Antimicrobial Efficacy of Henna Extracts

Kathem K. Al-Rubiay,[1] Nawres N Jaber,[2] Al-Mhaawe BH,[3] Laith K. Alrubaiy [4]

Abstract

Lawsonia inermis (henna plant) has been used in herbal medicine for ages. However, the medical benefits of this plant have been discussed in only a few publications. In this study, the antibacterial effects of water, alcoholic and oily extracts of Lawsonia inermis leaves against bacterial cultures isolated from various skin diseases were investigated and compared with Tetracycline, Ampicillin ,Gentamicin and Ciprofloxacin antibiotics. Cultures of Staphylococcus aureus Staphylococcus epidermidis *(Co-agulase negative staphylococci or CONS), ß-hemolytic streptococci and Pseudomonas aeruginosa species* were obtained from 74 (35 females, 39 males) patients with different skin infections who attended the Dermatology outpatient clinic in Basra General Hospital. The bacterial isolates were treated with L. inermis extracts in vitro. Alcoholic and oily extracts were more effective than the water extract which had no effects using standard method of NCCL, 2000.

Alcoholic extracts had the highest antibacterial activity with a MIC of 0.125-0.150 µg/ml against *ß-hemolytic streptococci* and against CONS was 0.125-175 µg/ml .Oily extracts had a MIC of 0.25-0.30 µg/ml against *Staphylococcus epidermidis (cons)*. Both alcoholic and oily extracts had the same MIC (0.5 µg/ml) on Staphylococcus aureus. However, alcoholic extracts were more effective on *Pseudomonas aeruginosa* with a MIC of 0.5-0.57 µg/ml than oily extract (MIC of 0.20-0.28 µg/ml). However, there were no statically differences between the effects of oily and alcoholic henna extracts (p= 0.050).

When comparing the extracts' MICs with those of antibiotics, alcoholic extracts showed pronounced antibacterial effects against the isolated bacteria in vitro but oily extracts had much similar MICs to those of antibiotics and there are significant difference between effect of both extracts and antibiotics p>0.050.

Key words: Skin diseases; Lawsonia inermis; Henna; Bacteria; Herbal Medicine.

Received: 30 July 2008
Accepted: 11 Sept 2008

From the [1]Department of Dermatology, Basra General Hospital; [2] Department of Microbiology, University of Basra; [3] Department of Pathology, University of Basra; [4] Department of Medicine, Bangor Hospital, UK.
Address Corresponding and reprint reqeust to: Dr. Laith Alrubaiy, Ysbyty Gwynedd NHS trust, Bangor, UK
Email: laithalrubaiy@gmail.com

Introduction

Henna or Hina (*Lawsonia inermis*, syn. *L. alba*) is a flowering plant, 2-6m in height. It is the sole species in the genus *Lawsonia* in the family Lythraceae.[1] Henna, *Lawsonia inermis*, produces a burgundy dye molecule, lawsone.[2] This molecule has an affinity for bonding with protein, and thus has been used to dye skin, hair, fingernails, leather, silk and wool. The dye molecule, lawsone, is primarily concentrated in the leaves. Products sold as "black henna" or "neutral henna" are not made from henna, but may be derived from indigo (in the plant *Indigofera tinctoria*) or *Cassia obovata*, and may contain unlisted dyes and chemicals.[1]

It is well known that plants have been used in traditional herbal medicine for many years.[3] In some parts of the world, plants and herbs are still the prime medicines used in medical treatment.[4-6] *L. inermis* is widely grown in various tropical regions in Asia, America and Africa. In Arabic, the word "henna" refers to *L. inermis*.[5,7]

The main uses of henna are as a cooling agent, astringent, anti-fungal and anti-bacterial herb for the skin and hair.[8,9] It has also been used as a dye and preservative for hair, skin and fingernails as well as leather and clothes.[8,9] Its core chemical components are 2-hydroxynapthoquinone (lawsone), mannite, tannic acid, mucilage and gallic acid. Out of these ingredients, the main one is 2-hydroxynapthoquinone (lawsone). About 0.5-1.5% of henna is made of lawsone. Its bioactive feature is thought to be due to its high protein binding capacity.[2,9]

The skin has a complex flora. Infections can result when there is a breakdown in the integrity of the skin or when the immune defense is compromised. Common skin infections include cellulitis, erysipelas, impetigo, folliculitis, and furuncles and carbuncles.[10] Many types of bacteria have ability to produce skin infections. *Staphylococcus aureus* is the most common cause of skin infections. It is frequently found in the nose and skin. About 20% of the population is long-term carriers of *S. aureus*.[11] The purpose of this study was to evaluate the antibacterial properties of henna extracts in vitro and to compare them with Tetracycline, Ampicillin, Gentamicin, and Ciprofloxacin antibiotics.

Methods

Sampling

Bacterial isolates were obtained from 74 patients (39 males, 35 females) with different skin infections who attended the

Dermatology out patient clinic at Basra General Hospital. Bacterial culturing and identification of bacterial species were done at the Department of Microbiology in the University of Basra using Colle J, *et al* method, 1996.[12]

Plant samples and extraction procedure

L. inermis were collected from private gardens in Abu Al-Khasib City in Basra. The leaves were left to dry at room temperature for 24 hours. The dried leaves were ground to a powder and were kept in dry containers. Three types of extract were prepared in the present study: oily, alcoholic and water-based extracts. The oil-based extract was prepared by mixing 50 gm of dried leaves powder of *Lawsonia inermis* with 500 mL of n-hexane for 24 hours. The solvent was then removed with a negative pressure to make 3 gm of oily henna extract. The alcoholic extract was prepared by mixing 25 gm of henna powder with 250 mL of 70% ethanol for 12 hours. This mixture was cooled and filtered by Buchner funnel and filter paper (Wattman No. 185). The solvent was dried and concentrated using Rotary evaporator at 50°C. Water-based henna extract was prepared in the same way except that distilled water was used instead of alcohol.

Studying the antibacterial activity of Henna extracts:

The antibacterial effects of henna extracts on four bacteria strains, namely: *Staphylococcus aureus, Staphylococcus epidermidis (coagulase–negative staphylococci (cons), ß-hemolytic streptococci* and *Pseudomonas aeruginosa* were studied. These bacteria were isolated from patients who attended the Dermatology outpatient clinic in Basra General Hospital. This was done using Agar-well diffusion method.[13]

1. **Determination of the inhibition zones (mm) and minimum inhibitory concentrations (MIC).**
   The inhibition zones (mm) and minimum inhibitory concentrations (MIC) of *L. inermis* (henna) extracts were assessed using Agar diffusion dilution method. Mullar Hinton agar was used with different diluted extract concentrations (10-0.03 µg/ml). 0.1 ml containing 10^5 CFU /ml (0.5 McFarland) were spread on the agar as described in NCCLS-2000[13] and Alwaili& Sloom, 1999.[14]
   Note: One typical species from each bacterial skin infection was selected for the study. The total isolates were 5 for each bacteria study.
2. **Comparing efficacy of henna extracts with antibiotics:**
   Tetracycline, Ampicillin, Gentamicin, Ciprofloxacin antibiotics were used in this study to evaluate the antibacterial efficacy of *L. inermis* (henna) extracts. Muller Hinton agar was used with different antibiotic concentrations (30 mcg) to measure the (MICs). Determination of MIC was carried out using Maki's method, 1985.[15]

Results

Seventy four patients (39 males and 35 females) with various skin infections were involved in this study (Table 1). The studied skin infections were impetigo, carbuncles, furuncles, infected eczema and infected wounds. The isolated bacteria were *coagulase-negative staphylococci, Staphylococcus aureus, ß-hemolytic streptococci* and *Pseudomonas aeruginosa.* When measuring the antibacterial activity of henna extracts, alcoholic and oily extracts were more effective than the water based extract which had no effects. The standard method of NCCL were used, 2000 [13] (Table 2).

Table 1: Distribution of skin diseases according to sex

Skin diseases	Males	Females	Total No.
Impetigo	15*	10	25
Carbuncle	5	7	12
Furuncle	6	6	12
Infected eczema	9	7	16
Infected wound	4	5	9
Total	39	35	74

*: P<0.050, SD: 6.987

Table 2: Inhibition zones diameters (mm) of *L. inermis* (henna) extract (10 mcg) against the bacteria isolated from skin diseases*

Bacteria	Numbers of tested isolates	Diameters of inhibition zone of oily extract	SD	Diameters of inhibition zone of alcoholic extract	SD	Diameters of inhibition zone of water extract
Staphylococcus aureus	5	28 ± 11*	2.54	30 ± 12.4	3.35	NA
CONS	5	25 ± 6.5	1.65	28 ± 8.2	4.32	NA
ß-hemolytic streptococci	5	23 ± 4.3	1.98	30 ±7.9	2.68	NA
Pseudomonas aeruginosa	5	15 ± 2.7	1.43	25 ±6.2	2.98	NA

NA: not affect (poorly on no growth)
SD: Standard Deviation; CONS: Co-agulase Negative Staphylococci
Inhibition zone: is the zone that does not show any bacterial growth.
NOTE: All comparisons were carried out with standard method of NCCL, 2000.[10]
*:P<0.050

Alcoholic extracts had the highest antibacterial activity with an MIC of 0.125-0.150 µg/mL against *ß-hemolytic streptococci* and against *coagulase-negative staphylococci* was 0.125-175 µg/mL. Oily extracts had an MIC of 0.25-0.30 µg/mL against *Staphylococcus epidermidis*. Both alcoholic and oily extracts had the same MIC (0.5 µg/mL) on *Staphylococcus aureus*. However, alcoholic extracts had more effect on *Pseudomonas aeruginosa* with a MIC of 0.5-0.57 µg/ml than oily extract 0.20-0.28 µg/ml. However, there was no statistical difference with p value ≥ 0.05 (Table 3). When comparing the extracts' MICs with those of antibiotics, alcoholic extracts showed pronounced antibacterial effects against the isolated bacteria in *vitro* but oily extracts had much similar MICs to those of antibiotics with a statistically significant difference between the effect of extracts and antibiotic p<0.050 (Table 4).

Table 3. The minimum inhibitory concentrations (MICs) (µg/mL) of alcoholic and oily extracts

Bacteria	Number of tested isolates	MICs of oily extract	SD	MICs of alcoholic extract	SD
Staphylococcus aureus	5	0.50-0.62*	0.012	0.500-0.570	0.021
CONS	5	0.25-0.30	0.011	0.125-0.175	0.015
ß-hemolytic streptococci	5	0.50-0.55	0.023	0.125-0.150	0.014
Ps. aeruginosa	5	0.20-0.28	0.140	0.500-0.570	0.014

* : p=0.050 : there are no statistical differences between effects of oily and alcoholic henna extracts
SD: Standard Deviation; CONS: Co-agulase Negative Staphylococci

Table 4. The minimum inhibitory concentrations (MICs) (µg/mL) of the antibiotics on the bacteria used in the study

Antibiotic	Concentrations (*mcg*)	Number of tested isolates	*Staph. aureus*	*CONS*	*ß-hemolytic streptococci*	*Pseudomonas aeruginosa*
Ampicillin	30	5	5*	0.50	0.125	> 5
Tetracycline	30	5	1	1	1	1
Gentamicin	30	5	0.125	0.25	1	1
Ciprofloxacin	30	5	0.500	0.50	5	2

Mcg = µg/ ml
*: p<0.050: there are statistical differences between antibiotics and henna extracts effects.
CONS: Co-agulase Negative Staphylococci
Note: One typical species from each bacterial skin infections was selected for the study. The total isolates were 5 for each bacteria study.

Discussion

Henna has many traditional and commercial uses, the most common being as a dye for hair, skin and fingernails, as a dye and preservative for leather and cloth, and as an anti-fungal. Henna body art is made by applying henna paste to the skin: the lawsone in the paste migrates into the outermost layer of the skin and makes a red-brown stain. Some pastes have been found to include: silver nitrate, carmine, pyrogallol, disperse orange dye, and chromium. These have been found to cause allergic reactions, chronic inflammatory reactions, or late-onset allergic reactions to hairdressing products and textile dyes.[16-18]

Henna contains Lawsone in about 0.5 to 1.5% of its ingredients. Lawsone (2-hydroxynapthoquinone) is the principal constituent responsible for the dyeing properties of the plant. However, henna also contains mannite, tannic acid, mucilage and gallic acid.[2,9] These substances are present in henna in the form of a mixture. Antimicrobial activity may be due to numerous free hydroxyls that have the capability to combine with the carbohydrates and proteins in the bacterial cell wall. They may get attached to enzyme sites rendering them inactive.[2] Water extracts did not show any antibacterial activity compared to alcoholic and oily extracts. This may be due to the lack of the solvent properties which plays an important role in antibacterial efficacy.[9]

The alcoholic extract showed the lowest MICs compared to other types of extracts and this may be due to the large quantity of active substances that were precipitated during the extraction process due to the effect of Hexane solvent itself.[5] When compared with antibiotics, alcoholic henna extracts showed more antimicrobial activity while oily extract had similar antibacterial

activity compared to those of antibiotic. We concluded that henna has an in-*vitro* antibacterial activity against the tested bacterial strains. These findings have also been mentioned in literatures.[5-7]

Conclusion

Henna leaf extracts have antimicrobial activity on the bacteria responsible for the common skin infections. Alcoholic and oily henna extracts have similar effects to some of the antibiotics commonly used in clinical practice.

References

1. Singh M, Jindal SK, Kavia ZD, Jangid BL. Khem Chand. Traditional Methods of Cultivation and Processing of Henna. Henna, Cultivation, Improvement and Trade: 21-14. Jodhpur, India: Central Arid Zone Research Institute, 2005.
2. Harborne SB, Baxter A. Phytochemical Dictionary. A handbook of bioactive compounds from plants. Tylor and Francis. London, 1995.
3. Blanks T, Brown S, Cosgrave B, Woody J, Bentley V, O' Sullivan N, et al. The Body Shop Book of Wellbeing Mind, Body, and Soul. Ebury Press London. 1998 p. 173-192.
4. Natarajan V, Venugopal PV, Menon T. Effect of Azadirachta indica (neem) on the growth pattern of dermatophytes. Indian J Med Microbiol 2003 Apr-Jun;21(2):98-101.
5. Hemem SS. Activity of some plant extracts against common pathogens in bacterial skin infection: thesis MSc, College of Education, Basra University, Iraq, 2002.
6. Muhammad HS, Muhammad S. The use of Lawsonia inermis linn. (henna) in the management of burn wound infections. Afr J Biotechnol 2005;4:934-937.
7. Habbal OA, Al-Jabri AA, El-Hag AH, Al-Mahrooqi ZH, Al-Hashmi NA. In-vitro antimicrobial activity of Lawsonia inermis Linn (henna). A pilot study on the Omani henna. Saudi Med J 2005 Jan;26(1):69-72.
8. Singh A, Singh DK. Molluscicidal activity of Lawsonia inermis and its binary and tertiary combinations with other plant derived molluscicides. Indian J Exp Biol 2001 Mar;39(3):263-268.
9. Kelmanson JE, Jäger AK, van Staden J. Zulu medicinal plants with antibacterial activity. J Ethnopharmacol 2000 Mar;69(3):241-246.
10. Stulberg DL, Penrod MA, Blatny RA. Common bacterial skin infections. Am Fam Physician 2002 Jul;66(1):119-124.
11. Kluytmans J, van Belkum A, Verbrugh H. Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks. Clin Microbiol Rev 1997 Jul;10(3):505-520.
12. Colle J, Fraser A, Marmion B, Simmans A. Mackie and McCartney. Practical Medical Microbiology. 14th ed. Churchill Living ston. New York, USA. 1996 p. 978.
13. NCCL. 2000. Antibiotic susceptibility methods. CLSI.
14. Al-Waili NS, Saloom KY. Effects of topical honey on post-operative wound infections due to gram positive and gram negative bacteria following caesarean sections and hysterectomies. Eur J Med Res 1999 Mar;4(3):126-130.
15. Maki HA. (1983). Isolation and Identification of pathogenic bacteria encountered in cases of wound infection with their Antibiogram pattern. M. Sc. Thesis, College of Medicine, Univ. Baghdad.
16. Stante M, Giorgini S, Lotti T. Allergic contact dermatitis from henna temporary tattoo. J Eur Acad Dermatol Venereol 2006 Apr;20(4):484-486.
17. Sosted H, Johansen JD, Andersen KE, Menné T. Severe allergic hair dye reactions in 8 children. Contact Dermatitis. Blackwell Publishing Limited 2006;54(Issue 2):87-91.
18. A Report of the Allergy Vigilance Network, (2007). European Annals of allergy and Clinical Immunology vol. 39, 6:189-192.