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Antimicrobial Activity of *Lawsonia inermis* L.

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Henna (*Lawsonia inermis* L.) is a small shrub frequently cultivated in India, Persia, and along the African coast of the Mediterranean Sea. Powdered leaves of this plant, in the form of a paste, are used both as a cosmetic and as a remedy for boils, wounds, and some mycotic infections in certain countries of the Middle East. The antibacterial activity of an aqueous extract of henna leaf and some properties of this extract are given in this paper.

The following bacteria were used: *Bacillus cereus*, *B. anthracis*, *Escherichia coli*, *Proteus vulgaris*, *Staphylococcus aureus*, *Erwinia carotovora*, *Agrobacterium tumefaciens*, and *Xanthomonas campestris*. Cultures of *X. campestris* and *A. tumefaciens* were obtained from the American Type Culture Collection. All others were from the Microbiology Department, Tehran University. A 10-ml amount of each bacterial suspension was prepared from the stock culture, and the turbidity was adjusted to give 65% light transmission at 650 mμ. This dilution resulted in approximately 150 million bacteria per ml.

The henna extract was prepared by adding 1 g of powdered dried leaves to 10 ml of sterile distilled water. After being shaken, the preparation was allowed to stand for 4 hr. It was filtered through cheesecloth, then through filter paper, and finally through a Sietz filter into a sterile 100-ml filter flask. Portions of the filtrate were autoclaved at 14 psi for 15 min.

Test plates were prepared by adding 0.2 ml of the bacterial suspension to a test tube containing 10 ml of melted nutrient agar at 50 C and pouring into the petri plate. After solidification, a 14-mm core of agar was removed from the center of each plate with a sterile cork borer, and 0.5 ml of henna extract was placed in each hole. There were six replicates for each treatment. Daily observations were made, and the zones of inhibition were measured in millimeters when a uniform growth of bacteria covered the agar surface.

Both gram-positive and gram-negative bacteria were inhibited. Inhibitory action was greatest against *B. anthracis* and least against *S. aureus*. No bacterial colonies were observed within the zone of inhibition in plates of *B. anthracis* and *X. campestris* when the plates were incubated at room temperature (23 C) for 8 to 10 days (Table 1).

In determining the activity of raw and autoclaved extract against *B. anthracis*, 0.2 ml of the bacterial suspension with an optical density of 0.20 was added to two test tubes, each containing 9 ml of nutrient broth. The suspension was carefully shaken to insure an even distribution of the culture. Then 1 ml of the raw or autoclaved extract was added to the two tubes, and tubes were again shaken. The antimicrobial activity of the extract against *B. anthracis* was increased by heat (Fig. 1). This is probably the reason that the paste is commonly used with hot water rather than cold.

TABLE 1. *Activity of henna extract against bacteria*

Bacteria	Gram stain	Diam of inhibition zone[a]
Bacillus cereus	+	20
B. anthracis	+	40
Escherichia coli	−	20
Proteus vulgaris	−	25
Staphylococcus aureus	+	15
Erwinia carotovora	−	20
Agrobacterium tumefaciens	−	30
Xanthomonas campestris	−	32

[a] Each value is the average diameter of six replicates.

The antimicrobial substance in henna is highly soluble in water, partially soluble in 70% ethyl alcohol, and heat-stable. The aqueous extract of this activity was fractionated by descending paper chromatography with *n*-butyl alcohol-acetic acid-water (4:2:1). The resulting chromatogram was then cut into 2-cm strips and bioassayed by plating on nutrient agar seeded with with *B. anthracis* (F. Malekzadeh, Phytopathology **56**:497, 1966). The section of the chromatogram with an R_F value of 0.66 gave an inhibition zone on the agar plate. When the chromatogram was sprayed with ferric chloride, this section turned deep blue, indicating the presence of phenolic compounds.

Although the activity in vitro of quinones has

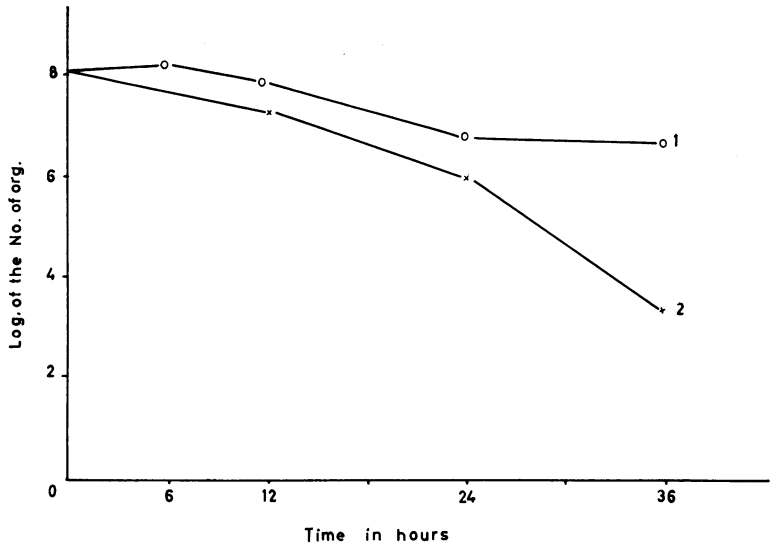

FIG. 1. *Activity of aqueous extract of henna against Bacillus anthracis (1) raw extract and (2) autoclaved extract.*

been investigated against *S. aureus* (G. Del Vecchio, Bull. Soc. Ital. Biol. Sper. **21**:1, 1946; W. B. Geiger, Arch. Biol. Chem. **11**:23, 1946; A. Willemart and R. Chaux, *Les Grands Fonctions de la Chimie Organique*, Dunod, Paris, 1958), this is believed to be the first time that the antimicrobial activity of the phenolic compounds present in henna has been tested against plant pathogenic bacteria.

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