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Antibacterial and phytochemical analysis of *Cassia occidentalis* L. seeds against respiratory tract pathogens

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Medicinal properties of plants make them potent to prevent or cure diseases. In present study, antibacterial activity of various extracts of *Cassia occidentalis* L. seeds was evaluated against three respiratory tract pathogens i.e. *Staphylococcus aureus* MTCC 1144, *Streptococcus pneumoniae* MTCC 655 and *Streptococcus pyogenes* MTCC 442. The shade dried seeds of *C. occidentalis* were crushed and extracted in petroleum ether (PET), acetone (ACE), methanol (MeOH) and aqueous (H_2O) by using Soxhlet apparatus. The antibacterial activity was examined by agar well diffusion method. Chromatographic separation was carried out on the active extract and efficacy of the resulting fractions was tested against the selected microorganisms. Amoxicillin was used as positive control to determine the sensitivity of the strains. The results showed that MeOH extract was more active than other extracts in its antibacterial activity. The zone of inhibition exhibited by MeOH extract against tested microorganisms ranged between 20.9±0.21 to 23.1±0.15 mm, respectively. Phytochemical screening revealed the presence of flavonoids, phenols, tannins, amino acids, saponins, glycosides, terpenoids and steroids. The investigation corroborates the traditional uses of *C. occidentalis* in the treatment of respiratory tract diseases.

Keywords: Antibacterial activity, Agar well diffusion method, *Cassia occidentalis*, Phytochemical analysis, Respiratory tract pathogens.

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Introduction

Plants are the pavement bricks of all the living organisms on the earth. They produce a wide range of secondary metabolites such as alkaloids, unsaturated fatty acids, flavonoids, phenols, tannins and terpenes that can be used to treat various chronic and infectious diseases[1]. In recent years, increasing strains of microorganisms throughout the world have developed resistance to large number of antibiotics that has created immense clinical problem and made the management of infectious diseases quite complicated[2]. The way to avoid antibiotic resistance of pathogenic species is by using plant based compounds rather than existing synthetic antimicrobial agents[3].

Respiratory tract infections are the most common ailment including allergies, asthma and chronic obstructive pulmonary disease (COPD). The common causative agents are *Escherichia coli*, *Klebsiella pneumoniae* responsible for nosocomial infections[4], *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Moraxella catarrhalis* for community acquired infections[5], *Enterobacter cloacae* and *Bacillus subtilis* which cause occupational asthma[6] respectively.

Cassia occidentalis L. belongs to the family Caesalpiniaceae, commonly known as *Kasondi* in Hindi, *Kasmard* in Sanskrit and *Coffee Senna* in English. It is a shrub, grows erect to a height of 1.8 m approximately. *Cassia* species have been used as traditional medicine in rain forest and other tropical areas for centuries and is a native plant of southern India. *C. occidentalis* is used to cure various diseases e.g. fever, menstrual problems, tuberculosis, liver complaints and as a tonic for general weakness and illness[7]. The roots, leaves, flowers and seeds have been employed in herbal medicine around the world[8]. An infusion of *C. occidentalis* bark is used in folklore for diabetes treatment[9].

The aim of this study was to examine the antibacterial and phytochemical aspects of various extracts of *C. occidentalis* against bacterial pathogens causing respiratory tract infections.

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Materials and Methods

Plant material

C. occidentalis was collected from Bharat Heavy Electrical Limited (BHEL) campus, Haridwar and it was identified at Botanical Survey of India, Northern Regional Center, Dehra Dun, Uttarakhand. Seeds were washed by running tap water to remove the adhering unwanted material and cut into small pieces, dried at room temperature and then powdered by using blender.

Preparation of extract

Plant extracts were prepared by immersing 200 g of powdered plant material in 600 mL of four different solvents i.e. petroleum ether, acetone, methanol and water, loaded in Soxhlet assembly and extracted for 72 h through successive method[10]. Plant extracts were filtered through Whatman No. 1 filter paper and crude extracts obtained by removing solvents in vacuum evaporator at 30ºC. Residues were stored at 4ºC until further use. Extracts were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 200 mg/mL for agar well diffusion method.

Microorganisms used

The bacterial strains causing respiratory tract infections i.e. *Staphylococcus aureus* MTCC 1144, *Streptococcus pyogenes* MTCC 422 and *Streptococcus pneumoniae* MTCC 655 were procured from Institute of Microbial Technology (IMTECH), Chandigarh. Bacterial strains were maintained at 4ºC of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from stock cultures to test tubes of Mueller Hinton broth (MHB) for bacteria that were incubated without agitation for 24 h at 37ºC.

Antibacterial activity

Agar well diffusion method was used to evaluate the antibacterial activity[10]. This method depends upon the diffusion of the tested material to such an extent that growth of the added microorganism is prevented entirely in a zone around the hole containing a solution of tested material[11]. Muller-Hinton Agar Media No. 173 (Hi media Pvt. Ltd., Mumbai, India) was used for antibacterial screening. 0.1 mL of diluted inoculum (10^5 CFU/mL) of test microorganisms were mixed in Muller-Hinton broth and poured in sterilized petri dishes. A cork borer (6 mm diam.) used to punch wells in medium and filled with 45 µL of plant extracts of 200 mg/mL final concentration of extracts. DMSO was used as negative control. Efficacy of extracts against bacteria was compared with a moderate-spectrum antibiotic amoxicillin (positive control). Each extract was assayed in triplicate. Plates were incubated at 37°C for 24 h in BOD incubator. The antibacterial activity was interpreted from size of diameter of zone of inhibition measured in millimetre (mm).

Column chromatographic separation

The lower end of a glass column was plugged with glass wool. The material was poured on the glass wool and air bubbles released was trapped with the flat end of a packed rod. The column was packed with wet silica gel by pouring the silica gel into the column in a stepwise manner. The side of the column was taped gently with a glass rod compaction of the particles. As silica gel settles, the column outlet was adjusted. The sample was drawn onto the absorbent and eluted with solvent mixture of benzene and chloroform in ratio (50:50). All fractions obtained were collected.

Phytochemical screening

The MeOH extract and fractions obtained were subjected to phytochemical analysis to determine the presence of following bioactive components by using standard qualitative methods[12].

Alkaloids

Test solution was acidified with acetic acid and a drop of Mayer's reagent was added. A white precipitate indicated the presence of alkaloids.

Flavonoids

On addition of conc. HCl in methanolic extract of material, a red colour appeared which indicated the presence of flavonoids.

Glycosides

Plant extract was filtered and sugar was removed by fermentation with baker's yeast. The acid was removed by precipitation with $Ba(OH)_2$. The remaining extract contained the glycosides. The hydrolysis of solution was done with conc. H_2SO_4 and after hydrolysis the presence of sugars was determined with help of Fehling's solution.

Steroids

The extract mixed with 3 mL chloroform and 2 mL conc. H_2SO_4 was poured from side of test tube and colour of the ring at junction of two layers was noted. A red colour showed the presence of steroids.

Saponins

Extracts were diluted with distilled water to 20 mL and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

Tannins

Extract was added in 1% ferric chloride and observed the colour. Bluish black colour appeared which disappeared on addition of dilute H_2SO_4 follow a yellow brown precipitate indicates the presence of tannins.

Results and Discussion

The data presenting to antibacterial activity of crude extract of *C. occidentalis* are shown in Table 1. *C. occidentalis* showed promising activity against tested microorganisms. MeOH extract was found most effective followed by H_2O, ACE and PET. It was highly active against *S. aureus* (23.1±0.15 mm) and lowest inhibition against *S. pneumoniae* (20.9±0.21 mm) in comparison to other solvent extracts.

Many of present findings on these extracts are in agreement with previous workers. Vaghasiya and Chanda[13] reported the antimicrobial activity of the MeOH and ACE extracts of fourteen plants belonging to different families against five Gram-positive bacteria (*S. aureus*, *S. epidermidis*, *B. cereus*, *B. subtilis*, *M. flavus*), seven Gram-negative bacteria (*P. aeruginosa*, *E. coli*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, *S. typhimurium*, *C. freundei*) and three fungi (*Candida albicans*, *C. tropicalis* and *Cryptococcus luteolus*). Sadiq *et al*[14] reported the *in vitro* antimicrobial screening of *C. occidentalis* against *S. aureus, P. aeruginosa, E. coli, S. typhi* and *Shigella* spp. The results showed that these extracts were effective against all the test organisms.

The phytochemical screening of MeOH and H_2O extracts showed the presence of flavonoids, phenols/tannins, amino acids, saponins, glycosides and steroids. In PET only steroids was present. While alkaloids and saponins were absent in ACE extract. Alkaloids test was negative in all extracts (Table 2). Arya *et al*[15] reported the presence of anthraquinone, carbohydrates, glycosides, steroids, flavonoids, saponins, phytosterols, gums and mucilages while alkaloids were absent in all the tested extracts of *C. occidentalis* leaves but in present investigation amino acids were also observed. The phytochemical constituents for the ethanol and water extract revealed the presence of tannins, cardiac glycosides, saponins and anthraquinone, while the fractions revealed the presence of tannins, terpenoids and anthraquinones[14]. Ranjithkumar *et al*[16] reported the presence of alkaloids in MeOH, H_2O, ethyl acetate (EA) extracts and absent in ethanol (EtOH) extract. Carbohydrate which constitutes the major edible part of this plant is present in all the four extracts. Glycoside is present only in MeOH, H_2O and cardiac glycoside has shown positive result for MeOH, H_2O and EA extracts.

Phytochemical analysis of the five fractions revealed that the fractions contained different components like steroids/terpenes, flavonoids, glycosides, tannins/phenols and saponins (Fig. 1). Glycosides were most effective against *S. aureus* (15 mm) followed by *S. pyogenes* (14 mm) and *S. pneumoniae* (11 mm). Among these phytoconstituents, glycosides were the most active constituents.

Table 2—The phytochemical screening of crude extracts of *C. occidentalis* seeds

S No.	Phytoconstituents	Solvents			
		PET	ACE	MeOH	H_2O
1.	Alkaloids	-	-	-	-
2.	Flavonoids	-	+	+	+
3.	Phenols/Tannin	-	+	+	+
4.	Amino acids	-	+	+	+
5.	Saponins	-	-	+	+
6.	Glycosides	-	+	+	+
7.	Steroids	+	+	+	+

+ = Present, - = Absent

Table 1—The inhibition zones diameters of various extracts of *C. occidentalis* seeds against respiratory tract pathogens

Pathogens	*Diam. of the inhibition zone (mm)				Positive Control (Amoxicillin)
	PET	ACE	MeOH	H_2O	
S. aureus	19.6±0.15	21.2±0.36	23.1±0.15	20.2±0.20	34.7±0.23
S. pneumoniae	15.8±0.16	19.2±0.17	20.9±0.21	17.8±0.18	31.9±0.18
S. pyogenes	17.2±0.20	17.7±0.18	21.7±0.25	19.8±0.13	34.8±0.10

*Zone of inhibition in millimetre (mm) in triplicate expressed as means and standard error of means, cork borer diameter: 6 mm

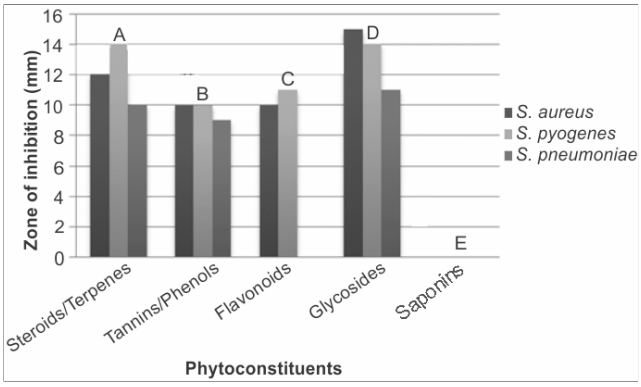

Fig. 1—Antibacterial activity of active constituents of MeOH extract of *Cassia occidentalis* at 50 mg/mL concentration. The bar diagram represents (A) Steroids/Terpenes (B) Tannins/Phenols (C) Flavonoids (D) Glycosides, and (E) Saponins. The maximum inhibition is noted at (D) 15 mm against *S. aureus*; (A) and (D) 14 mm against *S. pyogenes* and least inhibition at (B) 9 mm against *S. pneumoniae*; (E) No inhibition.

Conclusion

This study supports the traditional use of *C. occidentalis* and indicated that seeds of plant contain some major bioactive compounds inhibiting the growth of respiratory microorganisms thereby proving very effective source of derived drugs.

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