J. Sci. I. R. Iran

Vol. 1, No. 1
Fall 1989

Therapeutic Effects and *In Vitro* Activity of an Extract from **Lawsonia inermis**

F. Malekzadeh and P. P. Shabestari

Department of Biology, Faculty of Science, Tehran University, Tehran – Iran

Abstract

An extract of henna (Lawsonia inermia) was found to be active *in vitro* against gram (+) and gram (−) bacteria including **Bacillus anthracis, B.cereus, B. subtilis, Enterobacter aerugenes, Escherichia coli** 0128-B7, **E.coli** 055-B5, **E. coli** 0127-B8, **Proteus mirabilis, P. vulgaris, Pseudomonas aeruginosa Salmonella typhosa, S.paratyphi, Sarcina sp. Shigella dysenteriae** type 1, **S. dysenteriae** type 7, **S. dysenteriae** type 9, **Staphylococcus aureus hemolyticus, S. citreus** and **S. epidermidis** Exposed lesions on rabbits, guinea pigs and mice caused by subcutaneous inoculation of a beta hemolytic, coagulase positive strain of Staphylococcus aureus were healed within 8 days after exposure to a lyophilized extract of henna. The antimicrobial activity of the extract may be due to Lawsone, the dying principle of henna.

Introduction

Since the discovery of penicillin and its antimicrobial activity (7), interest has been centered on the search for other antimicrobial agents (1, 2, 9, 10, 11, 12, 14, 18). A number of plant extracts are known to have antimicrobial activity (1, 2, 4, 6, 10, 11, 17). Henna (Lawsonia inermis L.) is cultivated in India, Iran and in many northern African countries. The powdered leaves of henna have been used in these countries as a cosmetic and a remedy for skin infections (13). We show here that an extract of henna is active *in vitro* against 20 human pathogenic bacteria and cured Staphylococcus aureus infections in rabbits, guinea pigs and mice.

Materials and Methods

Leaf Extraction: To determine the solubility of the antimicrobial substance, water at different pH, 70% ethanol, acetone, and ether were used as solvents. Ample amounts of the powdered leaves of henna were soaked in 100 ml of the solvents and kept in the refrigerator for 8 hours, then strained through a cheesecloth and the residue was squeezed out in a hand press. Ethanol, acetone and ether were evaporated and the resulting extracts were filtered through a Sietz filter or autoclaved. These extracts were lyophilized and used in all experiments at a concentration of 1/10 in sterile distilled water.

In vitro Studies

Fresh cultures of the following bacteria were prepared from the stock cultures of the Biology Department: **Staphylococcus aureus hemolyticus, Sta. epidermidis, Sta. al bus, Sta. citreus, Sarcina Sp., Bacillus anthracis, B. subtilis, B. cereus, Escherichia coli** 0128-B7, **E. coli** 055-B5, **E. coli** 0127-B8, **Salmonella typhi, S. paratyphi, Shigella dysenteriae** type 1, **Sh. dysenteriae** type 7, **Sh. dysenteriae** type 9, **Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Enterobacter aerogenes.**

In all experiments 10 ml of each bacterial suspension was prepared from a 24 hr. culture and the turibidity was adjusted to give 65% light transmission at 650 nm. This dilution resulted in approximately 160 million bacteria per ml.

The filter paper disc-agar diffusion method was used for testing extracts for antimicrobial activity. Filter paper discs 7 mm in diameter (BBL) were placed in test tubes and autoclaved for 20 min. at 15 lbs pressure in^2 and then oven dried for 4 hrs at 65° C. Discs then were dipped into the test extracts aseptically, and the excess liquid was allowed to drain by allowing the lower edge of the disc to touch the side of the tube. Such discs with various concentrations of the extract were prepared and carefully placed on the surface of the seeded agar (fig 1). The disc in the center was wetted with sterile distilled water.

The test plates were incubated at 37° C for 48 hrs and the diameter of the zone of inhibition was measured to the nearest mm. by means of a celluloid millimeter ruler. A magnifying glass was used when needed.

Bacteria	Gram reaction	Dia of inhibition zone* m.m.					average value
		1	2	3	4	5	
Staphylococcus aureus haemolyticus	+	16	16	16	17	16	16
S. epidermidis.	+	15	14	15	15	14	15
S. albus	+	12	11	11	12	12	12
S. citreus.	+	11	12	12	11	12	12
Sarcine. Sp	+	15	15	16	15	15	15
Bacillus anthracis.	+	17	17	16	17	17	17
Bacillus subtilis.	+	11	12	12	12	12	12
Bacillus cereus.	+	10	10	10	9	10	10
Escherichia coli 0128-B7	–	9	8	8	9	9	9
Escherichia coli 055-B5	–	9	9	8	9	9	9
Escherichia coli 0127-B8	–	9	9	9	9	8	9
Salmonella typhosa.	–	10	10	9	9	10	10
Salmonella paratyphi.	–	9	10	10	10	10	10
Shigella dysenteria type 1	–	9	9	8	9	9	9
Shigella dysenteria type 7	–	9	9	8	9	9	9
Shigella dysenteria type 9	–	9	8	9	8	9	9
Proteus mirabilis.	–	11	11	11	10	11	11
Proteus vulgaris.	–	11	10	11	11	10	11
Pseudomonas aeruginosa.	–	*	*	*	*	*	*
Enterobacter aerugenes.	–	8	7	7	7	7	7

* Diameter of paper disc: 6 mm.

Table 1. Antimicrobial activity of lyophilized henna extract against bacteria assayed by paper disc method

Pharmacological Tests

In order to understand how to use the extract safely, the following tests were conducted:

1) The extract was applied as a 10% aqueous solution in a shaved area on the back of rabbits.

2) Amounts of 1, 2, 3 (up to 10 ml) of the 10% aqueous solution of the lyophilized extract were injected intraperitoneally in rabbits.

3) Pyrogenic tests were conducted in rabbits (1.8 – 2.0 kg). by injection of 1ml. per kg of body weight, according to the standard test of Pasteur Institute of Iran used for antibiotics, Sera, and other biological products.

4) For demonstrating the antigenic effect of the extract, 5 ml of the extract was injected in the rabbits, and injection was repeated every 3 weeks for a 2 month period.

The effects of the lyophillized extract on the healing of staphylococcal skin lesions in rabbits, guinea pigs and mice were investigated by a subscutaneous inoculation in a number of animals (20) with 8×10^8 beta hemolytic, coagulase positive strain of Staphylococcus aureus. Inoculation and dosage was calculated to produce a lesion in each animal. All inoculations were made within a 1 hr period from the same suspension of bacteria. Then the induced lesions were treated with the 4/10 concentration of the lyophilized extract by means of a cotton pad every 2 hrs. Similarly control animals were treated with sterile distilled water.

Characterization Studies

Chromatographic separation of the lyophilized extract and Lawsone (the dying principle of henna) was carried out with Watman No.1 paper (3.5×50 cm.) with n-butanol-acetic and water (4:1:2.2) as a descending solvent. The extracts were applied as spot (0.1 ml). After drying, chromatograms were cut into 2 cm strips and bioassayed by plating on nutrient agar seeded with **S. aureus**

Results and Discussion

Leaf extraction:

The results of leaf extraction by various solvents revealed that the active principle of henna is soluble in polar solvents and was readily extracted from henna leaves with aqueous Na_2CO_3 (pH 8). This extract showed great antimicrobial activity against **S. aureus,** while the ether extract gave no inhibition against **S. aureus.**

No	Time hr.	body weight	body temp. (normal)	ml. of Injection	Temperature measurements (hours)							Temperature change	
					10	11	12	13	14	15	16	−	+
1	9	1800	38.1	1.8	38.8	38.5	38.3	38.1	—	—	—	0	0.7
2	9	2000	38.3	2	38.9	38.5	38.4	38.2	—	—	—	0	0.6
3	9	2000	38.5	2	39.1	39	38.7	38.6	—	—	—	0	0.5
4	9	2100	38.1	2.1	38.8	38.6	38.3	38	—	—	—	0	0.7
5	9	1800	38.1	1.8	38.8	38.5	38.4	38.1	—	—	—	0	0.7
6	9	2000	38.2	2	38.9	38.6	38.4	38.1	—	—	—	0	0.7

Average temperature change: 0.6
Substance injected: Lyophilized henna extract in saline solution (conc: 1/10)
Route of injection: I.V.
FILE 76-069

Table 2. Pyrogenic test of the Lyophilized Henna Extract in Rabbits

No	Time hr.	body weight	body temp. (normal)	ml. of Injection	Temperature measurements (hours)						Temperature change	
					10	11	12	13	14	15	−	+
1	9	2100	38	2.1	38	38.1	38	38	—	—	0	0.1
2	9	2000	38.1	2	38	38	38.1	38.1	—	—	0	0
3	9	2050	38.4	2.05	38.2	38.3	38.4	38.5	—	—	0	0.1
4	9	2000	38.3	2	38.2	38.3	38.3	38.4	—	—	0	0.1
5	9	1800	38.1	1.8	38.1	38.1	38.2	38.1	—	—	0	0.1
6	9	1800	38.4	1.8	38.3	38.3	38.4	38.6	—	—	0	0.2

Average temperature change: 0.10
Substance injected: Lyophilized henna extract in saline solution (conc: 1/10)
Route of injection: I. M.
FILE 76-069

Table 3. Pyrogenic Test of the Lyophilized Henna Extract in Rabbits

In vitro studies:

When the extract was assayed *in vitro* against bacteria, the results revealed that both gram positive and gram negative bacteria were inhibited. Inhibitory activity was greatest against **S. aureus hemolyticus** and **B. anthracis** and least against **E. aerogenes** while **Ps. aeruginosa** was not inhibited at the concentration used (Table 1).

Pharmacological tests:

Application of the extract as a 10% aqueous solution in a shaved area on the back of rabbits indicated that it was non-irritating to the intact skin of rabbits.

In intraperitoneal injection (0.5 ml) of 10% solution of the lyophilized extract, the LD50 values for the guinea pigs were found to be in the range of 2 – 2.7 g per kg of body weight. In intravenous (I.V.) administration of the extract, body temperature rose 1 hr after injection, then dropped to normal within three hrs; while in I.M. administration, body temperature dropped slightly for 1 hr, then returned to normal. According to the standard test, since the average temperature change in I.M. injected animals was less than 0.6 degree and in I.V. injected ones 0.6 or higher, we may conclude that the lyophilized extract is pyrogenic intravenously, but non-pyrogenic when injected I.M. (Table 2, 3).

In the experiment determining the effect of the extract on the healing of staphylococcal skin lesions in the test animals, the results showed that the exposed lesions to the extract were healed within 8 days while in the control animals no evidence of healing was observed within 21 days (Fig, 2, 3, 4).

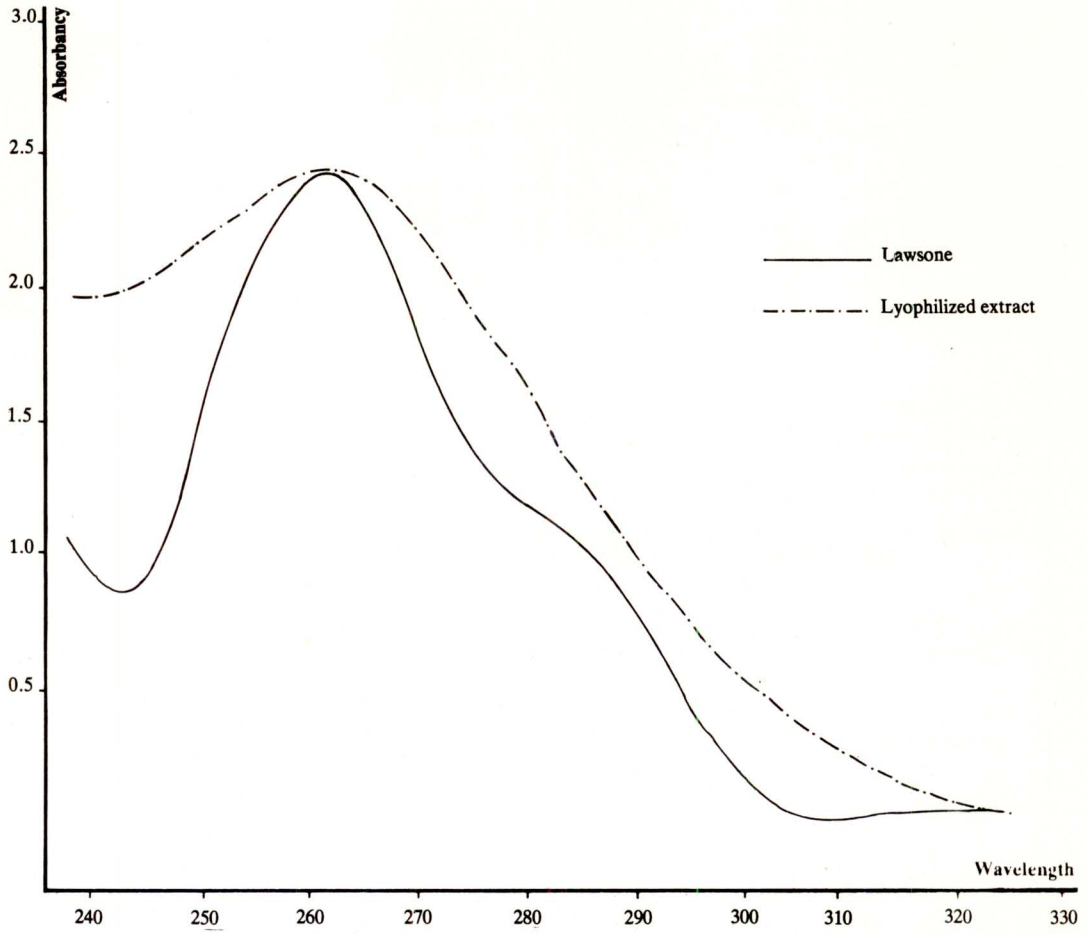

Fig (5) – Spectrophotometric analysis of the lyophilized extract and authentic Lawsone sample at 265nm by Beckman Du spectrophotometer

When the chromatograms were cut into strips (2 cm) and bioassayed by plating them on nutrient agar seeded with **S. aureus,** the results showed that part of the chromatogram with Rf value 0.71 gave an inhibition zone on agar plates. When the chromatograms were sprayed with 3% ferric chloride it gave a greyish blue color indicating that the spots with Rf value 0.71 on both chromatograms were phenolic substances.

Spectrophotometric analysis by Beckman Du spectrophotometer confirmed the similarity of the absorption peak of the lyophilized extract as compared with authentic Lawsone samples at 265 nm as shown in fig. (5).

Studies on the antimicrobial properties of the medicinal plants of the world have been investigated by many biologists (3,4,6,8,13,15,16,17). The mechanism of action and biological activity of substances isolated have drawn great attention (5, 7, 9, 10, 12). The antimicrobial activity of henna has been explored previously (9, 10). The purpose of this work was primarily to show the therapeutic effects of henna. The results showed the presence of this property in henna. Comparison of antimicrobial activity, solubity, Rf values (0.71), and the absorption spectra of the lyophilized extract and Lawsone showed that the

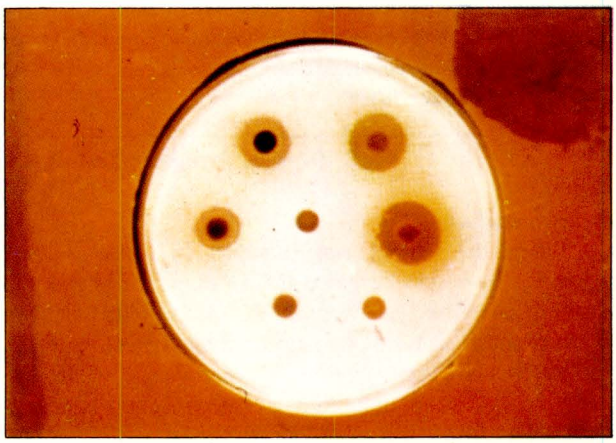

Fig 1:
Inhibition of syaphylococcus aureus
Growth by (Clockwise from discs showing the least inhibition): 0.0125, 0.025, 0.05, 0.1, 0.15 and 0.2 g/ml of Lyophylized henna extract using the filter paper disc-agar diffusion method.

Micrograph 1 (2000 ×)
Micrograph 2 (2000×)
Micrograph 3 (2000×)

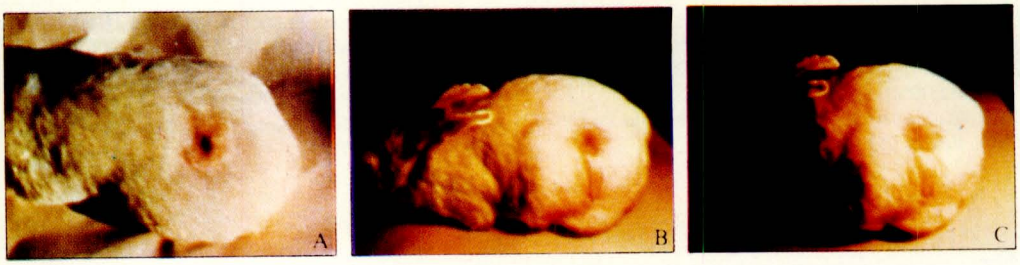

Fig 2:
Therapeutic effect of henna extract on healing of staphylococcal skin lesions in rabbit:
(A) Control animal 48 hours after bacterial infection.
(B) Control animal 8 days after bacterial infection.
(C) Treated animal after 8 days.
In each experiment 20 animals were used.

Fig 3:
Therapeutic effect of henna extract on healing of staphylococcal skin lesions in guinea pig:
(A) Control animal 3 days after bacterial infection.
(B) Control animal 8 days after bacterial infection.
(C) Treated animal after 8 days.

anitimicrobial activity of henna is possibly due to the presence of this substance. This substance can be used as a potential chemotherapeutic agent against bacterial and fungal infections in animals as well as humans without any harm.

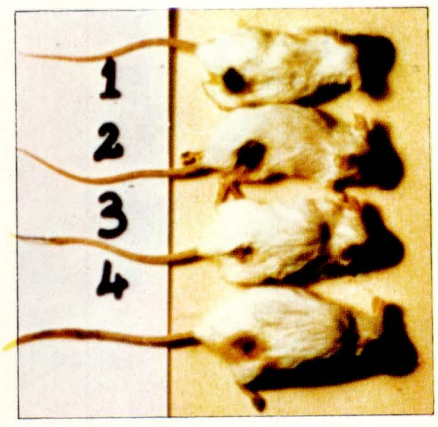

Fig 4:
Therapeutic effect of Lyophilized henna extract on healing of staphylococcal skin lessions in PR 8 – Kantlebury mouse.
(1) Control animal 2 days after bacterial infection.
(2) Treated animal after 4 days.
(3) Treated animal after 6 days.
(4) Treated animal after 8 days.

References

1. Arthuru, T.V. Hietaia P.K. & Oxa Wahlrees. Antimicrobial Substances in Cereals and Fodder Plants. *Arch. Biochem.*, **69:** 488 – 500 (1957).
2. Boll, P.M. Anibacterial Substances in Seed Plants Active against Tubercle Bacilli. *Antibiotics Ann.* 1955 – 1956, 255 – 256 (1955).
3. Borneman, W.S., D.E. Akin and W.P.Vaneseltine, Effect of Phenolic Monomers on Rumen Bacteria, *Appl. Environ. Microbiol.* **52;** pp 1331 – 1339 (1986).
4. Chesson, A.C, S.Stewart, and R.S. Wallace, Influence of Plant Phenolic Acids on Growth and Cellulolytic Activity of Rumen Bacteria. *Appl. Enviro. Microbiol.* **44:** 597 – 603 (1982).
5. Davidson P.M., and A.L. Branen; Antimicrobial Activity of Nonhalogenated Phenolic Compounds, *J. Food Prot.* **44:** 597–603 (1981).
6. Ferkas, G.L., & Z.Kiraly, Role of Phenolic Compounds in the Physiology of Plant Diseases and Disease Resistance *Phytopathol.* **44:** 105 – 150 (1962).
7. Frobisher Martin, Fundamentals of Microbiol., W.B. Saunders Co., Philadelphia, U. S.A. (1968).
8. Geiger, W.B. The Mechanism of Antibacterial Action of Qiunones and Hydrofiunones, *Arch. Biochem.* **11**: 23-30 (1940).
9. Herman Gershan & Large Shanks Fungitoxicity of 1, 4-naphtoquinones to Candida albicans and Trichophyton mentagrophytes, *Canad. Jour. of Microbiol* **21**: 9, pp 1317 – 1321 (1975).
10. Malekzadeh, F. Antimicrobial Activity of Lawsonia inermis L. *Applied Microbiol.* **16,**4:663 – 664 (1968).
11. Malekzadeh, F. An Antimicrobial Compound in Two Pistacia, Species, *Mycopathologia et Mycologia Applicata* **541**, pp 73 – 77 (1974).
12. McNew, G.D., and Burchfield, H. P. Fugitoxicity and Biological Activity of Qiunones. Contrib. *Boyce Thompson Institute* **16**: 357 – 374 (1951).

13. Osborn, E.M., On the Occurance of Antibacterial Substances in Green Plants. *The Brit. Jour. Exp. Path.* **24**: 227 – 231 (1943).
14. Owens, R. G., & Norati, H.M. Mechanism of Action of the Fungicide Dichlone (2,3-dichloro-1,4-naphto qiunones). *Contrib Boyce Thompson Institute* **19**: 463 – 482 (1958).
15. Suter, C.M. Relationship between Structure and Bactericidal Properties of Phenol. *Chem. Rev.* **28**: 269 – 299 (1941).
16. Viadimirtser, I. F., Bilich B. E., Cherkasor V. M., and Dyachenav, S.S. Antimicrobial Properties of some Naphtoqiunones Fiziel. Akt. Veshchestva, 2: 121 – 124, *Chem Abstr.* (1970) 73:1190 (1969).
17. Willenart, A., and Chaux R. Les grands Fonctions de la Chimie Organique, Dunod, Paris (1958).
18. Encyclopedia Britanica Vol. 11; Encyclopedia Britanica INC,. Chicago. p. 426 (1947)