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Antimicrobial activity of *Cassia* species

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Various plant parts of *Cassia italica* and C. siamea were successively extracted in petroleum ether, benzene, chloroform, alcohol and water. Similarly, the callus cultures of *C. italica* were also extracted in alcohol only. All these extracts and reference antibiotics (gentamycin/mycostatin) were screened against the select bacteria and fungi, and resultant inhibition zone (s) and activity index of crude drugs were measured.

BIOACTIVE principles are widely distributed among the higher plants. Though many possible sources of extractions and synthesis of antibiotics have been elaborately worked out but the search for a better, safer and an economic source is always necessitated. In this context, an attempt has been made towards the screening of various metabolite-rich fractions and bioactive compounds extracted from various plant parts from the selected Cassia species against a number of pathogenic bacteria and fungi. A glance to the literature indicate that many Cassia species have been investigated for their antibacterial[1-4] and antifungal[5-7] efficacy but there is no report on these activities of *C. italica* and *C. siamea*, except some work on antifungal efficacy in *C. siamea*[8].

EXPERIMENTAL

Plant materials

C. italica Mill. (Family : Leguminosae; sub-family: Caesalpinioideae) is a perennial and sub-tropical herb, while *C. siamea* Lamk., is an ornamental tree grown in gardens and along the avenues. The selected plant species were collected from the campus of Rajasthan University, Jaipur authenticated by the Herbarium, in the Department of Botany. The dried, powdered plant material of the various plant parts/calli of *C. italica* were used to evaluate the antibacterial and antifungal efficacy.

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Tissue culture

Seedling callus of *C. italica* established on Murashige and Skoog's (1962; MS) medium[9], supplemented with kinetin (Kn, 5 mg/l) and 2,4-dichlorophenoxy-acetic acid (2,4,-D, 2 mg/l) for over 30 weeks under continuous room light conditions (300 lux), 26±1° ; 55% relative humidity, maintained by frequent subculturings at 2 week interval upto 8 weeks and the growth was calculated (Growth Index = Final dry weight of tissue - Initial dry weight of tissue/Initial dry weight of tissue).

Extraction and isolation

50 g each of the dried and powdered test material was Soxhlet extracted successively with petroleum ether (60-80°), benzene, chloroform, alcohol and water for 24-36 h but, the callus was extracted with alcohol only. Various bioactive compounds were extracted and fractioned by using the established protocols for anthraquinones[10], sennosides[11], and flavonoids[12].

Microorganisms used

Bacteria: Both Gram +ve *(Staphylococcus aureus)* and

Table 1: Antibacterial and Antifungal Activities of Various Extracts and Bioactive Compounds of *Cassia italica*

Test Organisms		ETHANOLIC EXTRACTS Root	Stem	Leaves	Pods	Callus	SEQUENTIAL EXTRACTS (whole plant) PE	C_6H_6	$CHCl_3$	EtOH	Aq	METABOLITIES - RICH Fr. (whole plant) Anthr.	Flav.	Senn.
							A. BACTERIA							
E. coli	IZ+	12.00	±	8.00	9.00	8.00	10.00	7.00	9.00	8.00	±	29.00	±	14.00
	AI*	0.48		0.32	0.36	0.32	0.40	0.29	0.38	0.33		1.16		0.56
K. aerogens	IZ	-	-	-	-	-	±	±	8.00	9.00	±	15.00	-	10.00
	AI								0.32	0.36		0.63		0.42
P. vulgaris	IZ	8.00	8.00	9.00	-	-	9.00	±	7.00	8.00	-	14.00	-	9.00
	AI	0.31	0.31	0.34			0.34		0.27	0.31		0.58		0.38
S. aureus	IZ	8.80	9.00	-	-	10.00	-	8.00	-	7.00	-	26.00	±	15.00
	AI	0.33	0.37			0.42		0.32		0.28		1.08		0.63
							B. FUNGI							
A. flavus	IZ+	28.00	22.00	16.00	14.00	20.00	18.00	16.00	23.00	21.00	±	25.00		28.00
	AI	1.00	0.78	0.54	0.50	0.90	0.75	0.57	0.82	0.75		1.13		1.27
A niger	IZ	18.00	20.00	20.00	14.00	18.00	19.00	16.00	20.00	14.00	13.00	26.00		22.00
	AI	0.72	0.80	0.80	0.56	0.75	0.79	0.66	0.83	0.58	0.54	1.08		0.91
C. lunata	IZ	20.00	9.00	20.00	18.00	18.00	24.00	12.00	18.00	16.00	12.00	28.00		16.00
	AI	0.71	0.38	0.71	0.64	0.64	0.92	0.43	0.64	0.57	0.43	1.16		0.66
F. moniliforme	IZ	19.00	17.00	9.00	22.00	21.00	14.00	10.00	24.00	22.00	18.00	24.00		24.00
	AI	0.76	0.68	0.38	0.88	0.91	0.56	0.40	0.96	0.88	0.72	1.04		1.04

(+) Trace activity +IZ = Inhibition zone (in mm) including the diameter of disc (6 mm)

(-) Not measurable activity *Activity = $\frac{\text{Inhibition area of the test sample}}{\text{Inhibition area of the standard}}$

Abbreviation : PE = Petroleum ether; C_6H_6 = Benzene; $CHCl_3$ = Chloroform; EtOH Ethanol: Aq = Aqueous; Anthr. = Anthraquinones, Flav. = Flavonoids Senn. = Sennosides; Fr. = FRACTIONS.

Standards : Mycostatin = 100 Units/disc; Gentamycin - 10 ug/disc.

Gram-ve *(Escherichia coli, Proteus vulgaris, Klebsiella* aerogenes) obtained from SMS Medical College, Jaipur, were grown in Nutrient Broth medium and incubated at 37° for. 48 h followed by frequent subculturings to fresh medium were as the test bacteria.

Fungi: *Aspergillus niger; A. flavus, Fusarium moniliforme and Curvularia lunata*, obtained from the Laboratory of Microbiology and Mycology, Department of Botany, University of Rajasthan, Jaipur, grown on Potato Dextrose Agar medium and incubated at 27° for 78 h followed by periodic subculturings on to fresh medium were used as the test fungi.

Antimicrobial screening

Filter paper disc diffusion method[13] for assaying the activities of the test extracts (4 mg/disc) and reference

Table 2: Antibacterial and Antifungal Activities of Various Extracts and Bioactive Compounds of *Cassia siamea*

Test Organisms		ETHANOLIC EXTRACTS (whole plant)				SEQUENTIAL EXTRACTS (whole plant)					METABOLITES - RICH Fr (whole plant)		
		Stem	Leaves	Flowers	Pods	PE	C_6H_6	$CHCl_3$	EtOH	Aq	Anthr.	Flav.	Senn.
						A. BACTERIA							
E. coli	IZ+	9.00	9.00	8.00	9.00	9.00	8.00	±	7.00	-	27.00	-	9.00
	AI*	0.36	0.36	0.32	0.36	0.37	0.33		0.29		1.08		0.36
K. aerogens	IZ	-	-	-	-	±	7.00	8.00	9.00	-	17.00	11.00	12.00
	AI						0.28	0.32	0.36		0.71	0.46	0.50
P. vulgaris	IZ	7.00	8.00	7.00	7.00	-	7.00	9.00	8.00	-	19.00	-	10.00
	AI	0.27	0.31	0.27	0.27		0.27	0.34	0.31		0.79		0.42
S. aureus	IZ	8.00	7.00	9.00	8.00	7.00	±	9.00	7.00	-	28.00	9.00	8.00
	AI	0.33	0.29	0.37	0.33	0.28		0.36	0.28		1.16	0.37	0.33
						B. FUNGI							
A. flavus	IZ+	22.00	22.00	22.00	19.00	20.00	22.00	14.00	20.00	-	24.00	20.00	26.00
	AI*	0.78	0.78	0.78	0.64	0.71	0.78	0.54	0.71		1.09	0.71	1.18
A. niger	IZ	19.00	17.00	19.00	18.00	12.00	10.00	±	22.00	-	21.00	20.00	22.00
	AI	0.76	0.68	0.76	0.72	0.50	0.42		0.92		0.87	0.83	0.91
C. lunata	IZ	22.00	20.00	20.00	18.00	14.00	18.00	14.00	18.00		24.00	19.00	22.00
	AI	0.78	0.71	0.71	0.64	0.50	0.64	0.54	0.64		1.00	0.79	0.91
F. moniliforme	IZ	14.00	15.00	18.00	16.00	14.00	17.00	–	18.00		29.00	22.00	22.00
	AI	0.56	0.60	0.72	0.64	0.56	0.68	–	0.72		1.26	0.95	0.95

(+) Trace activity *IZ = Inhibition zone (in mm) including the diameter of disc (6 mm)

(-) Not measurable activity *Activity Index = $\frac{\text{Inhibition area of the test sample}}{\text{Inhibition area of the standard}}$

Abbreviation : PE = Petroleum ether; C_6H_6 = Benezene; $CHCl_3$ = Chloroform; EtOH - Ethanol; Aq = Aqeous; anthr. = Anthraquinones; Flav. = Flavonoids; Senn. = Sennosides; Fr. = FRACTIONS.

Standards : Mycostatin = 100 unit/disc; Gentamycin - 10 µg/disc.

drugs gentamycin (10 mg/disc) and mycostatin (100 units/disc) were used. The inhibition zone (s) and the activity index (AI = Inhibition zone (IZ) of the test sample/Inhibition zone of the standard drug) was calculated.

RESULTS AND DISCUSSION

The results of antimicrobial activities of various parts and bioactive compounds of selected plant species are down in Table 1 and 2 respectively.

While assessing the antibacterial activity, all the alcoholic and sequential extracts, though did not inhibit the growth of all the test bacteria but, did demonstrate significant activity against one or other. Nevertheless, the metabolite-rich fractions exhibited appreciable antibacterial activity widely and the anthraquinones were found to be the most active against all the select bacteria, with the maximum activity recorded against *E. coli* (IZ-29 mm).

Likewise, while studying the antifungal activity, the alcoholic and sequential extracts of the plant parts &/or calli inhibited the growth of almost all test fungi. However, the alcoholic extracts of *C. italica* (root : IZ-28 mm). exhibited greater antifungal activity > *C. siamea* (leaves and flowers: IZ-22 mm) against *A. flavus, F. moniliforme* and *C. lunata.*

Although, different bioactive compounds inhibited universally the growth of all the test fungi but, the anthraquinones of *C. siamea*, against F. moniliforme (IZ-29 mm) and sennosides of *C. italica*, against *A. flavus* (IZ-28 mm) exhibited maximum antifungal activity.

From the above findings thus, it is evidented that the exhibited pronounced activity against the microbes might yield important phytochemicals.

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