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Antimicrobial and Anti-parasitic Effect of Six Landraces of Henna *(Lawsonia inermis* L.)

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Abstract

Background: Henna is one of the plants that have been studied by traditional medicine practitioners for many centuries, and its description has been mentioned in many books. The main habitats of the plant are Iran, Pakistan, India, tropical, and subtropical regions of East Africa, and South Asia. This species is distributed in the western and southern areas of Iran, such as Hormozgan, Khuzestan, Kerman, Sistan and Baluchistan. In this study, the antibacterial and antiparasitic effects of six varieties of henna (*Lawsonia inermis* L.) Dalgan, Ordougah, Salmanyeh, Bam, Kahnooj, and Jiroft were investigated against three species of *Streptococcus agalactiae*, *Trichomonas vaginalis*, and *Pseudomonas aeruginosa, in vitro*.

Methods: The maceration method was used to prepare the hydroalcoholic extract. *S. agalactiae* PTCC1864, *P. aeruginosa* ATCC 27853, and *Trichomonas vaginalis* were prepared. The Folin-Ciocalteu method was applied to measure total phenol. Minimal inhibitory concentrations of *P. aeruginosa* and *S. agalactiae* were measured for all extracts, and the growth inhibition of *T. vaginalis* was reported for all extracts at 24, 48, and 72 hours.

Results: The MIC of the Bam landrace against *P. aeruginosa* and *S. agalactiae* was smaller than the other landraces, and the Bam landrace could 100% inhibit the growth of *T. vaginalis*. The total phenol of Bam was higher than others.

Conclusion: The different landraces of henna have different amounts of total phenols and rates of microbial and parasite inhibitory effects.

Keywords: *Lawsonia inermis*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Trichomonas vaginalis*, antimicrobial, anti-trichomoniasis

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Introduction

Many modern medicines are derived from herbal medicines, and the therapeutic effects of several native plants for several illnesses have been reported by herbalists (1,2). Natural products are one of the valuable sources of the traditional and synthetic herbal medicines used in the primary health care systems (3). According to the World Health Organization (WHO), there are currently 20 000 species of plants with medicinal properties used by more than 80% of the world's population to maintain their health (4). Many plants can grow in Iran, due to Iran's special climatic conditions and diversity of climate. Iran houses nearly 8000 plant species and is a huge potential reservoir of bioactive compounds (5).

Henna (*Lawsonia inermis* L.) has been employed in the Orient since ancient times for beauty and health purposes (6). Henna belongs to the Lythraceae family. This plant has shrubs about 2 m tall and young shoots with four ears. The flowering season of this plant is early spring to early autumn. The main habitats of the plant are Iran, Pakistan, India, subtropical and tropical areas of South Asia, and East Africa. In Iran, this species is distributed in the western and southern regions, such as Hormozgan, Khuzestan, Kerman, and Sistan and Baluchistan (7).

Henna is one of the plants that has been studied by traditional medicine practitioners for many centuries,

and its description has been mentioned in many books. In Persian medicine, henna is advised for uterine diseases especially Qoruh-e-Rahem which is similar to cervicitis in conventional medicine (8).

Kerman province and Sistan Baluchistan are located in the southeast of Iran. Kerman province has an area of 183 193 km^2 and Sistan and Baluchistan province has an area of 180 726 km^2. The climate of these two provinces is completely different in different areas due to the size of the region, the existence of low and high altitudes, and special climatic conditions (9). Considering the effect that climatic conditions have on the production of secondary metabolites of plants and considering that henna is cultivated in these two provinces, this study aimed to find the landrace that shows the best effect against some microorganisms causing cervicitis.

Accordingly, the antibacterial effects of the six henna landraces of Dalgan, Ordougah, Salmanyeh, Bam, Kahnooj, and Jiroft were investigated against three species of bacteria, including *S. agalactiae*, *T. vaginalis*, and *P. aeruginosa*, in the laboratory.

Methods

Chemicals

Gallic acid (Merck), Folin-Ciocalteu reagent (Merck), Lawson (2-hydroxy 1,4 naphthoquinone) (Merck), sodium carbonate (Merck), Muller Hinton agar (Merck), and NaCl blood agar (Merck), Ciprofloxacin (Farabi Company, Iran).

Collections of plant materials and extraction

The local landraces of *L. inermis* (henna), including Dalgan, Ordougah, Salmaniyeh, Bam, Kahnooj, and Jiroft were collected and identified by the Medicinal and Industrial Research Institute, Ardakan (voucher numbers SSU 0081, SSU 0079, SSU 0080, SSU 0068, SSU 0060, and SSU 0059, respectively). To obtain henna powder, the dried leaves were sieved.

The hydroethanolic extract was prepared using the maceration technique. The powders of leaves of *L. inermis* were passed through the sieve, and macerated with ethanol 80% for 72 hours. A magnetic stirrer was used for extraction at room temperature through occasional shaking. The Buchner funnel was used to filter the solution and concentrated in Laboratory conditions..

Determination of total phenol content (TPC)

The Folin-Ciocalteu technique was used to measure the TPC of extracts. Gallic acid (GA) was the standard. Seven concentrations of GA (10–200 µg/mL) were obtained and mixed with 0.5 ml of Folin-Ciocalteu reagent and sodium carbonate 7.5% after 3–8 minutes. The tubes were incubated for 30 minutes at room temperature and the absorbance reading was done at 760 nm. The experiments were performed in triplicate. TPC was assessed as mg of GA per gram by the equation taken from a standard curve of GA.

Bacterial strains, culture media, and growth conditions

The *S. agalactiae* PTCC1864 and *P. aeruginosa* ATCC 27853 commercial strains were employed and streaked on Blood agar and Nutrient agar plates, and then incubated at 37 °C for 24 hours. Bacterial suspension equivalent 0.5 McFarland standard was provided from the bacteria.

Inhibition zone determination

Streptococcus agalactiae and *P. aeruginosa* were passaged on Nutrient agar plates, followed by incubation (37 °C for 24 hours). Regarding the agar well diffusion technique, covering the Muller Hinton (MH) agar plate was done with bacterial suspension, and a sterile Pasteur pipette was used to create 6 mm wells. Afterwards, the wells were filled with 50 µL of different levels of plant extracts. The plates underwent incubation (37 °C for 24 hours). A well with antibiotic (ciprofloxacin) served as positive control and a well containing Ethanol 20% (extract solvent) served as negative control. The inhibition zones were determined with a ruler and noted (in mm) after 24 hours. The tests were conducted in triplicate.

Determination of minimal inhibitory concentration (MIC)

The MIC of extracts was determined using the broth micro-dilution test and performed in MH broth, Clinical Laboratory Standards Institute (10). Five dilutions of henna extracts (8–40 mg/mL) in MH broth were prepared and, 50 µL of each dilution was dispensed into the wells, inoculated with 25 µL of the bacterial suspension (10^8 CFU/mL (0.5 McFarland scale)) and 25 µL MH broth and mixing completely (final volume for wells was 100 µL). The plates were incubated at 37 °C for 24 hours. Then, MIC was considered the lowest dilution that was clear and indicated full suppression of bacterial growth.

The MBC was determined by incubating 10 µL of broth aliquots from each well in MH agar at 37 °C for 24 hours. All experiments were repeated in triplicate independently(11). All experiments were repeated in triplicate independently.

Anti-parasite screening

Vaginal swabs were collected from women with trichomoniasis attending healthcare centers in Yazd, Iran, for the isolation of *T. vaginalis*. The isolated *T. vaginalis* was cultured in TYI-S-33 medium, and stored in the parasitology research laboratory of the university until use. *T. vaginalis* cells were obtained from the logarithmic growth phase and a hemocytometer slide was used to estimate the number of cells. Afterwards, to assess the anti-*T. vaginalis* effects of *L. inermis* landraces 1×10^5/mL cells were used.

For screening of the anti-parasite activity of the extracts, 0.013 to 26.6 mg/mL of the extract concentrations were disposed of in phosphate buffered saline (PBS) and the microtubes were mixed. Then, each tube was filled with 100 µL of medium (10^5 live *T. vaginalis* microorganisms) and was incubated at 37 °C, and then the live parasites were calculated at 24, 48, and 72 hours. The tube was shaken, and a hemocytometer slide was used to calculate the live cells with 0.4% trypan blue. Metronidazole (50 ug/mL) was the positive control and PBS was the negative control. All tests were conducted in triplicate. The number of live parasites was compared with the positive and negative controls. The percent of growth inhibition (GI %) was calculated with the formula below:

Percent of growth inhibition = a–b/a ×× 100.

a: mean number of live parasites in the negative control tubes
b: mean number of live parasites in the tested tubes.

Statistical analysis

Data were evaluated for normality. They were analyzed by one-way ANOVA and Tukey's post hoc test (SPSS 16).

Results

TPC

The results of total phenol content are reported in Table 1. The Bam landrace had the highest amount of total phenol and the Salmaniyeh landrace had the lowest amount.

Antibacterial activity

The MIC of the Bam landrace against *P. aeruginosa* (6 mg/mL) and *S. agalactiae* (8 mg/mL) was smaller than other landraces. Salmaniyeh and Dalgan did not show any antibacterial effect against *S. agalactiae* (Table 2).

As shown in Table 3, the zone of growth inhibition (ZGI) (mm) of the Ordougah landrace against *P. aeruginosa* and *S. agalactiae* was 21.67 and 19.67 mm, respectively.

Anti-parasite activity

The anti-parasite activity results of henna extracts are shown in Figure 1. The anti-parasite effect of different concentrations of the Ordougah and Dalgan landraces showed a significant difference ($P<0.05$) in various time points, except for 48 and 72 hours after treatment for the Dalgan landrace (40 mg/mL) and 48 and 72 hours after treatment for Ordougah (20 mg/mL) ($P>0.05$).

The Bam and Salmaniyeh landraces inhibited the trichomonas 100% at 40 mg/mL. As Table 1 shows, the IC50 of the Salmaniyeh landrace was 0.15 mg/mL after 72 hours, which was the smallest IC50. After 24 hours, the IC50 of Bam and Salmaniyeh was 1.8 and 1.5 mg/mL, respectively.

Discussion

We compared the antimicrobial and anti-parasite effects of the Dalgan, Ordougah, Salmaniyeh, Bam, Kahnooj, and Jiroft landraces of henna. The Bam landrace had the best antimicrobial and anti-parasite effect. The Bam landrace also had the most phenol content. In a previous study on henna landraces, the relationship between total phenol content and antimicrobial effect was reported (12). There is a relationship between latitude and the biosynthesis of

Table 1. Total phenol and IC50 of henna landraces in 24, 48 and, 72 hours

Henna landraces	IC50 (mg/mL)			Total phenol (µg/mL)
	24 h	48 h	72 h	
Bam	1.8	1.2	1.3	303.2
Jiroft	2.2	1.7	1.8	196.3
Ordougah	5	2.5	0.3	190.04
Dalgan	5.8	2.5	0.31	171.5
Kahnooj	3	2	3.1	161.2
Salmanyeh	1.5	0.5	0.15	143.2

Table 2. Minimum inhibitory concentration (mg/mL) of henna landraces against *Streptococcus agalactiae* and *Pseudomonas aeruginosa*

Landraces	Bacteria											
	P. aeruginosa						*S. agalactiae*					
	Jiroft	Kahnooj	Bam	Salmaniyeh	Dalgan	Ordougah	Jiroft	Kahnooj	Bam	Salmaniyeh	Dalgan	Ordougah
A	---	---	3	---	---	---	---	---	3	---	---	---
B	---	6	6	---	---	---	---	---	6	---	---	---
C	8	8	8	8	8	8	8	8	8	8	8	8
D	10	10	10	10	10	10	10	10	10	10	10	10
E	20	20	20	20	20	20	20	20	20	20	20	20
F	30	30	30	30	30	30	30	30	30	30	30	30
G	40	40	40	40	40	40	40	40	40	40	40	40
H	C (-)											
L	C (+)											
M	Medium											

Conc.: concentration; C(+): positive control, ciprofloxacin; C(-): negative control, bacteria and medium; GBS: *Streptococcus B.*; Darker table cells show bacteria growth; ---: These concentrations have not been used for the landraces.

Table 3. Zone of growth inhibition (mm) of henna landraces against *Streptococcus agalactiae* and *Pseudomonas aeruginosa*

Conc. (mg/mL)	ZGI (mm) of *Pseudomonas aeruginosa*						ZGI (mm) of GBS					
	Landraces											
	Jiroft	Kahnooj	Bam	Salmaniyeh	Ordougah	Dalgan	Salmaniyeh	Ordougah	Dalgan	Jiroft	Kahnooj	Bam
3	--	--	7.3		---	---	---	---	---	--	--	7.3
6	--	--	12.8	---	---	---	---	---	---	--	7.5	8.3
8	0	0	13.6	---	14.33	0	0	10.67	7.33	7.3	13	10.6
10	0	7.3	13.7	7.33	15.67	0	7.33	12	8.67	9.7	15	13
15	--	--	14.3	---	---	---	---	---	---	---	--	15.3
20	10.6	7.6	15.6	8.33	17.67	10	8.33	14.33	10.67	15.7	18.3	18
30	11.6	8	18.3	9.67	19	10	9.67	16	12	17.7	19.3	--
40	13	11	14.3	11.33	21.67	11.33	11.3	19.67	13.67	18.7	21.6	20

ZGI: Zone of growth inhibition; GBS: group B *Streptococcus*.

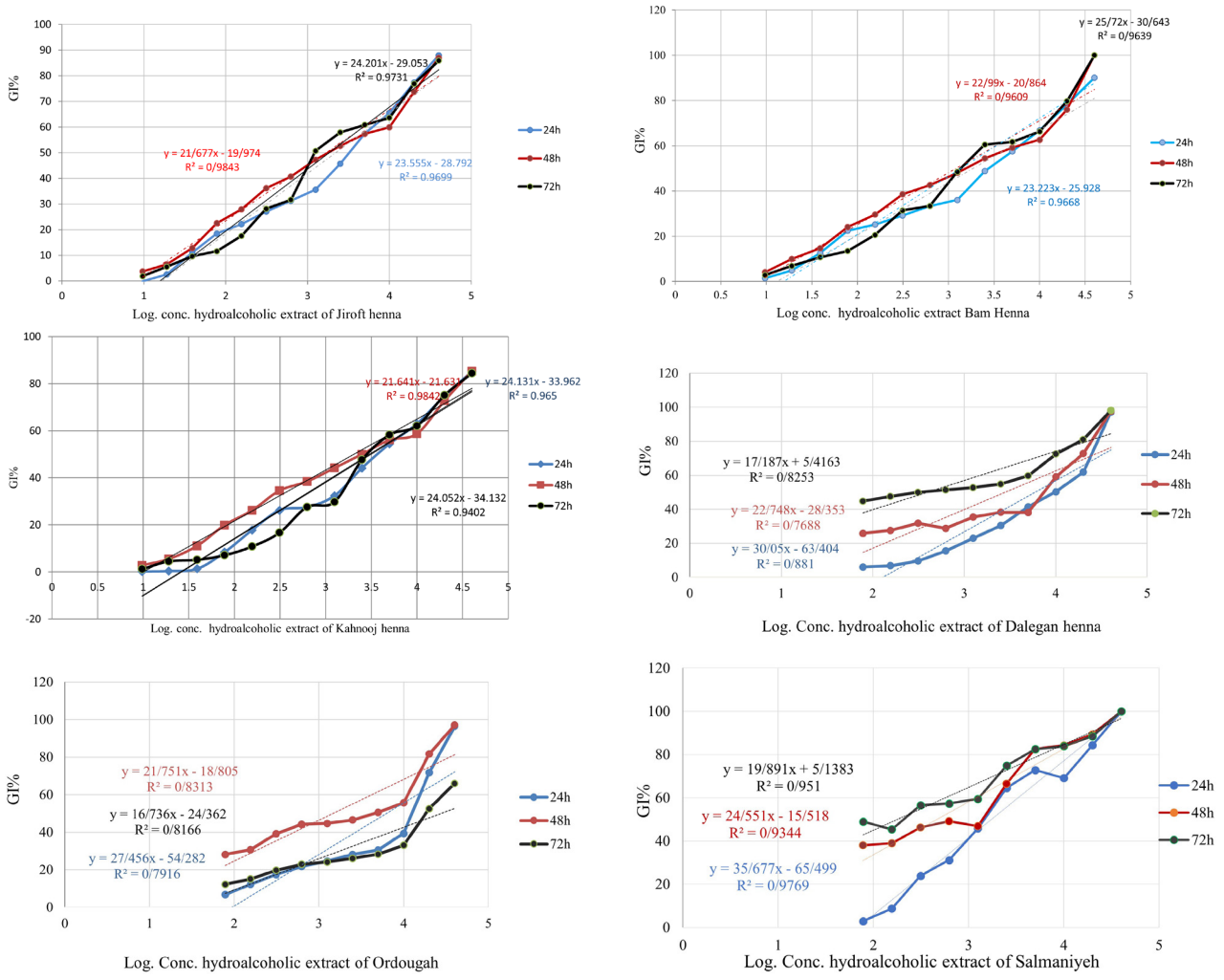

Figure 1. Growth inhibitory percent (GI%) of *T. vaginalis vs.* logarithm (Log) of concentration of extracts of henna landraces. GI%: percentage of growth inhibitory of parasites

secondary metabolites. The biosynthesis of flavonoids increases in more southern latitudes. The plants were gathered from Kerman and Sistan and Baluchestan provinces in Iran. Bam is more to the north than other cities, and the total phenols in the Bam landrace were more than others (13).

Flavonoids, like luteolin and apigenin, are found in henna. The antimicrobial effect of apigenin has been evaluated in many studies. Apigenin had no effect against *S. aureus* (ATCC 29213, 8325-4, BAA-1717, and wood 46). However, apigenin has reverse antibiotic (RA) activities against quinolone-resistant *S. aureus*. RA substances are

indifferent against antibiotic-susceptible bacteria but active against the relevant antibiotic-resistant bacteria. Some apigenin derivatives have high antibacterial activity against *Bacillus subtilis*, *S. aureus*, *Pseudomonas aeruginosa,* and *Escherichia coli* (14).

Regarding the anti-parasite activity of the studied extracts, the Bam landrace could inhibit the growth of the parasite 100%. Fatahi Bafghi et al showed that the Ghale Ganj landrace had less MIC (4 mg/mL) and its total phenol was higher than others (254 µg/mL) (12).

In Persian medicine, henna has been advised for uterine diseases. This study and another previous study (12) showed that henna may be useful for treating uterine diseases through its antimicrobial and anti-parasite effects. Trichomoniasis is the most prevalent, curable, non-viral sexually transmitted disease (15). *S. agalactiae* is the one of causes of serious neonatal infections and colonizes nearly 10%–30% of all pregnant women (16). Colonization of the vagina with *P. aeruginosa* has been reported (17). Therefore, due to its antimicrobial effects, henna can be used for developing new medicines to treat uterine infections. Because of its flavonoids and naphthoquinone constituents, henna has shown strong antimicrobial effects in many studies (18). The anti-inflammatory effect of henna can also be used to treat some inflammatory diseases, like cervicitis. Lawson as a naphthoquinone (NTQ) has an antimicrobial effect, especially against Gram-negative bacteria. It also has antiparasitic effects against malaria (19). Generally, as aromatic cyclic compounds, NTQs have a basic naphthalene skeleton and can be detected in many vegetative parts of higher plants, fungi, and algae. They have many pharmacological activities, including antibacterial, anticancer, anti-inflammatory, antiviral, and anti-trypanosome effects. Naphthoquinones have been reported to cause organelle disruption, intracellular ROS generation, and apoptosis leading to the death of the parasites (20).

Conclusion

We showed that henna could inhibit the growth of *T. vaginalis*, *P. aeruginosa,* and *S. agalactiae* and the rate of inhibition depended on the phenol content.

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Competing Interests

The authors declare that they have no competing interests.

Ethical Approval

Experimental research and field studies on plants (either cultivated or wild), including the collection of plant material, comply with relevant institutional, national, and international guidelines. The protocol used in this research was approved by the Ethics Committee of the Shahid Sadoughi University of Medical Science (Yazd, Iran) (approval No.: IR.SSU.MEDICINE.REC.1398.338, IR.SSU.MEDICINE.REC.1399.231).

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