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Research Article

Bioactive Nature of Flavonoids from *Cassia siamea* and *Lantana camara*

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ABSTRACT
Plant parts (leaves and flowers) of Cassia *siamea* and *Lantana camara* were collected, shade dried and powdered. Methanolic extract, free flavonoid and bound flavonoid extracts were prepared and screened for their antibacterial activity. Two pathogenic bacteria selected for the study were *Escherichia coli* and *Staphylococcus aureus*. Well established filter paper disc method was used for screening the extract for antibacterial activity. Free flavonoid of *Cassia siamea* was the most effective inhibitor against *S. aureus* (AI = 2.67) and bound flavonoid of *Lantana camara* also showed good efficacy against *S. aureus* where inhibition zone was of 22mm diameter.

***Key Words*:** *Cassia siamea, Lantana camara*, Flavonoids, Antibacterial, Extracts, Pathogenic Bacteria.

INTRODUCTION
Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many based on their use in traditional medicine. Various medicinal plants have been used for years in daily life to treat disease all over the world. Millions of rural households use medicinal plants in a self-help mode. Over one and a half million practitioners of the Indian system of medicine in the oral and codified streams use medicinal plants in preventive, promotive and curative applications. In recent years, the growing demand for herbal product has led to a quantum jump in volume of plant materials traded within and across the countries. Though India has a rich biodiversity, the growing demand is putting a heavy strain on the existing resources. According to an all India ethnobiological survey carried out by the Ministry of Environment & Forests, Government of India, there are over 8000 species of plants being used by the people of India

Plants remained, however, great sources of therapeutic agents until the beginning of the 20th century. With development of chemistry in the last century, plants have been looked upon as sources of new therapeutic agents (Kaushik 1989, Farombi 2003, Uniyal *et al.*, 2006). This investigation still continues and newer drugs of plants origin are being discarded every year. A large number of plants have been screened in last three decades for their chemical constituents as well as for pharmacologically active principles (Fabry *et al.*, 1998, Mahesh and Satish 2008). Flavonoids are used for their therapeutic properties several plants have been screened for their various antibacterial, antifungal, antiviral, and properties (Shahidi et al 2004).Anti microbial activity of flavonoids (Barnabas and Nagarajan, 1998) has also been worked out from different plant species *in vitro*. Agrawal (1980) screened flavonoids from in vitro tissue cultures of *Argemone mexicana* and for antimicrobial activity against some pathogenic bacteria. Similarly Noureddine *et al.*, (2005) also reported antimicrobial activity of phenolic compounds of various onions (*Allium cepa*) and garlic (*Allium sativum*) extracts.

MATERIALS AND METHODS
The present study deals with the extraction and screening of different extracts from aerial plant parts (leaves, flowers) of two medicinal plants namely *Lantana camara* and *Cassia siamea*. *Escherichia coli* and *Staphylococcus aureus* test bacteria were procured from Sawai Man Singh Medical College, Jaipur and were maintained on nutrient agar medium consisting of Agar- 15 gm, Beef extract- 3 gm, Sodium chloride- 5 gm, Peptone- 5 gm in 1 liter distilled water. A fresh suspension of test micro-organisms in saline solution was prepared from a freshly grown agar slant (fungal culture) before every antibacterial assay.

Extraction Procedure
Plants collected were washed in running tap water to remove dust. Aerial parts of the plants were shade dried and powdered separately for extraction. Each of the dried and powdered samples were soxhlet extracted with water and 80 percent methanol for 24 hours on water bath. For the extraction of free and bound flavonoids, the filtrate of 80 percent methanol was subsequently extracted in separating funnel with petroleum ether, ether and ethyl acetate. Petroleum ether fraction was discarded

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Table 1. Antibacterial Activity of *Cassia siamea* and *Lantana camara* plant extracts*

Test Material		Test bacteria			
		Escherichia coli		*Staphylococcus aureus*	
		I.Z. (mm)	A.I. (mm)	I.Z. (mm)	A.I. (mm)
A. 80% Methanolic extracts					
Lantana camara	Leaves	7	0.28	35	2.33
	Flower	8	0.32	8	0.53
Cassia siamea	Leaves	–ve	–ve	–ve	1
	Flower	8	0.32	9	0.6
B. Free flavonoids					
Lantana camara	Leaves	20	0.8	–ve	–ve
	Flower	7	0.28	9	0.6
Cassia siamea	Leaves	8	0.32	- ve	- ve
	Flower	9	0.36	40	2.67
C. Bound flavonoids					
Lantana camara	Leaves	22	0.88	22	1.46
	Flower	9	0.36	8	0.53
Cassia siamea	Leaves	10	0.4	- ve	- ve
	Flower	8	0.32	12	0.8

Abbrev. I.Z. - Inhibition Zone, A.I.-Activity Index

**Note-Inhibition Zone of standard (Streptomycin) against E. coli is 25mm. Inhibition Zone of standard (Streptomycin) against S .aureus is NIL. Inhibition Zone of standard Gentamycin against S. aureus is 15mm. Diameter of filter paper disc (6mm) included in inhibition Zone.*

due to its being rich in fatty substance. Ether fraction was used for free flavonoids and ethyl acetate fraction for bound flavonoids. Ethyl acetate fraction of each of the samples was hydrolysed further with 7 percent H_2SO_4 for two hours and was re-extracted with ethyl acetate. The fraction was washed with distilled water to neutrality and dried (Bhadauria 2001).

Antibacterial screening

The filter paper disc method (Gould and Bowie, 1952) was used for screening the extracts for antimicrobial activity. Standard size whatman filter paper discs (6mm in diameter) were saturated with the extract (0.04ml) and air dried at room temperature to remove any residual solvent that might interfere with the determination. The discs were then placed on the surface of sterilized nutrient agar medium that had been inoculated with the test bacteria (saline solution) and air dried to remove the surface moisture. The thickness of the agar medium was kept equal in all the petri-plates and the standard discs of Streptomycin and Gentamycin were used in each plate as control. Before incubation, the petri-plates were placed for one hour in a cold room to allow the diffusion of the compounds. They were incubated at 37^o C for 20-24 hours after which the zone of inhibition or depressed growth was measured. Each experiment was done in five replicates and the activity index was calculated for all of them.

RESULTS AND DISCUSSION

Different (Methanolic, Free flavonoids and Bound flavonoids) extracts screened showed growth inhibitory activity against one or both of the test bacteria (Table 1). The inhibition zone of extracts was compared with the inhibition zone produced by the standards (Streptomycin and Gentamycin).

In case of *Cassia siamea* best results were shown by the extracts of free flavonoids where the Activity index (A.I.) was found to be the maximum (2.67) against *Staphylococcus* aureus. Methanolic extracts and free flavonoids of *Cassia siamea* showed good results against *Staphylococcus aureus*, where activity indices were 1 and 0.60, respectively. Free flavonoids showed good efficacy against the both test bacteria. The activity indices of 2.6 and 0.36 were shown against *S. aureus* and *E. coli*, where inhibition zone were 40 and 9 mm, respectivey. Bound Flavonoids showed good response against both bacteria tested. Inhibition zones were 10 and 12 mm for *E. coli, S. aureus* and. Activity indices (0.4 and 0.8) respectively further proved good activity of bound flavonoids as compared to *Streptomycin* against *E. coli.*

In case of *Lantana camara* 80% Methanolic leaf extract of was effective against *E. coli* (AI=0.32) and *S. aureus* (AI=2.33) where inhibition zones were 8 and 35, respectively. Free flavonoids of *L. camara* showed good efficacy against both test bacteria. Bound flavonoids were the most effective inhibitors for both pathogenic test bacteria. The best response (AI=1.46) was shown against *S. aureus* where the inhibition zone was 22 mm. Best results were shown against *S.aureus* the inhibition zone and activity index were 35 mm and 2.33 respectively.

Earlier workers, Mishra et al (1983), Kambizi and Afolayan (2008), Negi and Pahwa (2010) observed the antibacterial activity of various medicinal plants from different countries and explained the traditional therapeutic uses of medicinal plants. Sharma *et al.,* (1998) screened *Cassia italica* for antibacterial activity against *Staphylococcus aureus* and *Escherichia coli.*Work on Flavonoids has been done by previous workers for antimicrobial activity. Harborne and Mabry (1982), Ahmad and Mohammad (1998) tested some flavonoids isolated from leaves and flowers of *Citrullus colocynthis* and *Lyceum barbarum* for their anti microbial activity against bacteria and fungi. In the present study, good antibacterial activity was shown by the extracts of *Cassia siamea* and *Lantana camara*, where the extracts of bound flavonoids were found to be the best inhibitors for the growth o*f Staphylococcus aureus* as compared to standards used. From the present findings, previous reports that *Lantana camara* has strong antibacterial property are further supported. A review of literature indicates that various investigators have studied antibacterial agents in the recent past, yet the work is very fragmentary. The present study is an extension of the similar type of work done till now.

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