In-vitro antimicrobial activity of Lawsonia inermis Linn (henna)

A pilot study on the Omani henna

Omar A. Habbal, *MD, PhD,* ***Ali A. Al-Jabri,*** *PhD,* ***Abdulghaffar H. El-Hag,*** *BSLT, FRMS,*
Zahra H. Al-Mahrooqi, *BSc, MSc,* ***Nasser A. Al-Hashmi ,*** *BTech.*

ABSTRACT

Objectives: To investigate the antimicrobial activity of henna's fresh and dry leaves and seeds obtained from Oman.

Methods: This study was carried out at the College of Medicine and Health Sciences, Sultan Qaboos University, Muscat, Sultanate of Oman during the period January-June, 2004.

Crude extracts of fresh and dry leaves and seeds were investigated for their antimicrobial activity against 3 standard bacterial strains namely: *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*. Eleven different bacterial strains were obtained from patients attending the Sultan Qaboos University Hospital, Muscat, Sultanate of Oman. In addition, one *Candida albicans (C. albicans)* species was used for testing the antifungal activity of the Omani henna sample.

Results: All fresh and dry leaves and seeds of the Omani henna demonstrated antibacterial activity against all 3 standard strains and the 11 patients' isolated strains. Henna dry leaves demonstrated the best *in-vitro* antimicrobial activity and in particular against *Shigella sonnei*. However, henna fresh and dry seeds failed to show any activity against *C. albicans*.

Conclusion: Omani henna does possess, *in-vitro* antibacterial activity against a wide spectrum of bacterial strains and *C. albicans*.

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Almost 50% of current pharmaceuticals are derived from the plant kingdom. Traditional healers have long used plants to prevent or cure infectious diseases. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, and have demonstrated *in-vitro* antimicrobial[1,2] and antiparasitic activities.[3,4] Henna is known to have medicinal properties.[5-7] Engravings of ancient Egyptians tell that they used henna for prophylactic and therapeutic purposes.[8] More recent findings demonstrated the usefulness of henna to treat headaches and skin diseases amongst many other usages in cosmetic preparations.[9] An aqueous preparation of this plant is used as a cosmetic, and the powdered leaves have been in use from the most ancient times in Eastern countries for dyeing the hair and the nails with a reddish-yellow tint. In the western world, henna has been used since 1890 for

From the Department of Human and Clinical Anatomy (Habbal, El-Hag, Al-Hashmi) and the Department of Microbiology and Immunology (Al-Jabri, Al-Mahrooqi), Sultan Qaboos University, Al-Khod, Muscat, *Sultanate of Oman.*

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Address correspondence and reprint request to: Dr. Omar A. Habbal, Head, Department of Human and Clinical Anatomy, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, Al-Khod, Muscat, Postal Code 123, *Sultanate of Oman.* Tel/Fax. +968 24415175. E-mail: Habbal@squ.edu.om

Table 1 - Antimicrobial activity of henna leaves and seeds at 50% concentrations.

Microorganisms	Henna Extract			
	Fresh Leaves	Fresh Seeds	Dry Seeds	Dry Leaves
Bacteria				
Escherichia coli	++	+	+	++
Pseudomonas aeruginosa	++	++	+	++
Staphylococcus aureus	+++	++	+++	+++
Enteropathogenic Escherichia coli	+	+	++	++
Shigella sonnei	+++	++	++	++++
Bacillus species	++	++	+	+++
Klebsiella pneumoniae	++	++	++	+++
Salmonella species	++	+	+	++
Bacteroides fragilis	+	+	+	+
Corynebacterium	++	++	++	++
Streptococcus pyogenes	+	+	+	++
Citrobacter freundii	+	+	+	++
Vibrio cholerae	++	++	++	++
Streptococcus Pneumoniae	+	+	+	++
Yeast				
Candida albicans	+	-	-	+

++++ = >40 mm zone of inhibition
+++ = 31-40 mm
++ = 21-30 mm
+ = 10-20 mm
- = 0 mm (no inhibitory activity detected)

tinting the hair. A brown resinoid component in henna is believed to have chemical properties similar to tannins and was named hennotannic acid.[10] This fraction was deployed both topically and internally in the treatment of jaundice, leprosy, smallpox, and some other skin infections.[3,4,11] Skin allergic and toxic reactions from henna have been reported.[11,12] Such reactions are usually due to impurities and additives rather than natural henna constituents.[13]

The species of henna is sometimes referred to as *Lawsonia alba* or *Lawsonia rubra* and is cultivated in India, the Middle East, Egypt and tropical America. Different species of henna are present and grown in the Sultanate of Oman, at the southeastern tip of the Arabian Peninsula. Omani henna is prevalent in Eastern and central areas of the Sultanate. In addition to its use as cosmetic, henna leaves are also used as a local anaesthetic,[14] anti-inflammatory, antipyretic and for treating mouth ulcers.[6] The antimicrobial and fungicidal[15,16] effect of henna has long been known. This preliminary study looks at the local plant in Oman and tests those effects on a variety of bacteria. In this study, we investigate the effect of the local henna on several bacterial species. This is a part of a wider project in which we envisage to test henna on a wider variety of bacteria, viruses and fungi.

Methods. Fresh and dry leaves and seeds of henna were obtained from one Omani henna sample. This henna sample was collected from Rumais area, Muscat region, Sultanate of Oman.

Henna leaves were cut into small pieces then crushed with a pestle and mortar. Seeds were crushed into fine powder with a pestle and mortar. A 100 g of leaves (fresh and dry) were soaked in 500 cc ethanol while 50 g of henna seeds (fresh and dry) were also soaked in 250 cc of ethanol for 3 days with frequent agitation. The mixture was filtered and the crude extract was collected. The crude extract was then distilled, at 48°C using a water bath. The semi-dry extract was collected for further drying in an oven at 37°C. The henna crude dry extract was then tested for its antimicrobial activity using standard antimicrobial assays. The crude extract was diluted into 50%, 25%, 12.5% and 6.25% of its original concentrations using sterile distilled water. Fourteen different bacterial strains were used to test the antibacterial activity of the henna sample. Three of those were laboratory standard microorganisms: *Escherichia coli*, *Staphylococcus aureus (S. aureus)* and *Pseudomonas aeruginosa*. The other 11 bacterial strains were isolated from patients attending the Sultan Qaboos University Hospital. In addition, one *Candida albicans (C. albicans)* species was used for testing the antifungal activity of the Omani henna sample. A sizable colony of each standard microorganism was emulsified in 4 ml distilled water, yielding about 1 x 106 and used to swab agar plates of diagnostic sensitivity test (DST), (Oxoid, England). Wells of 6 mm in diameter were made and 60 µl of each henna dilution was placed into each well with a chipped tip pipette. Each dilution was tested in triplicate. The plates were left at room temperature prior to incubation until the henna had seeped into the agar. After incubation for 24 hours at 37°C, the diameter of inhibition zones were measured in millimeters (mm) and the average was recorded. Penicillin and gentamicin were included as controls.

Results. In **Table 1**, fresh and dry leaves and seeds of the Omani henna sample demonstrated antimicrobial activity against the 14 different bacterial strains and *C. albicans*. However, the antimicrobial activity was not the same for all the microorganisms tested. Dry leaves showed the highest level of activity for all the bacteria strains tested and for the *C. albicans*. Leaves (fresh and dry) had the highest activity for the different microorganisms tested followed by seeds (fresh and dry). However, none of the fresh and dry seed samples showed detectable activity against *C. albicans*.

Figure 1 shows the anti-*Shigella sonnei* activity of the fresh and dry leaves and seeds. Henna dry leaves showed excellent anti-*Shigella sonnei* activity and demonstrated growth inhibitory activity up to a concentration of 6.25%. Dry leaves failed to demonstrate any detectable activity at this concentration. Fresh leaves also demonstrated anti-*Shigella sonnei* inhibitory activity up to 12.5% concentration, whereas, fresh seeds failed to show activity at this concentration. Both dry leaves and fresh leaves demonstrated better anti-*Shigella sonnei* inhibitory activity than fresh and dry seeds. Similar activities were noted for Staphylococcus aureus (**Table 1**). Other bacterial strains showed less antibacterial activities with the Omani henna dry and fresh leaves and seeds.

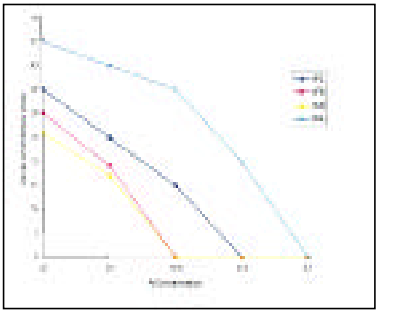

Figure 1 - Anti-*Shigella sonnei* inhibitory activity of the Omani henna. HFL - henna fresh leaves, HFS - henna fresh seeds, HDS - henna dry seeds, HDL - henna dry leaves.

Discussion. The most striking antimicrobial effect of henna is demonstrated by the inhibitory effect of all dilutions on both *Shigella sonnei* and *S. aureus*. This is reassuring since certain henna ingredients such as flavonoids, quinones and simple phenols have been reported to have antimicrobial activity on *Shigella sonnei*,[17] which support our findings. The dry leaves seemed to have stronger activity on the *Shigella sonnei* than the fresh leaves, which were shown to be more effective at higher concentrations. This may be due to the presence of certain natural constituents in the fresh leaves such as chlorophyll and water. We have noticed that the antimicrobial activity of the Omani henna sample, was generally more evident in the leaves of the plant rather than the seeds. The latter have only demonstrated a limited antibacterial activity and at higher concentrations. The anti-*C. albicans* activity is self evident as it demonstrated sensitivity to the leaves but not the seeds.

Quinones are present in henna,[2] these are aromatic rings with 2 ketone substitutions. They are ubiquitous in nature and are characteristically highly reactive. These compounds, being colored, are

responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin.[18] It is the presence of quinones in henna, which gives that material its dyeing properties.[2] The switch between diphenol (or hydroquinone) and diketone (or quinone) occurs easily through oxidation and reduction reactions. The individual redox potential of the particular quinone-hydroquinone pair is very important in many biological systems. Hydroxylated amino acids may be made into quinones in the presence of suitable enzymes, such as a polyphenoloxidase.[19] In addition to providing a source of stable free radicals, quinones are known to complex irreversibly with nucleophilic amino acids in proteins,[20] often leading to inactivation of the protein and loss of function. For that reason, the potential range of quinone antimicrobial effects is great. Portable targets in the microbial cell are surface exposed adhesions, cell wall polypeptides, and membrane bound enzymes. Quinones may also render substrates unavailable to the microorganism. In addition, they were shown to inhibit cell growth in culture.[21]

Leaves of the Omani henna are strikingly most effective against the spectrum of bacteria tested as compared to seeds. This is probably due to the inherent characteristics of the fully grown plants and the maturity of its chemically active constituents such as quinones. Such constituents would not have been established in seeds. Although, fresh leaves did demonstrate such bacteriostatic activities in general, these were less evident when compared with the effect of dry leaves. It is possible that the drying effect on the plant causes the active ingredients to be more concentrated than those in the green leaves, where water and other constituents are still present. We have concluded that Omani henna possesses antimicrobial activities both against a wide spectrum of bacterial strains and against *C. albicans*.

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