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ANTIMICROBIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *CASSIA ANGUSTIFOLIA* VAHL- *IN VITRO* STUDY

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ABSTRACT

The aim of the study was to evaluate and compare the antimicrobial activity of crude leaf extracts of *Cassia angustifolia* Vahl. The leaves of *Cassia angustifolia* Vahl. were taken from three different regions of India such as SR-1 from South India, SR-2 from Rajasthan and SR-3 from Haryana. Antimicrobial suspectibility testing of ethanolic and aqueous leaf extracts of *Cassia angustifolia* Vahl. were performed against 3 bacterial strains (*E. Coli*, *Bacillus substilis*, *Staphylococcus aureus*) and against 2 fungal strains (*Aspergillus niger*, *Candida albicans*) by Agar well diffusion method on nutrient agar medium. The proposed comparative study revealed that the ethanolic extract prepared from the sample SR-2 taken from Rajasthan found to be most effective against all microbial strains. This is the first such comparative report on antimicrobial activity of leaf extracts of *Cassia angustifolia* Vahl. taken from three different regions of India.

INTRODUCTION: *Cassia angustifolia* Vahl. (syn *Cassia senna*) in commerce is known as Indian or Tinnevelly Senna, is a well known traditional medicinal plant belonging to family Leguminosae [1, 2]. It is a valuable plant drug in ayurvedic and modern system of medicine for the treatment of constipation [3, 4]. The plant available in market from different regions is likely to vary in quality and therapeutic activity due to difference in various geographical regions, difference in method of cultivation, collection, storage and seasons of plants collected [5]. The purpose of the present study is to compare the *in vitro* antimicrobial activity of aqueous and ethanolic leaf extracts of *Cassia angustifolia* Vahl. Leaves were obtained from three different regions of India, first sample from South India (SR-1), Second from Rajasthan (SR-2) and third from Haryana (SR-3). Since diffusion method is used to investigate the antimicrobial activity of natural substances and plant extracts and hence for such comparative study the antimicrobial activity has been evaluated by Agar well diffusion method [6, 7] also known as cylindrical cup plate method.

MATERIALS AND METHODS:

Plant material: The leaves of *Cassia angustifolia* Vahl. were collected from three different regions of India such as SR-1 from South India, SR-2 from Rajasthan and SR-3 from Haryana and the voucher specimens submitted at the pharmaceutical department of Guru Jambheshwar University, Hisar.

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Extraction of Plant Material: Air dried coarsely powdered leaves were used for the antimicrobial screening. The ethanolic and aqueous leaf extracts were prepared from each samples of *Cassia angustifolia* Vahl by extracting the 25g of each air dried coarsely powdered sample of leaves with 100 ml of 90% v/v ethanol and distilled water respectively by using the soxhlet apparatus. The extracts were filtered through Whatman filter paper 42. The filterates were evaporated to dryness on the water bath. The prepared extracts were kept in desiccator and stored properly in refrigerator until required.

Culures of Microorganisms used: The bacterial strains *Escherichia coli* NCIM 2065, *Staphylococcus aureus* NCIM 2901, *Bacillus substilis* NCIM 2106 used for the proposed antibacterial study were obtained from the National Chemical Laboratory (NCL), Pune, Maharastra, India and the fungal strains *Aspergillus niger* NCIM 590, *Candida albicans* MTCC 227 for the antifungal activity were procured from the Institute of Microbial Technology (IMTC), Chandigarh, India.

Cultures of Media used: For the antibacterial activity and antifungal activity Nutrient Agar medium and Sabouraud's dextrose agar medium [8, 9] respectively were used. The culture medias used for the study were obtained from the HiMedia Bombay, India. The specified amount of culture media was dissolved in specified amount of distilled water. The media was heated to boiling for about 1 minute and thereafter the media was sterilized by autoclaving at 15 lb/square inch pressure at 121ºC for 15-20 min.

Antimicrobial activity: Aseptically inoculated 0.2 ml each seeded broth containing 10^6-10^7 cfu/ml of test organism onto sterilized nutrient agar media containing in the sterile petriplates and spreaded uniformly with the help of glass spreader. Allowed to solidify. 4 cavities or wells or cups of 6 mm internal diameter were made by punching with sterile stainless steel cork borer. Then these hole were numbered as a, b, c and d. To the first three cavities of each petriplates test samples of 2000 µg/ml concentration were added while in the fourth cavity pure solvent DMSO was added which was taken as a normal control. Three replicates were used for each drug extract. Standard solution of 1000 µg/ml of ampicillin trihydrate for bacterial assay and clotrimazole for fungal assay were introduced in well taken as drug control. All the work was carried out under the aseptic conditions. The plates were allowed to stand for 1 h for diffusion of solution and then incubated in incubator at temperature 37^{o}C ± 1^{o}C for 24 hrs for bacteria, 37^{o}C ± 1^{o}C for 72 hrs for the fungus *Candida albicans* and at 25^{o}C± 1^{o}C for fungal strain *Aspergillus niger* for a period of 7 days**.** The zone of inhibition formed around the cups in the form of transparent area after incubation was measured in millimeters.

RESULTS AND DISCUSSIONS: Both the ethanolic and aqueous extracts of each sample showed significant antimicrobial activity in terms of zone of inhibition against all the tested microbial strains used for the proposed study (**Table 1**). The ethanolic extract prepared from sample SR-2 taken from Rajasthan found to be most effective against all the tested bacterial and fungal strains and showed zone of inhibition of 14 mm against all bacteria and 12 mm against all fungal strains. The concentration of 1000 µg/ml of standard drugs ampicillin trihydrate for the bacterial assay showed maximum zone of inhibition of 27 mm against *B. substilis* followed by the *S. aureus* with zone of inhibition of 12 mm while the standard drug clotrimazole used for assay of fungi showed zone of inhibition of 42 mm against the tested fungi.

TABLE 1: ZONE OF INHIBITION OF EXTRACTS OF *CASSIA ANGUSTIFOLIA* VAHL. SAMPLES

Sample Name	Conc. (µg/ml)	Zone of inhibition (mm)				
		E. coli	*B. substilis*	*S. aureus*	*C. albicans*	*A. niger*
SR-1 Ethanolic	2000	13	12	12	11	10.5
SR-2 Ethanolic	2000	14	14	14	12	12
SR-3 Ethanolic	2000	12	14	13	11	10
SR-1 Aqueous	2000	12	14	12.5	12.5	11
SR-2 Aqueous	2000	13	15	13	12.5	11
SR-3 Aqueous	2000	14	15	13	13	11
Ampicillin trihydrate/Clotrimazole	1000	21	27	24	42	42

SR-1 Senna Raw (S.I); SR-2 Senna Raw (Rajasthan); SR-3 Senna Raw (Haryana); *E.coli=Escherichia coli*, *B. sustilis= Bacillus substilus*, *S.aureus=Staphylococcus aureus*, *C. albicans= Candida albicans*, *A.niger=Aspergillus niger*

The variation observed in the activity of the extracts prepared from different samples of *Cassia angustifolia* may be due to one or more reasons such as difference in geographical region, difference in the amount of active chemical constituents, difference in method of collection, preparation, storage and difference in the seasons of plant collected. From this comparative study it is of utmost importance to ensure the quality, uniformity and consistency of crude drugs used for the further uses.

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