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AN ANTIMICROBIAL ACTIVITY OF ANTHRAQUINONES FROM *CASSIA OCCIDENTALIS*

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ABSTRACT

Antimicrobials derived from plants have been receiving increasing attention in recent years. Antimicrobial activities of a number of phytochemicals have been reported. Many antibiotics are ineffective against several pathogenic organisms. About 90% of *S. aureus, E. coli, P. aeruginosa, S. pyogenus, C. albicans, A. niger and A. clavatus* have resistance against many antibiotics. In the present study, the effect of R-spirit, benzene and water extracts of a medicinal plants *cassia occidentalis* has been tested against β-Lactum resistant strains of *S. aureus, S. pyogenus, C. albicans* and *E. coli, P. aeruginosa A. niger and A. clavatus* in presence of antibiotics such as gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin. The alcoholic extract has shown the maximum antimicrobial activity and the active ingredients are found to be 4, 5-dihydroxy-9, 10-dioxo-4a, 9, 9a, 10-tetrahydro-anthracene-2-carboxylic acid and 1, 3, 8-trihydroxy-6-methyl-anthraquinone which are characterized by NMR, FTIR and Mass spectroscopy. The Minimum Inhibitory Concentration (MIC) for antibacterial activity of the compound was found 6.25 µg/mL against the β-lactum resistant strains of *S. aureus* and MIC for antifungal activity was found 50 µg/mL.

Key words: *Cassia occidentalis*, Emodin, Antibacterial activity, Antifungal activity, Rhein, Staphylococcus aureus.

INTRODUCTION

Plants have been valuable source of natural product for maintaining human health[1]. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs[2]. About 80% of population of developed countries use traditional drugs derived from the plants[3]. Medicinal properties of such plants need to be explored for developing better and more efficient medicines with fewer side effects.

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The use of phytochemicals as natural antimicrobial agents is gaining popularity. A number of studies proving the antimicrobial properties of plants have been reported[4]. Many plant's secondary metabolites have found application as natural antimicrobial agents[5]. However, very little is known about the molecular level mechanism of their actions. The antimicrobial compounds reported from medicinal plants include proteins, peptides, phenolics and anthraquinone derivatives compounds[6]. Antimicrobial properties of tannis, glycosides and essential oils from several plants have been reported[7]. The essential oils and their components are known to be active against a wide variety of microorganisms, including both Gram-negative and Gram-positive bacteria[8]. Their high antimicrobial activity is assigned to different terpenoids and phenols[9]. The problem of antibiotic resistance has been reported from many parts of the world[10]. Bacteria have genetics ability to acquire and transmit the resistance[11].

Cassia occidentalis is used in traditional medicine in the tropical areas of the world. In Peru, the roots are used as a diuretic and the decoction is made to treat fevers. The seeds are brewed into a coffee-like beverage to treat asthma and the flower infusion is used for bronchitis in the Peruvian Amazon[12,13]. In Brazil the roots are considered to be a tonic, febrifuge diuretic and are used against fevers, tuberculosis, anaemia, liver complaints, as a reconstituent for general weakness and illness. In south eastern Nigeria the leaves are used for fever. Different parts of this plant have been reported to possess anti-inflammatory, antihepatotoxic, antibacterial and antiplasmodial activities[14,15]. A wide range of the chemical constituents have been isolated from *cassia occidentalis,* including sennoside, anthraquinone glycosides, fatty oils, flavanoid glycosides, gallactomannan, polysaccharides and tannins[16]. In the present study, extract with active ingredients from the *cassia occidentalis* (family: Leguminaceae) and tested against a strain of *S. aureus, E. coli, P. aeruginosa, S. pyogenus, C. albicans, A. niger and A. clavatus* in presence of antibiotics such as gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin.

EXPERIMENTAL

Materials and Methods

S. aureus, E. coli, P. aeruginosa and S. pyogenus, C. albicans, A. niger and *A. clavatus* strains were obtained from the culture collection of the Shree Dhanvantary Pharmacy College and Pharmaceuticals Analysis & Research Centre, Taluk-Kim, Dist. Surat, India. The cells were found to be resistance to gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin. They were sub-cultured repeatedly in tryptone soy broth medium containing different β-lactam antibiotics such as gentamycin,

ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin. The concentration of antibiotics was gradually increased up to 5 µg/mL of broth. The microbial cell concentration in the tryptone soy broth medium was adjusted to 10^8 CFU/mL by using 0.5 McFarland turbidity standards. Each microbial suspension was placed on Muller-Hinton agar (MHA) medium. The antibiotics resistance was tested also using standard antibiotics discs[13]. The cells were found to be resistance to antibiotics, such as gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin[17]. The media were purchased from Sisco Laboratories and the antibiotics discs were from Himedia Laboratories, India.

Extraction of active ingredients

The spices of *cassia occidentalis* were collected from Campus of the Bhavnagar University, Bhavnagar, India. An expert in the field of Botany authenticated the plant. The raw material were crushed and extracted with water, R-spirit and benzene by using a Soxlet apparatus. The solvents were evaporated to obtain the dry fractions and the yield was found to be 5.2, 9.7 and 2.7%, respectively. The dry fractions were made into a suspension using 10% dimethyl sulphoxide (DMSO) in distilled water. The concentration of the material was made 1 mg/mL. The antimicrobial activity of the extracts was studied by disc diffusion assay. The filter paper (Whatman No. 1) disc of 6 mm diameter were soaked in 20 µL of the extracts and dried in an incubator at 40^{o}C to remove the solvent. MHA plates containing gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin were prepared[18]. The concentration of antibiotics in the plate was 4 µg/mL. The plates were inoculated with the antimicrobial cells culture of concentration 10^8 CFU/mL as described previously. Paper discs soaked in 10% DMSO and dried in an incubator at 40^{o}C were used as the control. For each extract, six separate discs loaded with about 50 µg per disc were used and the average value of the diameters of inhibition zones was taken and the results of MIC were given in Table 1 and Table 2. The R-spirit extract showed the maximum resistance and was fractionated further to identify the antimicrobial compound. Silica gel chromatography was performed to separate different components of the extract and the mixture of dichloromethane and methanol of varying ratio was used to elute the fractions. Purity of the components were tested using Thin Layer Chromatography (TLC). Each fraction was tested for antimicrobial activity using disc diffusion method as described above and the compound in the fraction showing the maximum activity was characterized.

Determination of MIC

Minimum inhibitory concentration (MIC) of active ingredients was determined by

serial dilution method. Test-tubes containing tryptone soy broth medium and the active ingredients of varying concentrations from 125 µ/mL, 250 µ/mL and 500 µ/mL were prepared. 2000 µg/mL of the standard test antimicrobial broth culture were added to each of the test tubes and the tubes were incubated at 37°C for 24 h. A positive control tube containing only the growth medium and the organism was also set-up. The MIC was found to be 4 µg/mL[19].

Identification of active ingredients

NMR, FTIR and Mass spectroscopic techniques were carried out to characterize the active ingredients. NMR, analysis was carried out on a Bruker AMX 400 instrument. The compounds were identified based on comparison of the mass spectra recorded on Micro mass 70-70E mass spectrometer. The spectrum and the database showed that the compound were 4, 5-dihydroxy-9, 10-dioxo-4a, 9, 9a, 10-tetrahydro-anthracene-2-carboxylic acid and 1, 3, 8-trihydroxy-6-methyl-nthraquinone[20]. The dry samples were milled with potassium bromide and an FTIR was taken on a Thermo Nicolet series instrument model No. IR 200.

RESULTS AND DISCUSSION

Antimicrobial activities of different solvents extracts of the plants are given in the Table 1 and Table 2. The R-spirit and water extracts showed the maximum activity. Maximum inhibition zone was given by R-spirit, while benzene was found to be ineffective. The purified active ingredients showed larger inhibition zones in presence of gentamycin, ampicilin, ciprofloxacin, chloramphenicol, norfloxacin, nystatin and greseofulvin, which might be due to its synergistic effect with the antibiotics. In the absence of antibiotics, the average inhibition zone was only slightly larger than that of the extract. This might be due to presence of negligible quantities of other compounds than the active ingredients in the extract. The MIC for antibacterial activity of purified active ingredients was 6.25 µg/mL against *S. aureus*, which was considered very well, while MIC for antifungal activity was found 50 µg/mL against the strain of *C. Albicans.* The active ingredients were characterized using spectroscopic techniques such as NMR, FTIR and Mass spectroscopy. The spectrum and the database showed that the compound were 4, 5-dihydroxy-9, 10-dioxo-4a, 9, 9a, 10-tetrahydro-anthracene-2-carboxylic acid and 1, 3, 8-trihydroxy-6-methyl-anthraquinone. The study showed of the antimicrobial ingredients from the *cassia ocssidentalis* is against resistant of S. *aureus, E. coli, P. aeruginosa* and *S. pyogenus, C. albicans, A. niger* and *A. clavatus*. The study also showed that 4, 5-dihydroxy-9, 10-dioxo-4a, 9, 9a, 10-tetrahydro-

anthracene-2-carboxylic acid and 1, 3, 8-trihydroxy-6-methyl-anthraquinone were contributing to their antimicrobial activities.

Table 1: Antibacterial activity of various fractions of *Cassia Occidentalis* in rectified spirit solvent compared with standard drugs as prescribed in the standard protocol

S. No.	Plant fractions	Minimal Bactericidal Concentration (MBC)			
		E. coli	***P. aeruginosa***	***S. aureus***	***S. pyogenus***
		MTCC 443	**MTCC 1688**	**MTCC 96**	**MTCC 442**
		Concentration of extract in μg/mL			
1	Fraction-1	12.5	12.5	6.25	12.5
2	Fraction-2	50	100	50	25
3	Fraction-3	12.5	25	25	50

Table 2: Antifungal activity of various fractions of *Cassia Occidentalis* in rectified spirit solvent compared with standard drugs as prescribed in the standard protocol

S. No.	Plant Fractions	Minimal Fungicidal Concentration (MFC)		
		C. albicans	***A. niger***	***A. clavatus***
		MTCC 227	**MTCC 282**	**MTCC 1323**
		Concentration of extract in μg/mL		
1	Fraction-1	50	250	50
2	Fraction-2	125	500	100

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