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EFFICACY OF LEAF EXTRACTS OF *CASSIA ANGUSTIFOLIA*(LINN.) FOR ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS

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ABSTRACT

The present investigation was undertaken to evaluate antimicrobial activities of different extracts (ethanol, methanol, petroleum ether and aqueous solutions) of *Cassia angustifolia* plant. Antimicrobial efficacy of various extracts of *C. angustifolia* was assessed by disc diffusion method against Gram positive bacteria- *Staphylococcus aureus* (MTCC *3160)*, Gram negative-*Escherichia coli* (MTCC 1652) and *Pseudomonas aeruginosa* (MTCC 647) and fungi- *Aspergillus niger* (MTCC282), *Aspergillus flavus* (MTCC 2456)*, Fusarium oxisporum*(MTCC349) and *Rhizopus stolonifer* (MTCC 2591). The ethanol extract exhibited highest zone of inhibition against *P. aeruginosa* (22.4±0.86mm) with low MIC value (14.8 mg/ml). Phytochemical screening of the extract showed the presence of alkaloids, flavonoids, carbohydrates, proteins, tannins and triterpenoids in *cassia angustifolia*.

KEYWORDS:*Cassia angustifolia*, antimicrobial activity, zone of inhibition, phytochemical analysis.

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INTRODUCTION

Medicinal plants are those plants which contain substances that can be used for the therapeutic purposes in one or more of its organ or substances which are precursors for the synthesis of useful drugs (Sofowora, 1982). These are a great source of medicines, especially in traditional system of medicines, which are useful in the treatment of various diseases (Bako et al., 2005). Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker et al., 1995). Antimicrobial activity has been reported in many plants by various workers viz., Sarin, 2005; Bansal et al., 2010; Chahal et al., 2010; Seth and Sarin, 2010 and Malwal and Sarin, 2011. *Cassia angustifolia* belongs to family Fabaceae and is commonly known as Indian Senna, Sonamukhi, Cassia Senna, Alexandrian Senna. *C. angustifolia* is used as a febrifuge, in splenic enlargements, anaemia, typhoid, cholera, biliousness, jaundice, gout, rheumatism, tumours, foul breath and bronchitis and probably in leprosy (Hayashi et al., 1980). Leaves are usefull in constipation, abdominal disorders, leprosy, skin disorders, leucoderma, splenomegaly, hepatomegaly, dyspepsia, cough, and bronchitis (Silva et al., 2008). The World Health Organization (WHO) approves senna leaf for short-term use in occasional constipation (WHO, 1999). Senna should not be used during pregnancy (Soyuncu et al., 2008). It has anti-inflammatory properties (Vanderperren et al., 2005), detoxification ability (Bournemouth, 1992) and also helps improve the function of the digestive system (Hoffmann, 1990). Cassia senna helps to reduce the nervous tension (Mills, 1993) and also helps in aiding the spleen and liver in production of blood and red blood cells (Spiller et al., 2003). The present study was undertaken to investigate the antimicrobial activity and phytochemical analysis of *C. angustifolia*.

MATERIALS AND METHODS

Plant material

Plants of *C. angustifolia* were collected from the campus of University of Rajasthan, Jaipur (RUBL21108). The leaves of *C. angustifolia* were separated, washed with running water to remove dust, shade dried and powered.

Preparation of extract

The powered leaves (500mg) of *C. angustifolia* were extracted with ethanol, methanol, petroleum ether and aqueous solution using soxhlet`s apparatus for 12-14 hours on a water bath separately. The organic extracts were separately filtered with Whatmann No. 1 filter paper and evaporated to dryness on water bath to obtain semi-solid mass. However, aqueous extraction is performed by using hot water maceration. The dried extract were stored at 5ºc in the refrigerator until used for further studies.

Antimicrobial Screening

Test microorganisms

Antimicrobial activity was evaluated against common pathogenic microorganisms, Gram positive bacteria- *Staphylococcus aureus* (MTCC *3160),* Gram negative- *Escherichia coli* (MTCC *1652), Pseudomonas aeruginosa* (MTCC *647)* and fungal strains *Fusarium oxisporum*(MTCC *349), Rhizopus stolonifer* (MTCC 2591), *Aspergillus niger* (MTCC *282) and Aspergillus flavus* (MTCC 2456). All the tested microorganism were obtained from Microbial Type Culture Collection and Gene Bank (MTCC), Chandigarh. The bacterial cultures were grown and maintained on Nutrient Broth medium at 37ºC for 24h while the fungal culture were maintained on Potato Dextrose Agar slants and incubated at 27ºC for 48h.

Antimicrobial activity

Antimicrobial assay of the crude extracts was performed agains seven tested pathogenic strains by disc diffusion method (Gould and Bowie, 1952). The nutrient agar plates and

potato dextrose agar plates were seeded with suspension (10^6 cfu/ml) of the bacterial and fungal strains vice-versa. The empty sterilized Whatmann No. 1 filter paper disc (6mm) were impregnated with 1 mg/ml of extracts dried and placed aseptically on seeded plates with the help of a sterile forceps. Finally, the sensitivity discs were pressed with forceps to make complete contact with the surface of the medium. Later on these plates were kept at room temperature for 30 minutes (pre diffusion time). The standard discs (6mm) impregnated with antibiotics gentamycin and nystatin (1.0mg/disc) were used as positive control. The plates were incubated at 37ºC for 24 hrs and 25ºC for 48 hrs for bacteria and fungi, respectively. The diameter of the inhibition zone (mm) was measured. The experiment was done in triplicate and the mean values (±SD) calculated for conclusion.

Determination of minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of various extracts against tested microorganisms was determined by broth dilution method (Basri and Fan, 2005). For broth dilution, 1 ml of standardized suspension of a strain (10^6 cfu/ml)was added to each tube containing extracts at various concentrations in nutrient broth medium. The tubes were incubated at 37ºC for 24h (for bacterial strains) and 25ºC for 48h (for fungal strains) and observed for visible growth after vortexing the tubes gently. The minimum inhibitory concentration (MIC) is taken as the lowest concentration of the extracts at which there is turbidity after incubation.

Qualitative Analysis of Phytochemicals

Specific qualitative tests were performed for the presence of phytochemicals viz., alkaloids, flavonoids, carbohydrates, proteins, tannins and triterpenoids in various plant parts of *C. angustifolia*.

RESULTS AND DISCUSSION

Plants have been utilized as medicines for thousands of years (Samuelsson and Bohlin, 2004). The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug resistant pathogens (Doss *et al*., 2009) so there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action (Parivuguna *et al*., 2008). Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanism of action. Contrary to the synthetic drugs, antimicrobial of plant origin not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (singh and kumar, 2011). In the present investigation, antimicrobial efficacy of the crude extract of *C. angustifolia* was quantitatively assessed on the basis of inhibition zone and minimum inhibitory concentration. The ethanol, methanol, petroleum ether and aqueous extracts of *C. angustifolia* exhibited varying degree of inhibitory effect against all tested pathogenic strains (Table 1 and 2). The most susceptible bacterium and fungi are *P. aeruginosa* and *Rhizopus stolonifer*. The inhibition zones (IZ) were in the range of 8.5±0.35 to 22.4±0.86 mm for most of the tested strains. Crude ethanolic extract of *C. angustifolia* leaves showed more pronounced antimicrobial activity than other extracts. The ethanol leaves extract exhibited highest zone of inhibition against *P. aeruginosa* and *R. stolonifer (*22.4±0.86mm and 20.1±0.62mm, respectively) with low MIC value (14.8 and 20.5 mg/ml, respectively). Presence of some important metabolites viz., alkaloids, flavonoids, carbohydrates, proteins, tannins and triterpenoids was also confirmed in various plant extracts by specific qualitative tests (Table 3).

Table 1
Antimicrobial activity of leaf of Cassia angustifolia (inhibition zone)

Microorganisms					
Bacteria		EtOH	MeOH	PE	Aqoeus
S. aureus	IZ	14.5 ±0.39	16.6 ±0.13	19.5±0.52	18.2 ±0.40
	AI	0.68	0.779	0.937	0.875
E. coli	IZ	16.3 ±0.89	15.1 ±0.19	13.2±0.31	14.2 ±0.42
	AI	0.721	0.668	0.68	0.731
P. aeruginosa	IZ	22.4 ±0.86	18.9± 0.11	22.2±0.59	20.7 ±0.52
	AI	1.032	0.87	0.932	0.869
Fungi					
A. niger	IZ	10.8±0.30	18.2±0.54	10.4 ± 0.56	9.8 ±0.42
	AI	0.843	0.875	0.684	0.644
A. flavus	IZ	10.3±0.45	17.1±0.52	14.5±0.25	15.2±0.91
	AI	0.83	0.909	1.169	0.808
F.oxisporum	IZ	15.0±0.43	17.3±0.35	10.4±0.21	8.5 ±0.35
	AI	0.842	0.831	0.584	0.664
R.stolonifer	IZ	20.1±0.62	13.5±0.41	10.3±0.63	12.6±0.34
	AI	0.975	0.733	0.5	0.684

Abbreviations: IZ= Inhibition zone (in mm) includes the diameter of disc (6 mm); Standards: gentamycin (1.0 mg/disc), nystatin (1.0 mg/disc); AI- activity index = IZ of test sample / IZ of standard. Values are mean of triplicate readings (mean ± S.D).

Table 2
Antimicrobial activity Cassia angustifolia(MIC)

Microorganisms						
		EtOAc	MeOH	PE	Aqoeous	Standard
Bacteria						
S. aureus	MIC	50	40.1	30.5	24.3	23.5
E. coli	MIC	45.9	51.2	44.9	50	33.2
P. aeruginosa	MIC	14.8	31	31.1	24.8	20.6
Fungi						
A. niger	MIC	53.6	49.6	51.6	33.7	9.3
A. flavus	MIC	35	1.1	27.1	37.2	10
F.oxisporum	MIC	33.8	59.6	34.9	30.5	30.7
R.stolonifer	MIC	20.5	68	44.7	49.6	19

Table 3
Phytochemical screening of various extract of C. angustifolia

Phytoconstituents	**Petroleum Ether extract**	**Benzene extract**	**Chloroform extract**	**Ethanol extract**	**Aqueous extract**
Carbohydrates	--	+	+	+	++
Proteins	--	--	--	+	++
Tannins	--	+	--	++	--
Flavonoids	--	--	+	++	+
Alkaloids	++	+	+++	+	--
Triterpenoids	+	--	--	+	--

CONCLUSION

The present investigation revealed that the various extract from leaves of *C. angustifolia* exhibited antimicrobial properties which explain the basis for its use in traditional medicines. However, petroleum ether extract exhibited significant inhibitory activity against tested pathogenic microorganisms.

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