Evaluation of Antimicrobial Activity of *Lawsonia Inermis* (Henna) Against Microbial Strains Isolated from Goat Skin/Leather

by

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Abstract

In the present work, antimicrobial activity of the aqueous extract from leaves of *Lawsonia inermis* (henna) has been evaluated. Two gram positive and five gram negative bacterial strains isolated from raw goat skins and three fungal species isolated from finished leathers have been utilized for the antimicrobial studies using aqueous extract from henna leaves. Gram-positive bacteria have found to be completely inhibited by henna at concentrations of 650 ppm and gram negative bacteria have been inhibited by henna concentration between 780 to 910 ppm. Growths of all fungal strains isolated have been inhibited by henna extract at a concentration of 1300 ppm. This study clearly demonstrates that henna offers good antimicrobial activity against the bacterial and fungal species.

Resumen

En el presente trabajo, la actividad antimicrobial del extracto acuoso de las hojas de *Lawsonia inermes* (jena) ha sido evaluada. Dos cepas bacterianas gram positivas, así como cinco gram negativas aisladas de pieles caprinas crudas, y tres especies micóticas obtenidas de cueros terminados, han sido utilizadas para estudios antimicrobiales utilizando extractos acuosos de hojas de jena. Bacterias gram positivas fueron completamente inhibidas por jena a concentraciones de 650 ppm, mientras que las bacterias gram negativas fueron inhibidas por concentraciones de jena entre 780 a 910 ppm. Crecimientos de todas las cepas micóticas aisladas fueron inhibidas por el extracto de jena a concentración de 1300 ppm. Este estudio claramente demuestra que jena ofrece buena actividad anti-microorganismos contra especies de bacterias y hongos.

Introduction

Currently, there is growing interest for the use of plant extracts of herbs and spices for the treatment of diseases and food preservation owing to their antioxidant as well as antimicrobial activity.[1-3] In recent times, major research activity in the leather process industry is focused on the use of natural materials for leather processing. In such an attempt, the authors have recently established the use of extract from *Lawsonia inermis* (henna) leaves for leather making.[4,5] In the present study, an attempt has been made to study the antimicrobial activity of henna extract against selected microbial species that could cause deterioration to skin and leather.

Lawsonia inermis is a small shrub or small tree with grayish brown bark. *Lawsonia inermis* is a member of the family Lythraceae, which consists of about 500 species widely spread in tropical regions with relatively few species in temperate region.[6] *Lawsonia inermis* is generally considered as a native of Africa and Asia. It is widely cultivated in tropical regions of the world in Sudan, Egypt, China and India.[7] Henna leaves have been extensively used for centuries as dye for nails, hands, hair and textile. Henna is also used in treating skin problems, headache, jaundice, amoebiasis and enlargement of the spleen.[8] The leaves of henna are chewed for curing mouth ulcer and inflammation of the tongue and gums in chicken and may be added to food to lower fever. Flower of *Lawsonia inermis* gives essential oil (Mehndi oil) long used in Indian perfumery. Leaves are said to be used as antiperspirant and sedative, expectorant and anti-inflammatory.[9,10] Leaf powder of this plant, in the form of a paste are used both as cosmetic and as remedy for boils, wounds and mycotic infections in certain countries of Middle East. Chemistry of the constituents of *Lawsonia inermis* has been of interest for many researchers and the occurrence of β- sistosterol glucoside, flavonoid,

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quinoids, naphthalene derivatives, gallic acid, coumarins, xanthones, luteolin and phenolic glycosides, lawsoniaside and laioside in the henna leaves have been reported.[8, 11-16]

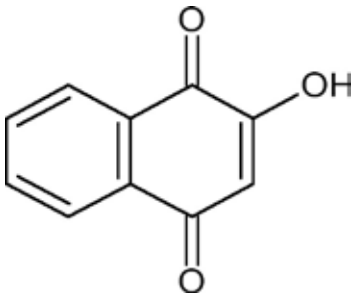


Fig. 1 Structure of 2-hydroxy–1,4- naphthaquinone (Lawsone)

Henna shows inhibition against both gram positive and gram negative microbes. In a study the inhibitory action of henna has shown to be high against *B. anthracis* compared to other bacterial species tested.[17] Lawsone as shown in Fig. 1 is the antimicrobial agent in henna. It is highly soluble in water, partially soluble in 70% ethyl alcohol and is heat stable.[17] Chloroform and ethanol extracts of henna leaves exhibit promising antibacterial activity against *Shigella* and *Vibrio Cholerae*.[18] Powdered leaves inhibit aflatoxin production by *Aspergillus parasiticus*. Leaf extract along with neem extract also showed antifungal activity against fungal pathogens of tobacco. Aqueous extract of the herb is used as ingredient of MELICON V, an antimicrobial veterinary herbal antiseptic, which is used for the treatment of wounds, cuts and other skin lesions in animals.[19] Bark extract exhibited absolute fungitoxic activity against ringworm fungi, *Microsporum gypseum* and *Trichophyton mentagrophytes*.[20-22] In the present study, an attempt has been made to study the anti bacterial properties of henna extract on the bacterial strains isolated from raw goat skins. Also, anti fungal properties of henna on fungal species isolated from finished leathers have been studied.

Materials And Methods

Materials

Microorganisms isolated from goat skin and finished leather was used in this study. All the chemicals and microbiological media used in the present study were of analytical grade. All microbiological media used were dehydrated media (Hi-Media, Mumbai).

Preparation of Henna Extract

Dried henna leaves sourced from Sudan have been used for the study. The required amount of ground henna leaves were soaked in water (1:10 w/v) at a temperature of 80±2˚C in a water bath for an hour, filtered through a piece of cotton cloth and then through Whatman No.1 filter paper. The supernatant was concentrated to 1/3rd of its volume and used for antimicrobial studies. The solid content of the henna extract was determined to be 35±1%.

Isolation and Screening of Bacterial Strain from Goat Skin

Fresh goat skin sample were procured from the local slaughter house. The sample (10 g) was homogenized using mortar and pestle and the homogenized samples were diluted appropriately with saline and plated on brain heart infusion (BHI) agar and incubated aerobically at 37˚C for 2-3 days. The bacterial colonies grown were picked up and transferred into BHI broth. The isolated strains maintained at 6˚C were propagated twice in BHI broth and used for further studies.

Bacterial Growth in the Presence of Henna

Appropriate quantities of henna extract from stock solution were transferred into different flasks containing 20 ml of melted nutrient agar to obtain final henna concentrations of 130, 260, 390, 520, 650, 780 and 910 ppm. A control sample was prepared by transferring an equivalent amount of sterile water to 20 ml of melted nutrient agar. 100 µl of each bacterium from isolated bacterial culture were inoculated into flasks under aseptic conditions. The medium was then poured into sterilized petri-plates in quadruplet and incubated at 37˚C for 20-24 h. The colonies developed after incubation were counted and expressed as colony forming units per ml of culture (cfu/ml). The inhibitory effect was calculated using the following formula,

$$\% \text{ inhibition} = (1- T/C) \times 100$$

Where T = cfu/ml of test sample and C = cfu/ml of control.

Isolation and Screening of Fungal Spores

Fungal species were isolated from two year old finished leather sample. The fungal species in the leathers were identified based on the colony morphology and further characterization using optical microscope.

Preparation of Spore Suspension

The spore suspensions from the respective species were prepared as described by Tamil Selvi *et al*.[23] Culture of the fungi was grown on potato dextrose agar (PDA) slants for 7 to 10 days at 28±2˚C until well sporulated. 10 ml of sterile water is added to the slants aseptically and the spores are dislodged with a sterile inoculating loop and serially diluted and used for further experiments.

Fungal Growth in the Presence of Henna

To the 10 ml aliquots sterilized and cooled yeast extract sucrose (YES) broth, 100 µl of the spore suspension were added individually. 1.25 mL from diluted solution from the henna stock was added and mixed well to the broth, making a final concentration of henna as 1300 ppm. This was incubated at 28±2˚C in dark for 168 h. A control containing 10 ml YES broth (without extract) was used. Mass of fungal spores was quantified for samples at different times. The growth of the respective fungal species in control at 168 h was assumed to be 100% and based on this, mold growth at previous time points and henna treated ones were quantified.

RESULTS AND DISCUSSIONS

Characterization of Bacterial Species Isolated from Goat Skins

Animal hides and skins are very good hosts for the growth of wide range of microorganisms derived from air, water, soil and other sources. When the animal is alive, most of these organisms have little effect on the skin, but once the animal is dead it acts as a perfect medium for the rapid growth of microorganisms. Presence of proteins and lipids facilitate the growth of microorganism on the raw hides/skins. Earlier literature reports the presence of 100 bacterial strains on the salted hides.[24] In the present study we have isolated only dominant bacterial colonies from the raw goat skins. The bacterial colonies isolated from goat skins were identified and characterized to species level based on colony morphology, staining and biochemical tests using the schemes outlined in Gerhard et al[25] and Bergey et al[26] and the same is presented as Table I. The isolated organisms were identified as *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, Proteus vulgaris, Yersinia enterocolitica*.

Anti-Bacterial Activity of Henna Against Bacterial Species Isolated from Goat Skins

Aqueous henna extract prepared from dry henna leaves have been tested for their anti-bacterial activity against the seven bacterial strains isolated from the goat skins. The % inhibition of the respective bacterial strains have been determined based on the difference between growth of bacterial colonies for control and henna treated (varied concentrations) medium and the data are presented in Fig. 2. From the figure it is seen that henna exhibited antibacterial activity against all the bacterial strains used for the study, however concentration of henna required for 100% inhibition varied depending on the bacterial strains. Concentration of henna required for 100% inhibition for the growth of various bacterial colonies have been determined and presented as Minimum Inhibitory Concentration (MIC) in Table II. Bacterial strains such as *Pseudomonas aeruginosa, Proteus vulgaris* and *Yersinia enterocolitica* required 910 ppm of henna solution for 100% inhibition of their growth. Bacterial strains such as *Klebsiella pneumoniae* and *E. coli* required 780 ppm of henna for complete inhibition and *Staphylococcus aureus* and *Bacillus subtilus* strains require even lower concentration (650 ppm) of

TABLE I
Morphological and biochemical characteristics of bacteria isolated from raw goat skins

Biochemical characteristics	*Staphylococcus aureus*	*E. Coli*	*Pseudomonas aerogenosa*	*Bacillus subtilus*	*Klebsiella pneumonia*	*Proteus vulgaris*	*Yersinia enterocolitica*
Gram staining	(+)	(-)	-	+	-	(-)	(-)
Motility	(-)	(+)	+	(+)	-	(+)	(-)
Indole	+	(+)	+	(+)	-	(+)	D
MR	-	+	+		-	+	(-)
VP	+	(-)	-	(+)	+	-	(-)
Catalase	(+)	(-)	(+)	(+)	(+)	(-)	(+)
Oxidase	(-)	(-)	(+)	No		(-)	(-)
Growth at 5% NaCl	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Growth at 10% NaCl	(-)	(-)	(-)	No	(-)	(-)	(-)
Growth at pH 5.7	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Acid from D-glucose		(+)	(+)	(+)	(+)	(+)	(+)
Acid from L-arabinose	(-)	(+)	(-)	(+)	+	(-)	(+)
Acid from D-mannitol	(+)	(+)	(+)	(+)	(-)	(-)	(+)
Acid from D-xylose	(-)	(+)	(-)	(+)	(-)	(+)	d
Acid from D – trechalose	(+)	(+)	(-)		(+)	(+)d	(+)
Gas from glucose	d	+ +	(+)	(-)	+	(+)/(+)	(-)
Hydrolysis of starch	Nd	+	(-)	(+)		ND	-
Hydrolysis of casein	Nd	-		(+)	(-)	+	+
Hydrolysis of gelatin	-	-		(-)		+	+
Reduction of Nitrate	(+)	(+)	(+)	(+)	+	(+)	(+)
Production of indol		(+)		(-)	-	(+)	d
Utilization of citrate	-	-	+	(-)	+	+	Nd

d → 75% positive; Nd → Not Determined

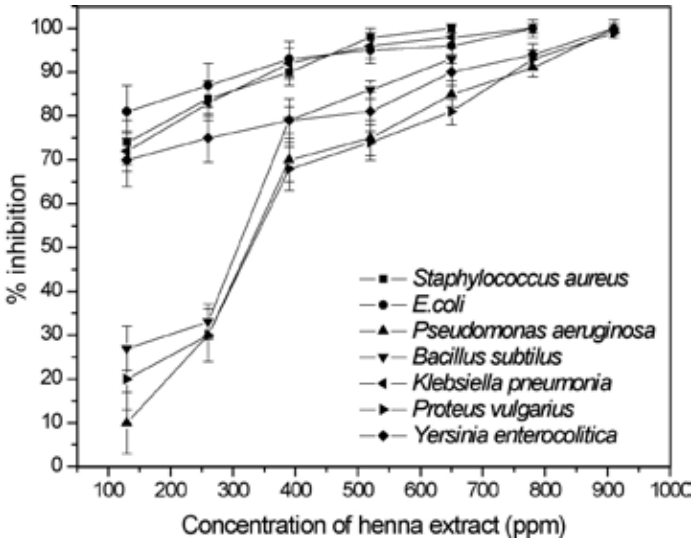


Fig. 2 Effect of initial concentration of henna extract on the growth of different bacterial strains

henna as MIC. In an earlier report by Malekzadeh,[17] the inhibition of *E. coli* and *Salmonella enteritidis* has been related to the presence of phenolic compounds and the activity of quinones. The site(s) and number of hydroxyl groups on the phenolic structure are thought to be related to their relative toxicity to microorganisms, with evidence that increased hydroxylation results in increased toxicity to microbes.[27] In addition, some authors have found that more highly oxidized phenols are more inhibitory.[28,29] The mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins.[30] Henna leaves are reported to have phenolic flavanoid compounds,[8] which could have facilitated the inhibition of bacterial species used in this study. The results clearly demonstrate that the antibacterial effects of henna extracts are more effective against gram-positive bacteria compared to gram-negative bacteria.

Characterization of Fungal Species Isolated from Finished Leather

The dominant fungal spores present in the moist finished leather have been isolated. Based on the colony morphology, three dominant fungal spores were identified and they are *Aspergillus niger, Aspergillus flavus,* and *Penicillium notatum*.

Anti-Fungal Activity of Henna Against Fungal Strains Isolated from Leather

The antifungal activities of henna extract against *A. flavus, A. niger* and *P. notatum* as a function of time have been studied for a period of 7 days. Spore suspension containing around 3.0×10^4 spores/ml of *A. flavus*, 2.6×10^4 spores/ml of *A. niger* and 2.9×10^4 spores/ml of *Penicillium notatum* have been used for the experiments. The growth of the fungi in control medium has been considered to be 100%. With reference to the control, growth profiles of fungi grown in henna medium have been quantified and are shown in Fig. 3. It could clearly be seen that at henna concentration of 1300 ppm no growth of *A. niger, A. flavus* and *P. notatum* have been observed up to 120 h. Thus it could be inferred that henna extract shows significant antifungal activity against the three species compared to control sample.

TABLE II

Minimum Inhibitory Concentration (MIC) of henna extract for different bacterial species

Bacteria	MIC (ppm)
Staphylococcus aureus	650
E.coli	780
Pseudomonas aeruginosa	910
Bacillus subtilis	650
Klebsiella pneumonia	780
Proteus vulgaris	910
Yersinia enterocolitica	910

Effectiveness of Henna as an Anti-Microbial Agent

Afzal et al[8] reported four flavonoid glucosides from the leaves of *Lawsonia inermis*. They have been identified as apigenin-7-glucoside, apigenin-4-glucoside, luteolin, luteolin-7-glucoside, luteolin-3-glucoside and Acacetin-7-glucoside (Fig. 4). The leaves of *Lawsonia inermis* contain phenolic glucosides, lawsoniaside (1,2,4-trihydroxynaphthalene-1,4-di-β-D-glucopyranoside) and laliioside (2,3,4,6-tetrahydroxyacetophenone-2-β-D- glucopyranoside).[16] Flavones are phenolic structures containing one carbonyl group. The addition of a 3-hydroxyl group yields a flavonol.[31] Flavonoids are also hydroxylatedphenolic substances but occur as a C_6-C_3 unit linked to an aromatic ring. Since they are known to be synthesized by plants in response to microbial infection,[32] it should not be surprising that they have found to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls. More lipophilic flavonoids may also disrupt microbial membranes.[33]

Analysis of air-dried henna leaves powder gave about 11% condensed tannin. Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity and a wide range of anti-infective actions, have been assigned to tannins.[34] One of their molecular actions is to complex with proteins mainly through non-specific interactions such as hydrogen bonding and hydrophobic effects, as well as by covalent bond interaction.[34,35] Thus, their mode of antimicrobial action, as described in quinones may be related to their ability to inactivate microbial adhesins, enzymes, cell envelope transport proteins, etc. In the present

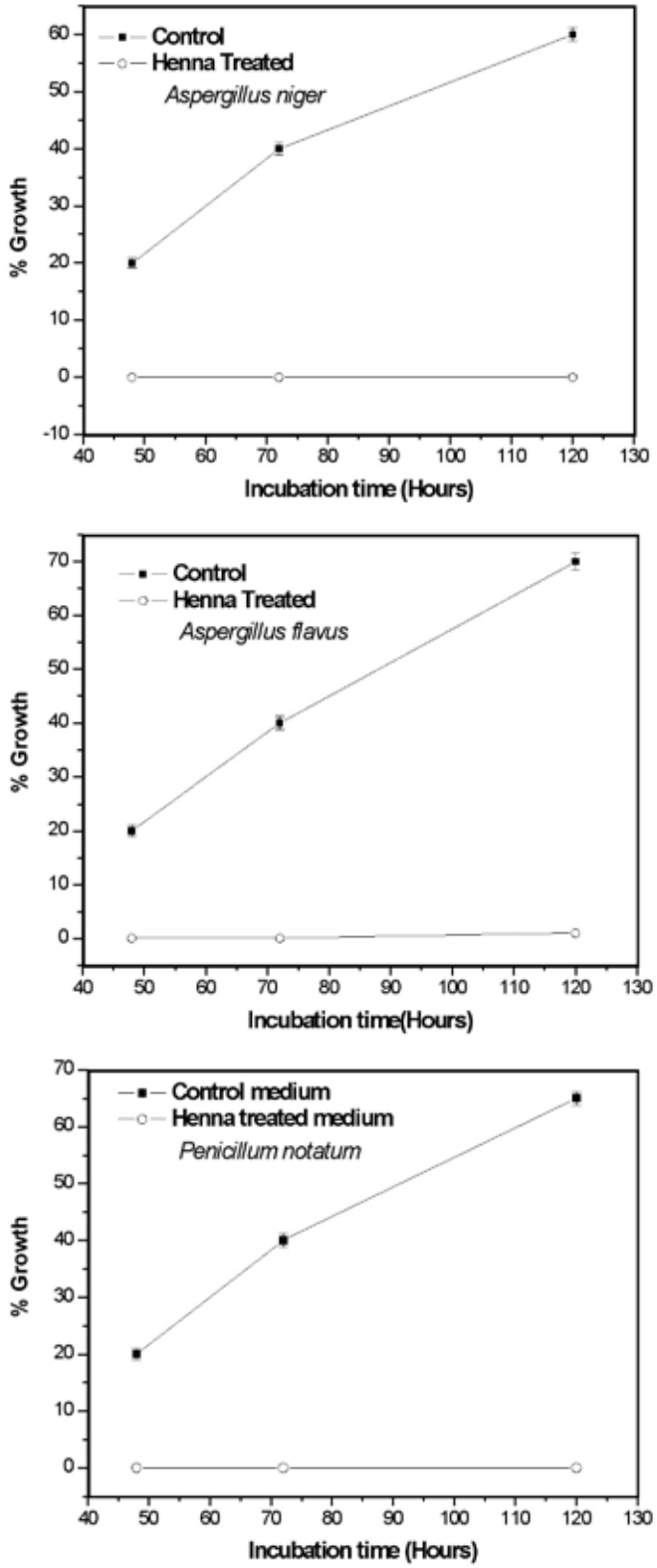
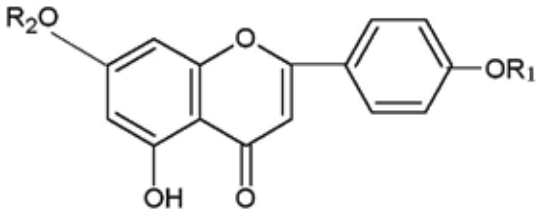


Fig. 3 Growth of fungal spores of *Aspergillus niger, Aspergillus flavus* and *Pencillum notatum* in henna treated medium.

work the effectiveness of henna, both against bacterial and fungal strain commonly found in hides and leathers respectively, has been demonstrated. This would further facilitate in establishing the use of henna for leather making.

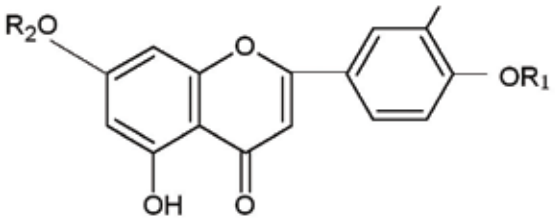


Apigenin – 7- glucoside $R_1 = H$; $R_2 = glucose$

Apigenin – 4′ - glucoside $R_1 = glucose$; $R_2 = H$

Afzal *et al* (1980)

(Leaves)



Luteolin $R_1 = R_2 = H$

Luteolin - 3′ - glucoside $R_1 = glucose$; $R_2 = H$

Luteolin -7- glucoside $R_1 = H$; $R_2 = glucose$

Afzal *et al* (1980), Mahmoud *et al* (1980)

(Leaves)

Fig. 4 Flavonoids as secondary metabolites isolated from *Lawsonia inermis* (Mahmoud *et al* 1980)

CONCLUSION

In the present study, an attempt has been made to evaluate the antimicrobial activity of aqueous henna leaves extract widely distributed in Sudan. It is seen that the aqueous henna leaves extract exhibited promising antibacterial activity against *Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae Escherichia coli, Pseudomonas aeruginosa Yersinia enterocolitica,* and *Proteus vulgaris*. The extract showed antifungal activity against the fungal strains identified in finished leathers *viz., Aspergillus flavus, Aspergillus niger* and *Penicillium notatum*. Hence, henna extracts could advantageously be used as a natural alternative ecobenign material for the preservation of leathers.

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