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ANTIBACTERIAL ACTIVITY OF SOME EXTRACTS OF *LAWSONIA ALBA*, *SOLANUM DULCAMARA* AND *ALLIUM SATIVUM* AGAINST FOUR PATHOGENIC BACTERIA

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Aerial plant parts of *Lawsonia alba, Solanum dulcamara* and *Allium sativum* were collected, shade dried and powdered. Pathogenic bacteria *Escherichia coli, Enterobacter aerogens, Proteus mirabilis* and *Staphylococcus aureus* were procured from Sawai Man Singh Medical College, Jaipur and were maintained on Nutrient agar medium. Well-established filter paper disc method was used for screening the extracts for antibacterial activity. Bound flavonoids were the most effective inhibitors for all pathogenic test bacteria. Best results were shown by the extracts of *Lawsonia alba,* especially the extract of bound flavonoids where the activity index (A.I.) was found to be the maximum (3.3) against *Staphylococcus aureus* in which the inhibition zone was 50 mm, much higher than Gentamycin (IZ=15mm).

Keywords : Activity index; Antibacterial; Bound flavonoids; Inhibition zone; Screening.

With the increased interest in antibiotics globally, more research on plants which can serve as a potential source of antimicrobial substances has been promoted. Numerous surveys[1-4] of plants throughout the world were carried out which have demonstrated the wide occurrence of active compounds in higher plants. In India various investigators have studied antibacterial agents in recent past yet the work is very fragmentary[6-10]. The present investigation was carried out in laboratory is an extension to study the antibacterial activity of different plant extracts against four human pathogenic bacteria.

For the present investigation plant material of *Lawsonia alba, Solanum dulcamara,* and *Allium sativum* were washed, shade dried and powdered for the purpose of extraction. *E. coli, E. aerogens, P. mirabilis* and *S. aureus* test bacteria were procured from Sawai Man Singh Medical College, Jaipur and were maintained on Nutrient agar medium (agar 15gm, beef extract 3gm, sodium chloride 5gm, and peptone 5gm, in one liter distilled water). A fresh suspension of test micro-organisms in saline solution was prepared from a freshly grown agar slant before every antibacterial assay.

Extraction procedure : Plant collected were washed in running tap water to remove dust. Aerial parts of plants were shade dried and powdered separately for extraction. Each of the dried and powdered samples was 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 due to its being rich in fatty substance. Ether fraction was used for free flavonoids and ethyl acetate fraction was used 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.

Antibacterial Screening : The filter paper disc method[11] was used for screening the extracts for antimicrobial activity. Standard size Whatman filter paper dics (6.0 mm diameter) were sterilised in an oven at 140^{0}C for one hour, saturated with the extract (0.04 ml) 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 sterilised Nutrient agar medium that had been inoculated with the test bacteria (using saline solution) and air dried to remove the surface moisture. The thickness of the agar medum was kept equal in all the petriplates and the standard disc (Streptomycin and Gentamycin) was used in each plate as a control. Before incubation, the petriplates were placed for one hour in a cold room (5^{0}C) to allow the diffusion of the compounds from the disc in to the medium. Plates were incubated at 37^{0}C for 20-24 hours after which the zone of inhibition or depressed growth could be easily measured. All the experiments were done in five

Table 1. Antibacterial activity of some plant extracts.

Test material	Test bacteria							
	E.coli		*P. micrabilis*		*E. aerogens*		*S. aureus*	
A. Water extract	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.	I.Z. (mm)	A.I.
1. *Lawsonia alba*	12	0.48	12	0.48	-ve	-	15	1.0
2. *Allium sativum*	-ve	-	10	0.4	-ve	-	-ve	-
3. *Solanum dulcamara*	-ve	-	-ve	-	-ve	-	7	0.46
B. 80% Methanolic extract								
1. *Lawsonia alba*	8	0.32	10	0.4	10	0.5	10	0.66
2. *Allium sativum*	11	0.44	10	0.4	-ve	-	12	0.8
3. *Solanum dulcamara*	-ve	-	8	0.32	-ve	-	10	0.66
C. Free flavonoids								
1. *Lawsonia alba*	12	0.48	13	0.52	15	0.75	12	0.8
2. *Allium sativum*	11	0.44	10	0.4	10	0.5	12	0.8
3. *Solanum dulcamara*	-ve	-	10	0.4	-ve	-	13	0.86
D. Bound flavonoids								
1. *Lawsonia alba*	25	1.0	28	1.12	20	1.0	50	3.3
2. *Allium sativum*	25	1.0	40	1.6	20	1.0	25	1.6
3. *Solanum dulcamara*	24	0.96	28	1.12	15	0.75	38	2.53

I.Z. = Inhibition Zone (in mm); A. I. = Activity Index

$$\text{A.I.} = \frac{\text{Inhibition zone of the sample}}{\text{Inhibition zone of the standard}}$$

Inhibition zone of standard (Streptomycin) against *E. coli* = 25mm
Inhibition zone of standard (Streptomycin) against *P. mirabilis* = 25mm
Inhibition zone of standard (Streptomycin) against *E. aerogens* = 20mm
Inhibition zone of standard (Streptomycin) against *S. aureus* = Nil
Gentamycin against *S. aureus* = 15mm
Diameter of filter paper disc (6mm) included in Inhibition Zone.

replicates and the activity index was calculated for each of them.

$$\text{Activity Index (A.I.)} = \frac{\text{Inhibition Zone of the Sample}}{\text{Inhibition Zone of the Standard}}$$

Different (water, methanolic and flavonoid) extracts screened showed growth inhibitory activity against one or more of the test bacteria (Table 1). The inhibition zones of the extracts were compared with the inhibition zone produced by the standards (Streptomycin and Gentamycin).

Best results were shown by the extracts of *L. alba*, especially the extract of bound flavonoids where the inhibition zone was found to be maximum (IZ = 50mm AI = 3.3) against *S. aureus* which was much higher than Gentamycin (IZ = 15mm). Inhibition zones of 25, 28, and 20mm were measured for *E. coli*, *P. mirabilis* and *E. aerogens*, respectively. Water and methanolic extracts of *L. alba* showed positive response against all the test bacterial except water extract which showed negative response against *E. aerogens*. Free flavonoids showed good results against all four bacterial.

Bound flavonoids of *A. sativum* were the most effective inhibitor for all pathogenic test bacteria. Inhibition zones of 40, 25, 20 and 25 mm were observed against *P. mirabilis, E. coli, E. aerogens* and *S. aureus*, respectively and activity indices were 1.6, 1, 1 and 1.6, respectively. Bound flavonoids of *S. dulcamara* showed best results (IZ = 38mm) against *S. aureus* where activity index was 2.53. Inhibition zone was more than two times larger than that of Gentamycin.

Work on flavonoids has been done by previous workers[12-16] for antimicrobial activity. Datta *et al.*[17] reported that petroleum ether extract of ripe fruits of *L. alba* was active against the three pathogenic bacteria (*S. aureus, E. coli* and *S. pyrogens*). From the present findings, previous reports that *L. alba, S. dulcamara* and *A. sativum* have strong antibacterial property, are further strengthened.

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