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IN VITRO TESTING OF THE LEAF EXTRACTS OF *LAWSONIA ALBA* FOR ANTIMICROBIAL PROPERTIES

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ABSTRACT

Leaf extracts (chloroform, ethanol and aqueous extracts) of *Lawsonia alba* were tested in *vitro* for their antimicrobial activity against seventeen strains of pathogenic bacteria and twelve fungi including seven pathogenic strains. Each of the extract showed good activity against most of the strains tested. The sensitivity of the bacteria and fungi to the extracts was compared to that of ampicillin, tetracycline, co-trimoxazole and griseofulvin sensidiscs.

Introduction

The plant *Lawsonia alba*, commonly known as Mehdi, belongs to the family of Lythraceae. It is a shrub and distributed throughout Bangladesh, Pakistan and India. This plant is known to have important medicinal properties, especially the leaves are used in scabies, leprosy, syphilis, gonorrhoea etc. (Kirtikar and Basu 1984, Chopra *et al.*, 1982). Seed oil of *L. alba* has been reported to have analgesic activity (Bagi *et* at, 1988). Some workers reported its antimicrobial activity (AM-el-Malek *et al,* 1973, Tripathi *et al,* 1978, Datta *et al,* 1989). In this paper antimicrobial property of the leaf extracts of *Lawsonia alba* against a wide range of bacterial and fungal strains have been discussed.

Materials and Methods

Extraction : Twenty five grams green leaves of *Lawsonia alba* was blended separately with chloroform, ethanol and distilled water in a waring blender and kept with occasional shaking for 24 hours. The extracts were then filtered and concentrated at low pressure at 45°C to yield 2.75 ml extract for each. The extracts thus obtained were passed through Milipore filter.

Microrganisms : Identified clinical strains of *Aeromonas hydrophilia*, *Shigella* and *Vibrio cholerae* were collected from the International Centre for Diarrhoea] Disease Research, Bangladesh (ICDDR'B), Dhaka and rest of the strains were obtained from the

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Sensitivity Test : The sensitivity of the microrganisms to the extracts was tested *in vitro* by disc diffusion method (Bauer *et al*., 1966). The Mueller-Hinton agar (DIFCO) plates were coated with the respective bacterial cultures (10^6 cells/ml Mueller-Hinton broth) using sterile cotton swabs. Twenty ml of sterile Sabouraud media (DIFCO) in each of twelve test tubes (at 40°C) were inoculated with freshly cultured fungal strains and transferred to sterile petridishes and then allowed to solidify. The extract impregnated discs and antibiotic sensidiscs were placed on the inoculated plates. The bacterial plates were incubated at 37°C for 24 hours and fungal plates were incubated at 25°C for 48 hours.

Results and Discussion

Seventeen strains of pathogenic bacteria and twelve fungi were investigated *in vit*ro for their sensitivity to the chloroform, ethanol and aqueous extracts of *Lawsonia albs* leaves. Most of the strains tested were found to have promising response to each of the extracts.

Inhibitory activity of the extracts to the bacteria was compared to that of standard ampicillin, tetracycline and co-trimoxazole (Table 1). Ethanol extract was found to have considerable antibacterial spectrum. This extract gave moderate inhibitory zones (25-12 mm) against fifteen out of seventeen strains of bacteria. Chloroform extract showed mild inhibition (23-10 mm) against fourteen strains and the aqueous extract was active on thirteen bacteria giving 18-10 mm inhibitory zones. No activity was recorded against *Klebsella* and *Proteus* species (Table 1). It is interesting to note that six strains were resistant to ampicillin, eight strains to tetracycline and nine strains to co-trimoxazole. *Shigella dysenteriae* type 1 (T-1683) was resistant to all of the three antibiotics while each of the extracts was active against this bacteria.

Chloroform and ethanol extract showed promising inhibitory activity against nine out of twelve fungal strains tested (Table 2), whereas aqueous extract was active against only five strains of fungi. Three strains of fungi were resistant to all extracts and the antibiotic tested. Chloroform extract showed prominent inhibitory zones (31-11 mm), followed by ethanol (30-11 mm) and aqueous extract (23-9 mm). Antifungal antibiotic griseofulvin used as a reference standard in this experiment was active against only one strain of fungus.

Recently Datta *et al*., 1989 reported that seed oil of *L. alba* has inhibitory activity against only four strains (*Staphylococcus aureus, Streptococcus pyogens, E. coli* and *Pseudomnas aeruginosa*) out of ten bacteria including *Shigella, Salmonella, V. cholerae* and *S. lutea.* In this study, the leaf extracts (chloroform, ethanol and aqueous) of this

plant showed significant inhibitory action against fifteen virulent strains, including all of the ten bacteria tested by Datta *et al*., 1989. Moreover it has been observed that the leaf extracts have prominent activity against the bacteria causing diarrhoea and dysentery such as *Salmonella, Shigella* and *Vcholerae.* This finding add support to the claim of Ayurved and Unani practitioners that leaf extract of *L.alba* is very much effective in curing diarrhoea and dysentery. It is evident from the present study that leaves of *Lawsonia alba* would he a good natural antibiotic for the treatment of certain bacterial and fungal strains.

Table 1

Antibacterial activity of the leaf extract of *Lawsonia alba.*
The values indicate zone of inhibition in mm.

Bacteria	Strain No.	C-ex	E-ex	A-ex	AM	CT	TC
Bacillus subtilis	QL40	20	23	18	-	-	12
Sarcina lutea	QL166	19	12	12	30	29	25
Staphylococcus aureus	QL102	12	14	9	12	-	-
Streptococcus faecalis	ATCC	18	24	12	15	-	17
Streptococcus B-hemolyticus		-	15	17	13	-	11
Aeromonas hydrophilia	5358	13	12	11	-	17	18
Aeromonas hydrophilia	5328	15	12	10	-	16	15
Esherichia coli (ETEC)	3.2	16	18	13	12	-	14
Klebsiella sp.	BTCC13	-	-	-	-	20	-
Proteus vulgaris	QL144	-	-	-	18	-	-
Pseudomonas aeruginosa	QL147	10	13	-	13	10	-
Salmonella typhi		18	20	11	12	18	-
Shigella dysenteriae Type 1	T-1683	14	23	16	-	-	-
Shigella flexneri	2361	20	17	12	-	-	-
Shigella sonnei	9989	12	9	-	12	15	-
Shigella bodyii	4152	15	16	11	9	12	15
Vibrio cholerae	Ogawa CC^{+}	23	25	16	-	-	18

C-ex = Chloroform extract (20 μl/disc)
E-ex = Ethanol extract (20 μl/disc)
A-ex = Aqueous extract (20 μl/disc)
- = No inhibition

AM = Ampicillin (10 μg/disc)
CT = Co-trimoxazole (25 μg/disc)
TC = Tetracycline (30 μg/disc)

* Each μl of the extract contained approximately 100 μg.

Table 2

Antifungal activity of the leaf extracts of *Lawsonia alba*
The values indicate zone of inhibition in mm.

Fungi	Strain No.	C-ex	E-ex	A-ex	GF
Aspergillus fumigatus	BTCC488	17	14	11	-
Aspergillus flavus	EDCL	25	28	17	-
Aspergillus niger	QL107	-	-	-	-
Candida albicans	EDCL	16	25	23	-
Candida krussi	BTCC345	-	-	-	-
Curvullaria Sp.	BTCC486	20	14	-	nd
Rhizopus arrizae	UICC138	14	15	-	-
Rhizopus oriaza	UICC110	12	13	-	-
Rhizopus oligosporum	UICC116	11	14	-	-
Saccharomyces cereviceae	BTCC335	11	11	9	6
Saccharomyces carlbergensis	ATCC8090	-	-	-	-
Tricoderma sp.	BTCC487	31	30	20	-

C-ex = Chloroform extract (20 μ l/disc) GF = Griseofulvin (25 μg/disc)
E-ex = Ethanol extract (20 μl/disc) - = No inhibition
A-ex = Aqueous extract (20 μ l/disc) nd = Not done

* Each μl of the extract contained approximately 100 μg.

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