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I*n vitro* antimicrobial activity of *Lawsonia alba* (Dan-gyi)

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Water soluble crude extract of *Lawsonia alba* (Dan-gyi) was tested against two isolates of *Entamoeba histolytica* and fourteen strains of pathogenic bacteria. The growth patterns of amoebae in cultures were determined at time intervals of 12 and 24 hours respectively after exposure to the diluted extract. The *in vitro* bactericidal and bacteriostatic activities of *L. alba* extract were also tested by agar disc diffusion technique and by serial tube dilution technique. The Minimum Amoebicidal Concentration (MAC) of the crude extract of *L. alba* was found to be 100µg/ml. The extract of *L. alba* was found to possess antibacterial activity on some pathogenic bacteria. Out of fourteen strains of bacteria tested, six strains were inhibited at the concentration of 0.5 mg/ml and two strains were inhibited at 1mg/ml concentrations.

INTRODUCTION

Lawsonia alba (Syn: *Lawsonia inermis*; common name Henna, Mehndi, Mendika; Myanmar name: Dan-gyi) is a perennial shrub which grows widely in Myanmar. Henna is the Persian name and is native to Asia and Mediterranean coast of Africa and now thrives in warmer climates all over the world. The history and origin of Dan-gyi (Henna) are hard to track, with centuries of migration and cultural interaction, it is difficult to determine where particular traditions began. There are some historic evidences that Dan-gyi originated in ancient India. Others believe that Dan-gyi was introduced to India in the 12th century. This plant has been used for at least 5,000 years as a cosmetic and for its natural healing properties. There is also a documentation that Henna was used in ancient Egypt to stain fingers and toes of the Pharaohs prior to mummification. Dan-gyi is also used as a medicinal plant because of its attributed antibacterial, antifungal, astringent, anti-hemorrhagic, hypotensive and sedative effects. It has also been used as a folk remedy against headache, jaundice and leprosy in many world cultures [1, 2].

In this present study, a water soluble extract was prepared from mature leaves of Dan-gyi and its *in vitro* activities on two isolates of *Entamoeba histolytica* and fourteen strains of bacteria were tested.

MATERIALS AND METHODS

Extraction of leaves

Air dried leaves (370 gm) were refluxed for two hours in two liters of petroleum ether and filtered. The leaves residue were dried in air and extracted with two liters of 50% ethanol and filtered. Fifty percent alcoholic solution was concentrated under reduced pressure at low temperature. This procedure was carried out in Soxlet apparatus after removal of chlorophyll and waxy substances according to the method of Hanke and Talaat (1961) [3]. Fractionations were made to remove pectate after treatment with calcium hydroxide. The total extract weight obtained was approximately 40 gm. The

alcoholic substance was again eluted with two liters of distilled water and concentrated under reduced pressure. Weight obtained after concentration was approximately 30 g. This water soluble extract was used throughout the experiment. The extract was initially dissolved in sterile double distilled water and the pH was adjusted between 6.8 to 7.2. The desired concentrations were obtained by dilution.

In vitro amoebicidal activity

Stool samples containing active trophozoites of *Entamoeba histolytica* were collected from dysenteric patients attending Infectious Diseases Hospital, North Okkalapa, Yangon. Out of five samples collected, only two samples showed satisfactory growth and they were isolated to be free from other symbionts such as *Blastocystis hominis.* These two samples were designated as DMR/Eh 005 and DMR/Eh 006 and cultured continuously in two biphasic media [4,5]. Amoebicidal activity of the extract of *L. alba* was assayed by the method of Brackett and Blenick, 1947 [6] with minor modifications by Myint Oo and co-workers, 1972 [7]. Emetine hydrochloride (Biochemical Research, USA) and metronidazole (Myanmar Prarmaceutical Factory, Yangon) were used as control drugs. Before each experiment, the test and control cultures were ascertained to contain approximately equal numbers of trophozoites. Ten replicate countings of amoeba were made at 12 and 24 hours intervals according to the method described by Myint Oo 1989 [8].

Screening of in vitro antibacterial activity

Screening of antibacterial activity was done by the use of impregnated filter-paper discs. The discs, 8 mm in diameter, and punched from filter papers of Whatman Grade 17 was sterilized by autoclaving followed by drying in an oven. These were then impregnated with diluted extracts and fractions of *L. alba* extract (A & B) and then allowed to dry at room temperature under sterile condition. Test organisms used were *Escherichia coli* (O127/B2, WR2/69), *Klebsiella pneumoniae* (DMR), *Proteus mirabilis* (IM1), *P. vulgaris* (IM1), *Pseudomonas fluorescens* (WR2/69), *P. aeruginosa* (W12/69), *Salmonella typhi* (ATCC 992V), *Shigella boydii* (NHL), *S. dysenteriae* (NHL), *Shigella shigae* (NHL), *Staphylococcus aureus* (WS/69), *S. epi-dermidis* (WR1/69) and *Vibrio cholerae* ElTor (IM1). A few colonies of the pure cultures were inoculated into 2–4 ml of Trypticase soy broth. These tubes were incubated for 2-4 hours in a waterbath at 37˚C to produce a bacterial suspension with barely visible to moderate cloudiness. This contained approximately 10^5 to 10^7 organisms per milliliter of suspension. The organisms were then streaked onto the surface of Oxoid Sensitivity test agar with a swab. After the inoculum had dried, the dried discs were placed on the medium. A control disc (no drug), impregnated with solvent only and known standard control disc of tetracycline and ampicillin were also placed onto the medium,. After overnight incubation at 37˚C, the zones of inhibition were measured. The bactericidal or bacteriostatic action and the minimum inhibitory concentration were also determined by serial tube dilution method.. Comparison of three media namely Oxoid Sensitivity Test agar, Trypticase agar and Nutrient agar were also determined.

RESULTS

Table 1 shows the *in vitro* amoebicidal concentrations of emetine, metronidazole and water soluble extracts of *L. alba.* Two strains of *E. histolytica* grown in two types of media were used for comparative study. The minimum amoebicidal concentrations were not significantly different between variation of strains and culture medium in this study. The mean growth rates of two strains of *E. histolytica* after exposure to extracts of *L. alba* (100µg/ml), metronidazole (20µg/ml) and emetine hydrochloride (20µg/ml) are shown. Each drug was added after 24 hours of cultivation in two types of

media. A significant inhibition of water soluble extract of *L. alba* was observed at the concentration of 100µg/ml.

Table 2 shows the mean diameters of zone of inhibition by *L. alba* extract. Only *Pseudomonas fluorescens* was not inhibited by the extract of *L. alba.*

Table 1. Minimum amoebicidal concentrations of *L. alba* extract and control drugs on two isolates of *Entamoeba histolytica* grown in two different media *in vitro*

Isolates of *E. histolytica*	Types of Medium	Minimum Amoebicidal Concentration (µg/ml)		
		Emetine HCl	Metronidazole	*L. alba* extract
DMR/ Eh 005	Dobell & Laidlaw (1926)	25	50	150
	Rao (1951)	25	25	100
DMR/ Eh 006	Dobell & Laidlaw (1926)	25	50	150
	Rao (1951)	25	50	150

Table 3 represents the Minimum Inhibitory Concentrations (MICs) of *L. alba* extract on some pathogenic bacteria. It was observed that the extract of *L. alba* inhibits the growth of *Proteus mirabilis, Proteus vulgaris, Shigella flexneri, Staphylococcus aureus* and *Vibrio cholerae* ElTor at the concentration of 500µg/ml, whereas *Escherichia coli*, *Shigella boydii,* and *Shigella schmitzi* were inhibited only at the concentrations of 1 mg/ml. Table 4 represents the comparison of zones of inhibition in three different medias. Oxoid Sensitivity Test agar (OSTA) and Trypticase Soy agar (TSA) showed no significant variation but the inhibition zones were markedly smaller in some bacteria grown on Nutrient agar (NA).

DISCUSSION

In 1961, Hanke and Talaat [3] studied the biochemistry and physiology of *L. alba* . They had also conducted a clinical

Table 2. *In vitro* bactericidal activity of *Lawsonia alba* extract measured by paper disc method

Sr No	Organisms	Diameter of zone of inhibition (mm)				
		Lawsonia alba extract			Control drugs	
		Crude	Fraction A	Fraction B	Ampicillin	Tetracycline
1	*Escherichia coli* O127/ B8;	11	NT	NT	0	16
2	*Klebsiella pneumoniae*	12	NT	NT	15	15
3	*Proteus mirabilis*	22	17	17	20	10
4	*Proteus vulgaris*	21	11	18	14	10
5	*Pseudomonas fluorescens*	0	12	23	NT	NT
6	*Salmonella typhi*	14	10	10	20	24
7	*Shigella boydii*	16	NT	NT	20	16
8	*Shigella dysenteriae*	21	NT	NT	30	14
9	*Shigella flexneri*	12	NT	NT	30	13
10	*Shigella schmitzi*	NT	12	10	28	14
11	*Staphylococcus aureus aureus*	24	NT	NT	14	14
12	*Vibrio cholerae* ElTor	15	12	10	24	30

NT=Not tested

trial in. intestinal amoebiasis. The dried powder of leaves, given to the patients suffering from amoebic dysentery was found to be effective and no sign of toxicity was observed. As far as the authors are aware, there is no published report on *in vitro* amoebicidal activity of the extract of *L. alba.* However, it could be a useful herbal agent in clinical practice after further purification and a series of *in vivo* testings.

In 1968, Malekzadeh *et. al.* [9] used aqueous extract to search for its inhibitory effect on eight species of pathogenic bacteria (*Baciillus cereus, Bacillus anthracis, Escherichia coli, Proteus vulgaris, Agrobacterium tumafaciens, Santhomisus campestris, Staphylococcus aureus and Erwinia carotoroa*). This aqueous extract was active against all eight species of bacteria at the concentration of

100µg/ml. Out of eight species of bacteria tested by Malekzadeh [9], three different strains of bacteria were also included in our study.

Table 3. Minimum Inhibitory Concentrations of *Lawsonia alba* extract on some pathogemic bacteria

Sr. No.	Tested bacteria	Minimum Inhibitory Concentration (mg/ml)
1	*Escherichia coli* O127/B8; WR2/69	1.0
2	*Klebsiella pneumoniae,*DMR	NT
3	*Proteus mirabilis*, IM1	0.5
4	*Proteus vulgaris,* IM1	0.5
5	*Pseudomonas fluorescens,* WR2/69	NT
6	*Salmonella typhi,* ATCC 992V	NT
7	*Shigella boydii,* NHL	1.0
8	*Shigella dysenteriae,* NHL	NT
9	*Shigella flexneri,* NHL	0.5
10	*Shigella schmitzi,* NHL	1.0
11	*Staphylococcus aureus aureus,* WS/69	0.5
12	*Vibrio cholerae* ElTor, IM1	0.5

NT=Not tested

Table 4. Comparison of growth of bacterial species on three different media

Sr No.	Tested bacteria	Diameters of zones of inhibition (mm)		
		OSTA	TSA	NA
1	*Escherichia coli* O127/B8; WR2/69	22	23	18
2	*Klebsiella pneumoniae,* DMR	NT	NT	NT
3	*Proteus mirabilis,* IM1	22	24	18
4	*Proteus vulgaris*, IM1	28	30	21
5	*Pseudomonas fluorescens,* WR2/69	NT	NT	NT
6	*Salmonella typhi,* ATCC 992V	16	14	12
7	*Shigella boydii,* NHL	20	NT	16
8	*Shigella dysenteriae,* NHL	NT	NT	NT
9	*Shigella flexneri,* NHL	NT	27	21
10	*Shigella schmitzi,* NHL	NT	24	23
11	*Staphylococcus aureus aureus,* WS/69	NT	19	16
12	*Vibrio cholerae* ElTor, IM1	15	15	12

OSTA = Oxoid Sensitivity Test Agar
TSA = Trypticase Soy Agar
NA = Nutrient Agar
NT = Not tested

Although the extraction method was not the same, extract of *L. alba* showed marked inhibition on these three bacteria (*E. coli, P. vulgaris & S. aureus*) used in our study. It has also been proven in a study in Myanmar that *L. alba* extract exerted the acceleration of wound healing activity in *in vivo* experimentally induced wound infections in rats [10]. From this study, extract of *L. alba* showed promising results of *in vitro* antimicrobial activity on *E. histolytica* and some pathogenic bacteria. Thus, further purification and investigation should be carried out to obtain a potent antimicrobial herbal agent from *L. alba.* The active substance of the plant is believed to be "lawsone" (2-hydroxy-1, 4 - naptho-quinone) which could be isolated from leaves and other parts of the plant.

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