14]. Alcohol is a polar solvent and therefore all these secondary metabolites get extracted in it, hence this fraction showed inhibition of all test organisms. Phytochemical screening of pod also indicated presence of phenols, flavonoids, tannins and glycosides in various fraction of pods.

Conclusions

On the basis of this study, it can therefore be concluded that alcohol fraction of *C. obtusifolia* pod

can be used to develop a broad-spectrum antibacterial agent against urinary pathogens. Separation and structural elucidation of the compounds responsible for antimicrobial activity and a study of their mode of action is in progress.

Acknowledgement

The authors are thankful to Director, I.M.S., B.H.U., Varanasi (U.P.) for providing pharmacological activity. We are thankful to principal, S.G.R.P.G. College, Dobhi Jaunpur (U.P.) for providing necessary facilities to complete the work.

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