

# Formulation, Physicochemical, and Microbial Assay of Henna Oil Vaginal Suppository Formulated with Polyethylene Glycol Bases

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Received: 07 August 2018

Revised: 22 September 2018

Accepted: 04 November 2018

## What's Known

- We know that in Persian Medicine resources, henna is suggested for the treatment of uterine diseases, including cervicitis, uterine diseases, tumefaction of uterine, uterine rectification, infection, prevention of pregnancy, and enhancement of sexual desire. In most cases, it is used vaginally, but there has been no study reporting the use of henna oil vaginal suppository and evaluating its physicochemical properties.

## What's New

- This study provided the formulation of a henna oil vaginal suppository and examined its physicochemical and antimicrobial properties. We found that polyethylene glycols were a good basis for the production of henna oil vaginal suppositories.

## Abstract

**Background:** Persian Medicine is one of the oldest and richest complementary and alternative options in the field of medicine and has a comprehensive medical system. Henna oil is recommended in Persian Medicine for the treatment of numerous women's diseases such as cervicitis. To date, henna has been used for many medical purposes, including astringent, bleeding, cardioinhibitory, hypotension, and relaxation. Accordingly, the present study aimed to provide the formulation of a henna-oil-based vaginal suppository and examine its physicochemical and antimicrobial properties.

**Methods:** The present study was approved and performed in accordance with the regulations of Research Council, Kerman University of Medical Sciences, Kerman, Iran, in July 2016. Different percentages of henna oil, glycerin, and gelatin, as well as henna oil and polyethylene glycol 400 and 4000, were mixed to achieve a formulation with proper appearance features and, particularly, without any oil leakage from the suppository surface. Uniformity of weight, uniformity of content, disintegration time, and dissolution test of the suppositories were evaluated. The growth-inhibiting activity of the suppositories and aqueous extract of henna was evaluated against bacteria, including the Gram-positive bacterium *Gardnerella vaginalis*, *Neisseria gonorrhoeae*, and group B *streptococcus*.

**Results:** The formulations had a smooth appearance without any cracks or fractures. Disintegration times for glycerogelatin and polyethylene glycol suppositories were 60 and 10 min, respectively. 40% of the drug was released from polyethylene glycol suppositories after 60 min, but glycerogelatin suppositories had no release after three hours. Minimum inhibitory concentration (MIC) of suppositories and aqueous extract were 0.4 mg/mL and 0.01 mg/mL, respectively.

**Conclusion:** Polyethylene glycol suppositories had acceptable physicochemical properties, and the henna extract and suppositories inhibited the three studied pathogens.

Please cite this article as: Khazaeli P, Mehrabani M, Mosadegh A, Bios S, Zareshahi R, Moshafi MH. Formulation, Physicochemical, and Microbial Assay of Henna Oil Vaginal Suppository Formulated with Polyethylene Glycol Bases. Iran J Med Sci. 2020;45(3):207-213. doi: 10.30476/IJMS.2019.44965.

**Keywords** • Henna • Suppositories • Uterine cervicitis • Polyethylene glycols

## Introduction

The use of herbal medicines is popular in advanced countries as well as developing countries, due to their therapeutic and biological

effects, high safety, and low prices. The World Health Organization estimates that 80% of the population make use of traditional medication for primary essential health care. This understanding made pharmaceutical industries consider natural resources as an important source for new drugs.<sup>1</sup> Persian Medicine is one of the oldest and richest complementary and alternative options in the field of medicine and has a comprehensive medical system. It is a systematic and holistic approach aimed at keeping healthy and treating diseases. Herbal medicines use has long been common among non-professional therapists in Iran and many other countries such as Greece, India, and the Arab countries.<sup>2</sup>

Investigating the diagnosis of causes and treatment of diseases in Persian Medicine has a rich background. Scientists in the field studied and evaluated most of the medical issues; then, based on these theories, they became more experienced in the treatment. The effectiveness of methods of this medicine have been proved and experienced along with its low side effects.<sup>2</sup> Persian Medicine Scholars have carefully described various organs of the body, including the uterine and female genital organs. They have also explained many of uterine diseases and their treatment. They used henna, topically or orally, for the treatment of various diseases, namely skin and hair diseases, uterine diseases, and burns.<sup>3,4</sup>

*Lawsonia inermis* is a type of Lythraceae family. Leaves, stems, flowers, roots and seeds have been used in various ways in Persian Medicine.<sup>5</sup> Henna is one of the world's most widely used herbs; a great amount of which is in tropical and subtropical areas. Henna is a 6-7 m long shrub and grows in tropical Africa (Morocco, Egypt, Tunisia and Algeria) in Iran henna grows in Kerman and Sistan. It is a native herb in Kerman and Sistan-Balouchestan provinces in Iran and contains carbohydrates, proteins, flavonoids, tannins, alkaloids, terpenoids, quinones, coumarins, xanthines, and fatty acids.<sup>6</sup> This plant is used for many medical purposes, including astringent, bleeding, cardioinhibitory, hypotension, and relaxation.<sup>7</sup> In Persian Medicine resources, Henna is suggested for the treatment of uterine diseases including Qoruhe-e-Rahem (cervicitis), Wajà-e-rahem (uterine diseases), Waram-e-rahem/tumefaction of uterine, islah-e-rahem/uterine rectification, uterine bad smell (infection), prevention of pregnancy, and enhancement of sexual desire. In most cases, it is used vaginally (Homoul or Fatilah). Henna is most commonly used for Wajà-e-rahem (uterine diseases) treatment. Moreover, it is

recommended for increasing sexual desire.<sup>4, 8, 9</sup> Razi and Ibn Sina, famous Iranian physicians, also found henna and henna oil useful for the treatment of uterine diseases; Razi suggested its oil for Wajà-e-rahem and Ibn Sina suggested using the mixture of Henna oil and egg whites, vaginally, for the treatment of Qoruhe-e-Rahem.<sup>10, 11</sup> Numerous studies have shown that women tend to use herbal remedies to treat such conditions as dysmenorrhea, menopausal symptoms, menstrual disorders, mood disorders, and osteoporosis prevention during pregnancy.<sup>5, 12</sup> Moreover, henna oil is recommended in Persian Medicine for the treatment of numerous women's diseases such as cervicitis.<sup>10</sup> Accordingly, the present study aimed to provide the formulation of a henna-oil-based vaginal suppository. The suppositories were examined in terms of weight change, liquefaction time, brittleness resistance, uniformity of content, and the release or dissolution rate in order to investigate their physicochemical properties.

## Materials and Methods

The present study was approved and performed in accordance with the regulations of Research Council, Kerman University of Medical Sciences, Kerman, Iran, in July 2016.

### Chemicals

Polyethylene glycol 400 (PEG400) (Merck, Germany), polyethylene glycol 4000 (PEG4000) (Merck, Germany), Tween 60, Span 60 (Merck, Germany), glycerine (Sigma-Aldrich, Germany), gelatin (Sigma-Aldrich, Germany), sesame oil (Ardakan, Samar Oil)

### Plant Collection

Henna was collected from a region near Kerman called Shahdad where henna is cultivated. A herbalist named and typed the plant. A sample of the herb was kept in the herbarium of Kerman Pharmaceutical Faculty with the herbarium number KF 1408. The leaves were then separated from the plant, ground by a mill (Assan Toos Shargh, Iran), and passed through mesh 40.

### Henna Oil Preparation

Henna oil was prepared according to the methodology of Persian Medicine called Qarabadin (Pharmacopeia). For this purpose, 50 g of plant powder was soaked with 300 mL distilled water in a beaker overnight and heated to 90 °C for one hour on a heater. It was then filtered using the Buchner funnel vacuum and the

henna extract was mixed with an equal amount of sesame oil, which was placed again on the heater until the aqueous extract was completely evaporated and only the oil remained.<sup>13</sup>

Given the objective of the study, it was decided to use water-soluble bases, as they dissolve and are released better in the aqueous environment of the vagina. Different percentages of oil, glycerin, and gelatin, as well as oil and polyethylene glycol 400 and 4000, were mixed to achieve a formulation with proper appearance features and, particularly, without any oil leakage from the suppository surface.

The suppositories were produced in the weight range of 1.9 to 2.5 g. For this purpose, the displacement factor was first obtained using the formula  $f=d/(a-c)$ , where  $d$  is the effective amount of active ingredient and  $a-c$  is the amount of displaced excipient. The suppository bases were then melted on a water bath. One percent of Tween and Span 60 were used in both polyethylene glycol and glycerogelatin bases. However, the glycerogelatin base was excluded from the study, as it was impossible to be mixed with methanol.

#### Physicochemical Evaluation

The displacement values of henna oil suppository in each suppository base were calculated.

#### Uniformity of Weight

According to the British Pharmacopoeia, 20 suppositories were randomly selected and weighed and their average mass was determined.

#### Uniformity of Content

In order to examine and ensure the uniformity of active ingredients in the formulations prepared according to the British Pharmacopoeia, 10 PEG suppositories were randomly selected. Each suppository was put in a 50 mL volumetric flask and placed on a water bath until it was completely melted. The flask was then centrifuged at 9000 g for 15 minutes. The absorbance of the under natant was determined by ultraviolet spectrophotometer at a wavelength of 350 nm against the methanol blank. The amount of sample drug was calculated based on the calibration curve of luteolin in methanol. A drug-free suppository was simultaneously tested as the control.

#### Disintegration

The liquefaction time of suppositories was measured by the Krowczynski method.<sup>14</sup> To do this, a glass tube was manufactured and used

in accordance with the Krowczynski's design at the School of Pharmacy (figure 1). The suppository was first placed inside the glass tube on a small tank of distilled water. Water (37 °C) was circulating around the tube. About 30 g glass rod was placed on the suppository, and the time for the complete softening of the suppository and the downward movement of the glass rod until reaching the opening of the distilled water tank was recorded. The experiment was repeated three times for suppositories and the standard deviation of liquefaction time was calculated.

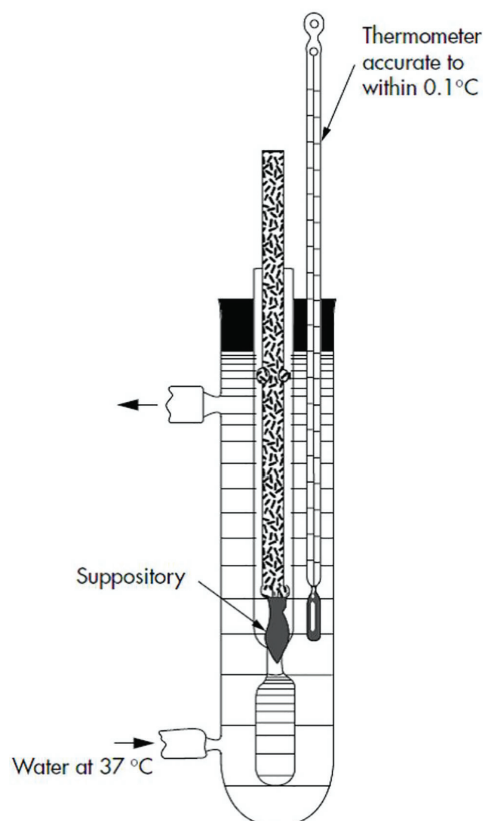


Figure 1: The suppository disintegration time device is shown schematically.

#### Dissolution Test

An *in vitro*, drug release test was carried out using a Dissolution Tester USP-25, apparatus 1 (Erweka, Germany). The medium consisted of 250 ml methanolic phosphate buffer (50:50), pH 7.4, maintained at  $37 \pm 0.5$  °C and the paddles were rotated at 150 rpm. Aliquots were withdrawn at 10, 20, 30, 40, 50, 60, and 90 min. Samples were then centrifuged and suitably diluted and the amount of Luteolin was determined by spectrophotometric measurement at 350 nm using an appropriate blank. The data presented are the average of three determinations. The mean and standard deviation of the drug released was calculated and the percentage of releasing was plotted against time.

**Antimicrobial Assay**

The growth-inhibiting activity of the suppositories and aqueous extract of henna was evaluated against bacteria, including the Gram-positive bacterium *Gardnerella vaginalis* ATCC 14018, *Neisseria gonorrhoeae* ATCC 31426, group B *streptococcus* ATCC 13813.

The bacterial strains were prepared from stocks obtained from the Pasteur Institute (Tehran, Iran). Bacterial strains were aerobically cultured at 35 °C for 24 hours in Brain heart infusion (Merck, Germany).

**Determination of Minimum Inhibitory Concentration (MIC)**

The minimum inhibitory concentration was determined through the broth macro-dilution test, known also as the tube dilution test. To this end, a suspension containing 0.5 McFarland ( $1.5 \times 10^8$  cfu/ml) was prepared from each of the microbes examined. The medium used was blood agar. The PEG suppository was melted and 1 mg of aqueous extract was added to the separated tubes and verification was done. Then, each of the microbes was cultured. The tubes were then incubated at 35 °C for 24 hours. A tube containing a sesame oil suppository and a tube containing a base suppository (suppository without any oil) were also considered. Then, the tubes were examined with naked eyes, and the last tube that showed transparency was recorded as the MIC. Ciprofloxacin was considered as a comparative control. A tube containing culture medium and

bacteria was considered as a positive control and a tube containing only the culture medium and henna oil was considered as a negative control.

**Results**

Due to the fact that our active ingredient was oil, suppository formulation was very hard. Most of the formulations showed leakage or did not have a smooth appearance. The formula number nine and 1 (table 1) did not leak from the base and had a smooth appearance without any cracks or fractures.

The mean weight of polyethylene glycol suppositories and glycerol-gelatin suppositories are presented in table 2. The disintegration time, displacement factor, and mean weight of suppositories are given in table 3. The results of the release test polyethylene glycol suppositories are shown in figure 2. glycerol-gelatin suppositories had no release after three hours. The results of the Beer-Lambert law validity test (figure 3) showed that there was a linear relationship between the 3-30 µg/mL luteolin in methanol and the absorbance read at 350 nm, indicating the validity of the Beer-Lambert law in the specified range of concentrations ( $r=0.99$ ).

**Antimicrobial Assay**

The anti-microbial effect of suppository is reported based on the amount of oil in suppositories in table 2. The MIC of PEG

**Table 1:** Components of various formulations of suppositories

	Glycerin (%)	Henna oil (%)	Gelatin (%)	Water (%)	PEG400 (%)	PEG4000 (%)	Tween & span 60 (%)
1	29.05	24.40	15.29	30.50	---	---	1
2	12.50	50	3	---	---	---	1
3	31.05	24.80	32.90	31	---	---	1
4	15.50	37.26	32.90	31	---	---	1
5	---	20	---	---	---	---	---
6	---	28	---	---	---	---	---
7	---	28.45	---	---	---	---	---
8	---	30.40	---	---	21.73	43.47	1
9	---	16.60	---	---	16.60	66.60	1

PEG: Polyethyleneglycol

**Table 2:** Minimum inhibitory concentration and minimum bactericidal concentration of aqueous extract and polyethyleneglycol suppository versus the three bacterial species examined

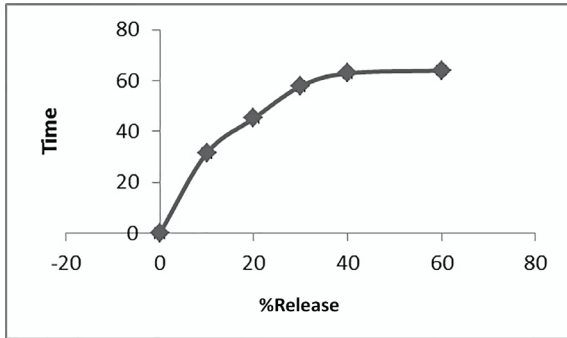
Microorganisms		<i>Gardnerella vaginalis</i>	Group B <i>streptococcus</i>	<i>Neisseria gonorrhoeae</i>
Suppository	MIC	0.4 mg /mL	0.4 mg/mL	4 mg/mL
	MBC	4 mg /mL	4 mg/mL	40 mg/mL
Aqueous extract	MIC	0.01 mg/ml	0.1 mg/ml	0.01 mg/ml
	MBC	0.1 mg/ml	1 mg/ml	0.1 mg/ml
Ciprofloxacin	MIC	0.4 µg/mL	0.05 µg/mL	0.5 µg/mL
	MBC	4 µg/mL	0.5 µg/mL	5 µg/mL

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration

**Table 3:** disintegration, displacement value, and mean of mass of suppositories

Factors	Supp PEG	GI
Displacement factor	0.32 mg	1.16 mg
Weight	2.4±0.1 g	2.3±0.01
Disintegration time	60 min	10 min

PEG: Polyethylenglycol; Supp: Suppository; GI: Glycerogelatin



**Figure 2:** 40% of luteolin is released from suppositories at 60 min.

suppositories and aqueous extract of henna against *G. vaginalis*, *N.gonorrhoeae*, and Group B *streptococcus* are reported. Sesame oil and base of suppository did not have any antimicrobial effects.

### Discussion

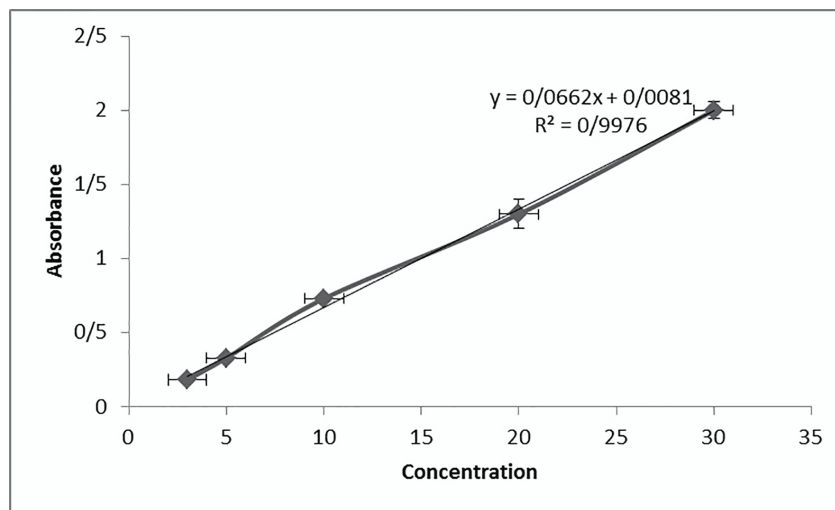
The present study aimed to prepare a vaginal suppository formulation for henna oil and study its physicochemical and antimicrobial properties. One formulation of PEG suppositories had acceptable physicochemical properties and the henna extract and suppositories inhibited the three studied pathogens.

According to the dissolution test results of the polyethylene glycol-base suppository, 40% of the drug was dissolved in 60 minutes whereas

the glycerogelatin-base suppository did not dissolve in the buffer medium and could not be performed in the methanolic buffer, so they failed the release test.

Other studies have also examined various formulations of herbal medicines for the treatment of vaginal diseases. Saqafi and others developed a vaginal suppository formulation from dill fruit extract and investigated its effect on candidiasis. They reported that the suppository containing 2% dill extract had the same effect as the clotrimazole vaginal tablet.<sup>15</sup> Nick Akhtar and others developed a vaginal suppository from myrtle extract and examined it on patients with human papilloma virus. They reported a negative Human Papillomavirus test in 92% of the patients.<sup>16</sup> Paramar and others determined the MIC of clotrimazole suppositories against *Staphylococcus*, *Bacillus*, *Aspergillus niger*, and *Candida* as 16, 8, >64, and >64 µg/mL, respectively.<sup>17</sup> The results of these studies are comparable with those of the present study in terms of antimicrobial effects on a number of active microorganisms in the uterus such as *C. albicans* and human papillomavirus; however, their main focus was on the clinical outcomes of suppositories rather than their physicochemical properties.

Hashmi and others examined a vaginal cream containing aqueous extract of henna and lead monoxide along with an herbal medicine on patients with cervicitis and reported a 26.7% improvement.<sup>18</sup> Similar to the present research, in their study, they used the henna aqueous extract in a vaginal drug form, but mixed with a mineral substance (lead monoxide), along with an oral drug. Thus, it cannot merely reflect the effect of henna on the treatment of cervicitis or the inhibitory effect of henna on cervicitis-inducing pathogens. On the other hand, the current study provided this formulation and examined it on



**Figure 3:** The Luteolin (3-30 µg/mL) absorption Curve was linear

patients based on Indian medical resources. In addition to various physicochemical properties, this study examined the antimicrobial effect of the suppository *in vitro*, in the hope of performing clinical studies in the future.

Talwar and others performed *in vitro* investigations on antimicrobial effects of a vaginal suppository prepared from *Azadirachta indica*, *Sapindus mukerossi*, and *Mentha citrate*, and reported its inhibitory effect on *Escherichia coli*, several fungi, including *C. albicans*, and herpes virus. This study did not determine MIC, but the suppository contained three different herbs; therefore, it had an inhibitory effect on a range of fungi and bacteria. The formulation of the present study merely consisted of a single plant.<sup>19</sup>

Glycero-gelatin bases are recommended in pharmaceutical references for producing vaginal suppositories.<sup>20</sup> The utilized glycero-gelatin base in the present study was found to have a problem in the dissolution test, which requires further investigations, while the polyethylene glycol base did not face this problem; thus, it is recommended for subsequent investigations.

In this study, 100 µg/mL aqueous extract and 400 µg/mL suppositories had an inhibitory effect on *Gardnerella*. Jones and others performed an *in vitro* investigation on the MIC of Triple sulfa in *Gardnerella vaginalis*, which resulted in the growth inhibition of this pathogen at a concentration of 25,000 µg/mL.<sup>21</sup>

In the present study, the inhibitory effect of the aqueous extract on all microbes occurred in lower concentrations. Muhammad and others reported the inhibitory effect of the henna aqueous extract on *Strep. B*. They stated that 10 mg/mL henna aqueous extract had no growth inhibitory effect in the disk diffusion test, but the effect was observed at a concentration of 30-80 mg/mL and increased with increasing concentrations.<sup>22</sup> In the present study, the henna aqueous extract had an inhibitory effect on *Strep. B* at a concentration of 0.01 mg/mL. The results of their study and those of the present study are significantly different. This difference maybe related to the difference in the plant growth area and the impact of the area on the plant effects. The aqueous extract of henna had a similar inhibitory effect on *Gardnerella* and *Strep. B*, but a higher concentration was required (0.1 mg/mL) for *Neisseria gonorrhoea*. Generally, the suppository needed higher concentrations than the aqueous extract for growth inhibition of the studied pathogens. This may be due to the fact that all extract contents did not enter the oil, making the oil weaker than the extract.

Similar to the present research, herbal compounds were used in some other studies

to prepare the suppositories, but those studies focused on the clinical effects of the product. In addition, they used the alcoholic extract for the dill suppository and the combination of aqueous extract and essential oil for the myrtle suppository; in addition, they did not investigate the physicochemical properties of the suppositories.<sup>15, 16, 18</sup>

Vinta and others performed a similar study to prepare lactobacillus suppositories, which produced only one formulation from three bases of glycero-gelatin, polyethylene glycol, and cocoa butter. They reported on its physicochemical and antimicrobial properties against *E. coli*.<sup>23</sup> The present study did not investigate different formulations produced from various bases, rather, it investigated the producibility and the physicochemical properties of suppositories prepared from an oily Persian Medicine product.

The main limitation of the present study was that other suppository bases were not tested. The henna suppository with other types of PEGs should be made and compared to this formulation.

## Conclusion

The results of this study showed that Polyethylene glycols (PEGs) could be regarded as a good basis for the production of henna vaginal suppositories. Further studies need to be carried out to investigate the clinical and pharmacokinetic effects of PEGs.

## Acknowledgment

This article is extracted from a PhD thesis by Rahele Zareshahi, Kerman Medical University. We are grateful to the experts at the Kerman Pharmaceutical Research Center, and particularly, Ms. Mohammadi who helped us in this project.

**Conflict of Interest:** None declared.

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