



Identification of Luteolin in Henna (*Lawsonia inermis*) Oil, a Persian Medicine Product, by HPTLC and Evaluating Its Antimicrobial Effects

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Abstract

Background and objectives: Persian scholars such as Avicenna and Zakariya al-Razi have pointed out many uses for henna oil. The present study aimed to propose a method for standardization of this oil. **Methods:** The luteolin content has been evaluated quantitatively by HPTLC method to standardize the henna oil. The oil sample was applied on silicagel plate and the bands were developed using CHCl₃-MeOH (95:5). The plate was scanned at λ 254 nm. The minimum inhibitory concentration was determined through the broth macrodilution test to examine the antibacterial activity of the oil. **Results:** The retention factor of the sample zone of luteolin standard was 0.6 ± 0.01 . The concentration of luteolin in henna oil was 56.57 ± 0.66 $\mu\text{g/mL}$. MIC of Henna oil against *Gardnerella vaginalis* and *Neisseria gonorrhoeae* was 87 $\mu\text{g/mL}$ and against Streptococcus was 870 $\mu\text{g/mL}$. **Conclusion:** According to the results of this study, henna oil contains the luteolin. Further studies are needed to identify other henna oil compounds and their effects. *Gardnerella vaginalis*, *N. gonorrhoeae*, Group B streptococcus are among the pathogenic agents of cervicitis. The application of henna oil for treatment of uterus diseases in Persian medicine can be justified given the antimicrobial effects of henna oil on these three species of bacteria.

Keywords: henna oil; HPTLC; *Lawsonia inermis*; Luteolin; Lawsone

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Introduction

Oils are among the oldest forms of medicines used in Persian medicine, with Iranian medical scholars believing their effects to be stronger than those of plants. These traditional drug forms were prescribed alone or in combination with pills, powders, or herbal extracts. Oils are suitable formulations that are recommended for the treatment of the brain, nerve, uterus, and stomach

diseases. In Persian medicine, topical treatment of the nervous system is believed to be more effective than oral medications due to changes occurring during digestion [1]. Vegetable oils are generally known as “Dohn” or “Adhan”, and their preparation methods are specified in books called “Qarabadin”, which include instructions for preparation of compound medications [2].

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Henna oil is a product obtained from the henna plant. In Persian medicine reference books, henna oil has been used in combination with other medicines to treat various diseases. Iranian scholars such as Avicenna and Zakariya al-Razi have pointed out many uses for henna oil, including relieving arthralgia, strengthening the hair, relieving pain, and treating uterine diseases [3-5]. However, in Persian medicine reference books, henna oil is most commonly recommended as a treatment or supplementary treatment for uterine diseases and arthralgia. Today, the plant is also used for many medicinal purposes, e.g. as stringent, for hemostasis, hypotension, and sedation. Many studies have reported various effects for henna, such as antimicrobial, antihyperglycemic, antioxidant, and wound healing properties [6,7].

Henna, *Lawsonia inermis* L. (Lythraceae), is known as a medicinal plant in Asia and the Mediterranean region. It is a globally used herb that is widely available in tropical and subtropical areas. The plant is a 6-7-meter long shrub in tropical Africa (Morocco, Egypt, Tunisia, and Algeria) and Asia (India, Iran, etc.). Henna is native to Iran in Kerman and Sistan and Baluchestan provinces [8]. It contains carbohydrates, proteins, flavonoids, tannins, alkaloids, terpenoids, quinones, coumarins, xanthenes, and fatty acids [9].

The present study aimed to propose a method for standardization of henna oil in order to carry out more studies on its therapeutic effects. The antimicrobial effects of henna oil were then investigated on the bacterial species *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, and group B *Streptococcus*. Since Persian medicine scholars have prescribed henna oil for the treatment of uterine diseases, especially infectious cases, the above three microorganisms were selected given their significant role in the incidence of cervicitis and infectious uterine diseases [7].

Material and Methods

Chemicals

Reference standard luteolin was purchased from Rot (Germany), agar and Twin 80 were purchased from Merck (Germany), and analytical grade solvents were obtained from SD Fine Chemicals.

Plant collection

Henna was collected from a region near Kerman

called Shahdad where henna is cultivated. A sample of the herb was kept at the Herbarium of Kerman University of Medical Sciences, Faculty of Pharmacy, Kerman, Iran with the herbarium number KF 1408. The leaves were then separated from the plant, ground by a mill, and passed through 40-mesh sieve.

Henna oil preparation

Henna oil was prepared according to the methodology of Persian medicine called "Qarabadin" [4]. Fifty g of plant powder was soaked in 300 mL distilled water overnight and then heated to 90 °C on a heater for one h. It was then filtered using the vacuum Buchner funnel. The henna extract was mixed with an equal amount of sesame oil (Samar, ardakan). The extract was heated again for complete evaporation of aqueous extract and remaining the oil only.

High performance thin layer chromatography (HPTLC)

Preparation of standard luteolin solution: a stock solution of luteolin was prepared by dissolving 10 mg of accurately weighed luteolin in methanol reaching a volume of 100 mL. The working solution of luteolin was prepared by appropriate dilutions of the stock solution with methanol.

Chromatographic conditions: stationary phase: precoated silica gel 60 F₂₅₄ HPTLC aluminum plates (20 cm × 7 cm) were obtained from Merck Company (Germany).

Solvent system: the standard solutions were produced at concentrations of 12, 24, 36, 48, and 60 µg/mL and they were used in triple 10 µL volumes (6 mm band length), with 15 mm from the bottom of the plate. The application rate was set automatically. The bands were developed using CHCl₃-MeOH (95:5). The plate was scanned at λ 254 nm. Peak areas were recorded for tracks and a calibration curve was established for luteolin by plotting the peak area (y axis) against the amount of luteolin in µg (x axis).

Sample preparation

Although lawsone is the main ingredient of henna, this substance is not water-soluble. Henna oil is prepared using its aqueous extract; therefore, the probability of lawsone into the oil is very low, as confirmed by results of Thin Layer Chromatography (TLC). Thus, henna oil was standardized by the luteolin marker as a flavonoid in henna [10].

The henna oil sample was mixed with an equal volume of methanol, stirred for an hour, and centrifuged at 4000 rpm for 13 min to make a transparent extract. Ten microliter of the oil extract with each standard concentration was then poured on the plate three times. To measure the device accuracy, a certain amount of luteolin (12 and 48 μg) was added to the oil extract and its concentration was determined (table 1).

Antimicrobial evaluation

The growth-inhibition activity of the samples was evaluated against Gram-positive bacterial species viz. *Gardnerella vaginalis* ATCC 14018, *Neisseria gonorrhoeae* ATCC 31426, and Group B *Streptococcus* ATCC 13813.

The bacterial strains were prepared from stocks from the Pasteur Institute of Iran (Tehran) and then aerobically cultured at 35 °C for 24 h in Brain heart infusion (BHI) (Merck, Germany).

Determination of minimum inhibitory concentration (MIC)

MIC was determined using the broth macrodilution test, also known as the tube dilution test. A tube containing medium and tween 80 without henna oil was considered as the negative control. The experiment was repeated 3 times. Ciprofloxacin was considered as the positive control [11].

Results and Discussion

The identity of luteolin in all samples was evaluated by comparison of the retention factor (R_f) of the sample and luteolin standard, viz. 0.6 ± 0.01 (figure 1).

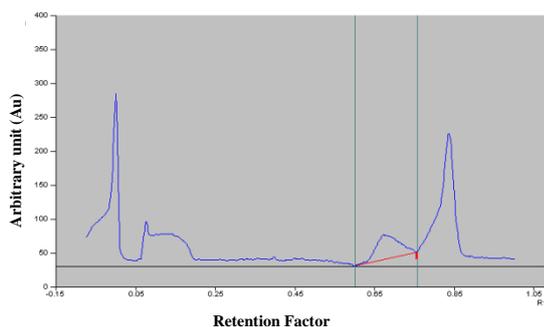


Figure 1. HPTLC chromatograms of methanol solution of luteolin

The concentration of luteolin in henna oil was 56.57 ± 0.66 $\mu\text{g}/\text{mL}$. A strong linear correlation was found between the surface under the curve and the applied standard concentration with a

confidence interval of $r=0.9948$ and a linear regression equation of $y=14.742x+492.8$.

The recovery rates were 114.7 and 108.81, respectively (table 1).

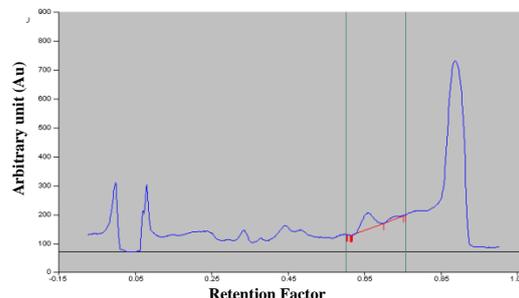


Figure 2. HPTLC chromatograms of methanol extract of henna oil

Table 1. Results of the recovery of HPTLC method for quantification of luteolin in henna oil

	Expected ($\mu\text{g}/\text{mL}$)	Found \pm SD ($\mu\text{g}/\text{mL}$)	Recovery (%)
Mixture 1	41.71	48 ± 2	115
Mixture 2	53.71	63.41 ± 2	118

Mixture 1: 1 mL standard solution (12 $\mu\text{g}/\text{mL}$) added to 2 mL oil extract; Mixture 2: 1 mL standard solution (48 $\mu\text{g}/\text{mL}$) added to 2 mL oil extract

The antibacterial action of henna oil against *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, and Group B *Streptococcus* has been shown in table 2. Henna oil showed similar inhibitory effects on *G. vaginalis* and *N. gonorrhoeae* at a concentration of 87 $\mu\text{g}/\text{mL}$, whereas the oil showed a comparatively weaker inhibitory effect on *Streptococcus* at 870 $\mu\text{g}/\text{mL}$.

According to the results of this study, henna oil contains the flavonoid luteolin as a flavon, which is among low-polarity flavonoids extractable with non-polar solvents (figure 2). Henna contains luteolin-7-O- glycoside. Glycosides are typically water soluble and are extracted from plants using water; therefore, they are expected to exist in the oil; however, lawsone is not water soluble due to its acidic property ($\text{pK}_a=4$) [12,13]. Since henna aqueous extract was used to prepare the oil, lawsone was not detected in the extract or in the oil. Therefore, luteolin was used as a for oil standardization [10]. Similarly, Zargarani et al. used flavonoids and Folin-Ciocalteu method to standardize chamomile oil. Similar to henna oil, chamomile oil was prepared by heating the aqueous extract of chamomile flower in sesame oil [14]. The presence of luteolin in henna oil can increase the probability that some polyphenolic compounds and flavonoids of henna leaves may be present in the oil.

Table 2. MIC and MBC of henna oil for the three experimental microorganisms

		<i>Gardnerella vaginalis</i>	<i>Neisseria gonorrhoeae</i>	Group B <i>Streptococcus</i>
Henna oil	MIC	87 µg/mL	87 µg/mL	870 µg/mL
	MBC	870 µg/mL	870 µg/mL	8700 µg/mL
Positive control (ciprofloxacin)	MIC	0.4 µg/mL	0.05 µg/mL	0.5 µg/mL
	MBC	4 µg/mL	0.5 µg/mL	5 µg/mL

MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration

The analgesic effects of henna oil pointed out in Persian medicine references are probably related to the anti-inflammatory effects of flavonoids. Avicenna, al-Razi, and other Persian medicine experts recommended henna oil for the treatment of infectious uterine diseases through vaginal application [5,15]. *Gardnerella vaginalis* and *N. gonorrhoeae*, and Group B *Streptococcus* are among the causing pathogens of such diseases as cervicitis. This application of henna oil can be justified given its antimicrobial effects on these three species of bacteria [7].

To the authors' knowledge, no study has yet been carried out on the antimicrobial effects of henna oil; however, antimicrobial effects of henna plant have been studied extensively. There are documentations of henna antimicrobial effects since 1968, and studies in this area are still ongoing. The aqueous, methanol, ethanol, and even acetone extracts of various parts of henna plant have shown inhibitory effects against various human pathogens such as Gram-positive (*Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*) and Gram-negative (*Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*) bacteria [13]. Sesame oil had no antimicrobial effects against none of the microbes examined in this study.

In the present study, henna oil provided less resistance to *Streptococcus* than towards the other two microbes. On the other hand, Mohammad et al. investigated the effect of chloroformic extract of henna against *Streptococcus*, and reported that henna extract showed antimicrobial effects at high concentrations [16].

Despite the fact that a large number of different phytochemicals can be isolated from henna, few studies have investigated the antimicrobial effects of the pure compounds. Gallic acid and lawsone are respectively responsible for antimicrobial effects of aqueous and chloroformic extracts of this plant against *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus citreus*,

Sarcina lutea, *Staphylococcus faecalis*, *Corynebacterium pyogenes*, *Corynebacterium* sp., *Shigella dysenteriae*, *Shigella flexneri*, *Escherichia coli*, *Klebsiella aerogenes*, *Mycobacterium phlei*, *Salmonella paratyphi*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* [14]. The present study suggested luteolin is an appropriate factor for standardization of henna oil and the antimicrobial effects of the oil has justified its use for treating women diseases in Persian medicine. Further studies are needed to identify other henna oil compounds and their effects.

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Author contributions

Payam Khazaeli, Mitra Mehrabani, Soodabeh Bioos, Ahmad Mosadegh and Mohammad Hasan Moshafi designed different parts of study and Rahele Zarehahi performed all tests. Rahele Zarehahi and Payam Khazaeli analyzed the data and wrote the manuscript. All authors have read and approved the final manuscript.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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Abbreviations

HPTLC: High Performance thin layer chromatography; TLC: thin layer chromatography; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; BHI: brain heart infusion