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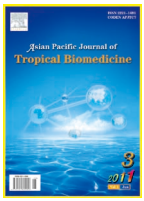

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Antibacterial activity of *Lawsonia inermis* Linn (Henna) against *Pseudomonas aeruginosa*

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ABSTRACT

Objective: To investigate the antibacterial activity of henna (*Lawsonia inermis* Linn) obtained from different regions of Oman against a wide array of micro-organisms. **Methods:** Fresh henna samples were obtained from different regions of Oman as leaves and seeds. 100 g fresh and dry leaves and 50 g of fresh and dry seeds were separately soaked in 500 mL of ethanol for three days, respectively, with frequent agitation. The mixture was filtered, and the crude extract was collected. The crude extract was then heated, at 48 ℃ in a water bath to evaporate its liquid content. The dry crude henna extract was then tested for its antibacterial activity using well-diffusion antibiotic susceptibility technique. Henna extracts were investigated for their antibacterial activity at different concentrations against a wide array of different micro-organisms including a laboratory standard bacterial strain of *Pseudomonas aeruginosa* (NCTC 10662) (*P. aeruginosa*) and eleven fresh clinical isolates of *P. aeruginosa* obtained from patients attending the Sultan Qaboos University Hospital (SQUH). 2-Hydroxy-p-Nathoqinone-Tech (2-HPNT, MW=174.16, $C_{10}H_6O_3$) was included as control (at 50% concentration) along with the henna samples tested. **Results:** Henna samples demonstrated antibacterial activity against all isolates but the highest susceptibility was against *P. aeruginosa* with henna samples obtained from Al-sharqyia region. **Conclusions:** Omani henna from Al-sharqyia region demonstrates high *in vitro* anti-*P. aeruginosa* activity compared with many henna samples from different regions of Oman.

1. Introduction

Pseudomonas is a gram negative rod micro-organism that belongs to the family Pseudomonadaceae. These pathogens are widespread in nature, inhabiting soil, water, plants, animals and humans. *Pseudomonas aeruginosa* (*P. aeruginosa*) is an important cause of infection in patients with compromised host defence mechanisms. It is the most common pathogen isolated from patients hospitalized longer than a week and is a common cause of nosocomial infections such as pneumonia, urinary tract infections and bacteremia[1]. Bacteremic pneumonia occurs in patients with neutropenia following chemotherapy and in patients with acquired immunodeficiency syndrome (AIDS)[1]. Pseudomonal bacteremia occurs in association with malignancy, chemotherapy, AIDS, burn wound sepsis, and diabetes[1]. Predisposing conditions include placement of intravenous lines, severe burns, urinary tract catheterization, surgery, trauma, and premature birth[1]. Infections with *P. aeruginosa* are complicated and can be life threatening[1]. The bacterium is resistant to many antibiotics and disinfectants, which makes it a difficult pathogen to treat.

Traditional healers have long used plants to prevent or cure infectious diseases. Almost 50% of current pharmaceuticals are derived from the plant kingdom. Plants are rich in a wide variety of secondary metabolites polyphenols, such as tannins, terpenoids, alkaloids, and flavonoids, which have been demonstrated to have *in vitro* antimicrobial properties[2-5].

Henna [*Lawsonia inermis* (*L. inermis*) Linn] is known to have medicinal properties[4,6,7]. Different species of henna are present and grown in Oman, at the south-eastern tip of the Arabian Peninsula. Omani henna is prevalent in Eastern

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and central areas of Oman.

The antimicrobial[8] and fungicidal[9,10] effect of henna has long been known and more recent studies in our laboratories confirmed previous observations[4]. In this present study we investigated the effect of the local Omani henna on several bacteria including *P. aeruginosa* and other clinical laboratory isolates. This is one part of a wider project in which we envisage to test henna on a wider variety of bacteria, viruses and fungi.

2. Materials and methods

2.1. Plant materials

The plants (as fresh leaves and seeds) were harvested from different localities (Nakhal, Salalah, Musandam, Nizwa, Khabora, Wadi and Bani-Awf) of Oman in July 2008 and identified based on ethnomedical data and interview with local communities in parallel with the Pharmacognosy Department, Faculty of Pharmacy, Sultan Qaboos University. The leaves and seeds were collected and washed thoroughly with water and air dried under shade and ground using a pestle and mortar.

2.2. Extraction of plant material

The air-dried materials of leaves (100 g) and seeds (50 g) were ground to a fine powder with a pestle and mortar. 100 g fresh and dry leaves and 50 g of fresh and dry seeds were extracted respectively, with 50% ethanol (600 mL) for three days, with frequent agitation. The ethanol extract was filtered through Whatmann No. 1 (Whatmann International Ltd, Maidstone, UK) paper and the filtrates were collected and heated in water-bath at 48 ℃ to evaporate its liquid content. The residue was dried further over night in an oven at 37 ℃. The extracts were preserved at −20 ℃ until use.

2.3. Test organisms

A wide array of different micro-organisms (*Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *P. aeruginosa*, *Bacillus* spp, *Klesiella pneumoniae*, *Salmonella* spp, *Shigella sonnei*, *Citrobacter frewndii*, *Vibrio cholerae*, *Neisseria meningitides*, *Haemophilus influenzae*, *Aeromonas hydrophila*, MRSA, *Micrococcus* spp, *Corynebacterium diphtheriae*, *Candida albicans*, *Cryptococcus neoformans*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Bacteriodes fragilis*, *Clostridium perfringens*), were initially screened for their susceptibility to the henna extracts at 50% concentration using the well diffusion method. *P. aeruginosa* (NCTC 10662), was used as the standard organism. This strain is used routinely for the antibiotic susceptibility tests of all *Pseudomonas* isolates in Sultan Qaboos University Hospital (SQUH). In addition, eleven clinical strains of *P. aeruginosa* isolated from patients attending the SQUH were used, for investigating the antibacterial activity of the different Omani henna samples, in parallel with the standard organism.

2.4. Antimicrobial assay

The well-diffusion assay[11] was used to determine the antimicrobial activity of the investigated extracts. Nutrient agar (OXOID LTD, Basingstoke, Hampshire, England) was prepared by dissolving of 27 g in water. One colony of each organism was emulsified in 4 mL of distilled water, to give approximately 1.0×10^4 CFU/mL. This was subsequently used to swab agar plates of diagnostic sensitivity test (DST, Oxoid, England). Wells of 4 mm in diameter were made and 60 μL of each henna "crude extract" dilution at different concentrations (50%, 25%, 12.5%) was prepared using sterile distilled water and placed into each well with a chipped tip pipette. Each dilution was tested in triplicate. Plates were kept for 2 h in refrigerator to enable prediffusion of the extracts into the agar. Then, the plates were incubated overnight (18 h) at 37 ℃. Ampicillin, gentamicin and amphotericin B were used as positive control. Negative controls were performed using 2-Hydroxy-p-Nathoqinone-Tech (2-HPNT, MW=174.16, $C_{10}H_6O_3$) (at 50% concentration) along with the henna samples tested. At the end of the incubation period the antibacterial activity was evaluated by measuring the inhibition zones (diameter of inhibition zone plus diameter of the well).

3. Results

Most of the henna samples obtained from different parts of Oman demonstrated low to intermediate activity against a wide range of micro-organisms (Table 1). However, fresh leaves obtained from Al-sharqiya region had the highest anti-*P. aeruginosa* activity (Figure 1). The antibacterial activity was higher than the 2-HPNT control used (Figure 1). All the four samples obtained from Al-sharqiya region, and at 50% concentration, demonstrated high anti-*P. aeruginosa* activity compared with the control.

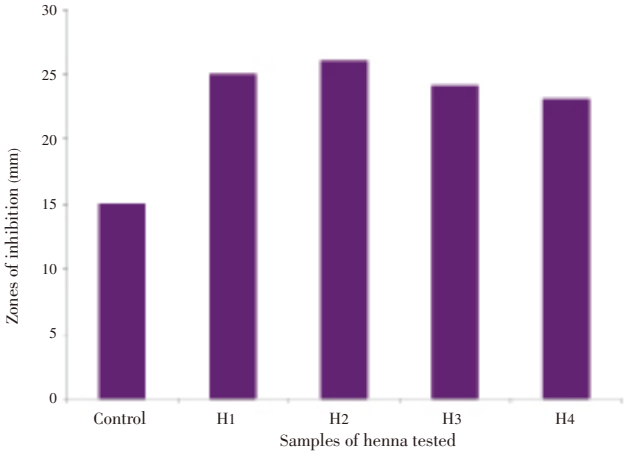

Figure 1. Antibacterial activity of henna fresh leaves against *P. aeruginosa* from Al-Sharquia region of Oman.
Control: 2-HPNT (2-Hydroxy-p-Nathoqinone-Tech.); H1-H4: Henna samples obtained from Jalan Bani Bu Ali, Sur, Badyia and Ibra, respectively. The samples H1-H4 were tested at 50% concentration. Zones of inhibition were measured in millimetres (mm) diameter and the average of three experiments was shown.

Fresh leaves, dry leaves, fresh seeds and dry seeds of the Omani henna samples demonstrated antibacterial activity against the standard strain of *P. aeruginosa*. Moreover, when using the clinical isolates of *P. aeruginosa*, the anti-*P. aeruginosa* activity was demonstrated up to 25% concentration of the fresh henna samples tested (Figure 2). All the anti-*P. aeruginosa* activity, at 50% concentration, was shown to be higher than the 2-HPNT control used. The antibacterial activity of the henna samples was still evident at 25% concentration, although the activity at this

Table 1
Antimicrobial activity of henna obtained from different regions of Oman against an array of different micro-organisms (cm).

Micro-organism	Nakhal	Salalah	Musandam	Nizwa	Khabora	Wadi Bani-Awf	2HPNT*
Staphylococcus epidermidis	2.0	2.5	2.8	2.5	2.2	3.5	3.5
Staphylococcus aureus	2.0	2.1	2.0	1.9	2.5	4.0	4.0
Escherichia coli	1.0	1.3	1.5	1.0	0.0	2.5	2.5
P. aeruginosa	1.2	1.8	1.8	1.2	0.0	3.5	3.5
Bacillus spp.	2.7	2.5	3.0	2.5	1.5	4.0	4.0
Klebsiella pneumoniae	1.5	1.4	1.4	1.1	0.0	1.5	1.5
Salmonella spp.	1.0	1.3	1.5	1.2	0.0	3.0	3.0
Shigella sonnei	1.5	1.5	1.8	1.0	1.0	3.4	3.4
Citrobacter frewndii	1.2	1.7	2.0	1.5	0.0	1.7	1.7
Vibrio cholerae	2.5	3.0	2.8	3.0	1.5	3.5	3.5
Neisseria meningitidis	2.0	2.5	2.2	2.6	1.0	2.6	2.6
Haemophilus influenzae	1.7	2.2	2.0	2.5	1.9	3.0	3.0
Aeromonas hydrophila	2.0	2.5	2.0	3.0	2.2	4.0	4.0
MRSA	2.9	3.0	2.4	3.0	2.5	4.0	4.0
Micrococcus spp.	2.0	2.5	2.4	3.0	1.5	3.5	3.5
Corynebacterium diphtheriae	1.0	1.5	1.0	1.5	2.0	1.0	1.0
Candida albicans	1.0	1.9	1.5	2.0	1.0	2.5	2.5
Cryptococcus neoformans	1.5	2.5	2.5	2.9	1.5	2.9	2.9
Streptococcus pyogenes	2.0	2.5	2.0	2.0	1.2	1.5	2.5
Streptococcus pneumoniae	2.0	2.1	2.5	2.0	1.0	2.0	2.5
Bacteriodes fragilis	1.0	2.0	1.5	1.5	1.2	2.0	4.0
Clostridium perfringens	3.0	3.0	3.5	3.0	4.5	3.0	4.5

*Control: 2-HPNT (2-Hydroxy-p-Nathoqinone-Tech).

concentration was slightly lower than the control used (Figure 2). At concentration of 12.5 % the anti *P.aeruginosa* activity was not detected.

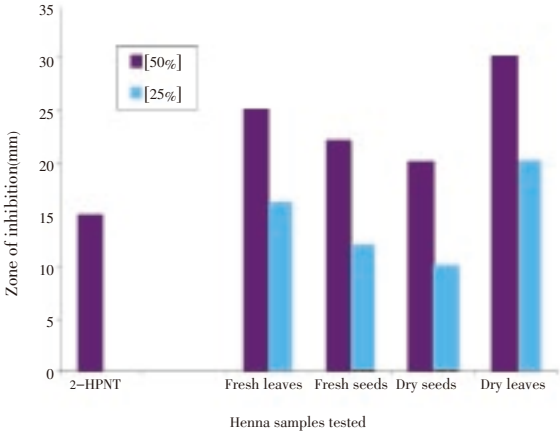

Figure 2. Anti-*P. aeruginosa* activity of Omani henna leaves and seeds at different concentrations.
2-HPNT: 2-Hydroxy-p-Nathoqinone-Tech, used as control.
Zones of inhibition were measured in millimetres (mm) diameter and the average of three experiments was shown.

Dry leaves and fresh leaves demonstrated high activity against *P. aeruginosa* both at 50% and at 25% concentrations and the activities were higher than the tested control. Moreover, fresh seeds and dry seeds demonstrated higher activity compared with the control only at 50% concentration of the henna samples tested. At 25% concentration, the anti-*P. aeruginosa* activity was still evident but lower than the control (Figure 2).

4. Discussion

In this study we have shown that henna samples from different regions of Oman demonstrated antibacterial activity against a wide range of different bacterial strains with the highest antibacterial activity being demonstrated against *P. aeruginosa* organisms. *P. aeruginosa* is an opportunistic pathogen that rarely causes disease in healthy people. This bacterium has minimal nutritional requirements and can tolerate a wide variety of physical conditions. *Pseudomonas* species are both invasive and toxigenic[1]. The pathogenesis of *P. aeruginosa* infections is multifactorial and complex. *P. aeruginosa* is the fourth most commonly isolated nosocomial pathogen, accounting for approximately 10% of all hospital-acquired infections[12]. It is found on the skin of some healthy persons and has been isolated from the throat and stool of non-hospitalized patients[13]. The gastrointestinal carriage rates among hospitalized patients increases to 20% within 72 h of admission. Internationally, *P. aeruginosa* is common in immunocompromised patients with diabetes.

Although infections caused by *P. aeruginosa* are treatable and potentially curable, acute fulminant infections, such as bacteremic pneumonia, sepsis, burn wound infections, and meningitis, are associated with extremely high mortality rates[14]. Treatment of *P. aeruginosa* is usually through intravenous multiple antibiotic combinations, and unfortunately it does not always work. Pseudomonal infections are usually treated with a combination of anti-pseudomonal antibiotics such as cephalosporin, carbapenems, quinolones and aminoglycosides. However, with the emergence of antibiotic resistance, resulting in treatment failures, henna, among other natural products, may be a cheap alternative method for the treatment of pseudomonal infections.

Henna is widely used throughout Arabia including Oman. In addition to its use as a cosmetic, henna leaves

are also used for fevers, as a local anesthetic[15], anti-inflammatory and for treating mouth ulcers[7]. The most striking antimicrobial effect of henna is demonstrated by the inhibitory activity against *P. aeruginosa*. It is interesting to note that antibacterial activity of some Omani henna was much higher than the tested control (2-HPNT). Our *in vitro* results indicate a possible important role for henna in the *in vivo* treatment of *P. aeruginosa*.

Although the fresh and dry seeds demonstrated antibacterial activity against *P. aeruginosa*, our fresh henna leaves demonstrated the highest anti-*P. aeruginosa* activity. Leaves of the Omani henna are strikingly most effective against the spectrum of bacterial isolates tested as compared with seeds. This is probably due to the inherent characteristics of the fully grown plants and the maturity of its chemically active constituents such as quinines. Such constituents would not have been established in seeds. Although fresh and dry seeds demonstrated antibacterial activities, these were less evident when compared with the effect of fresh and dry leaves. This may be attributed to the added presence of chlorophyll, which is one of the constituents of fresh leaves that are known to possess antimicrobial activity[4,16,17]. Henna leaves contain up to 5% by weight of the compound (2-hydroxy-1,4-naphthoquinone).

Quinones are present in henna[3]. These are aromatic rings with two ketone substitutions. They are ubiquitous in nature and are characteristically highly active. These compounds, being coloured, 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 that gives the material its dyeing properties[3]. The switch between diphenol (or hydroquinone) and 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. Hydroxilated 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].

We have concluded that some Omani henna possess high antibacterial activities against *P. aeruginosa*. Further work is required to further demonstrate this activity using different henna samples from different locations outside Oman. The possibility of using a cream or soaps incorporating henna active ingredients may be of great advantage for hygiene purposes for both physicians and patients in hospitals especially in intensive care units or infectious disease units where immuno-compromised patients are treated.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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