TRADITIONAL AND INTEGRATIVE MEDICINE

Trad Integr Med, Volume 8, Issue 1, Winter 2023

Original Research

# Development, Characterization, and *In Vitro* Antimicrobial Activity of *Lawsonia inermis* L. Leaves Hydroalcoholic Extract-Based Vaginal Suppositories

**Fakeha Firdous K.[1], Khaleequr Rahman[1]*, Arshiya Sultana[2], Shamsiya Khan[1]**

[1]*Department of Ilmul Saidla (Pharmacy), National Institute of Unani Medicine, Kottigepalya, Magadi Main Road, Bengaluru, India*
[2]*Department of Amraze Niswan wa Ilmul Qabalat (Obstetrics and Gyneacology), National Institute of Unani Medicine, Kottigepalya, Magadi Main Road, Bengaluru, India*

**Received:** 12 May 2022 **Revised:** 12 Jul 2022 **Accepted:** 13 Jul 2022

## Abstract

*Lawsonia inermis* L. (henna) leaves have good antimicrobial, anti-inflammatory and wound healing properties. It is used topically in the form of hamul in gynaecological infections in traditional medicine. However, due to the unpleasant dosage form, it has very low patient compliance. Hence, in this study hydroalcoholic extract-based vaginal suppository of henna leaves was formulated and evaluated for various parameters. Multiple batches of suppository were prepared using polyethylene glycol (PEG)-4000, PEG-400, Tween-80, Span-60, distilled water and henna hydroalcoholic extract. Based on the organoleptic character and optimization tests, the final batch was selected. The final batch was evaluated for various physicochemical parameters and antimicrobial activities. Batch containing extract 30%, PEG-4000 37.38%, PEG-400 19%, distilled water 4.55%, Tween-80 and Span 60 4.535% each, passed the disintegration time (11.45 min), mucoadhesiveness (90°) and melting point (37 °C) test and selected as an optimized batch. The mean weight of a suppository was 1.56±0.03 g. Secondary plant metabolites in henna leaves and suppository were comparable. Various molecules identified in leaves were also noted in the suppository on GC-MS. Heavy metals and microbial contamination were within the permissible limit. Zone of inhibition (at 50 µg/mL) and minimum inhibitory concentration for *E. coli*, *S. aureus*, *S. pyogenes, P. aeruginosa* and *C. albicans* were 20 mm, 18 mm, 20 mm, 16 mm, 19 mm and 20 µg/mL, 10 µg/mL, 25 µg/mL, 25 µg/mL, 45 µg/mL, respectively. This study indicated satisfactory physicochemical parameters and antimicrobial potential of the hydroalcoholic extract-based vaginal suppository of henna. Hence, it may be considered as a better alternative to its traditional dosage form.

**Keywords:** Antimicrobial herb; *Hamul*; Henna; Unani medicine; Vaginal suppository

## Introduction

In Unani medicine, various topical dosage forms such as *hamul, farzaja, fateela, shiyaf*, *marham*, and *aabzan*. are available to treat gynaecological disorders effectively [1]. Out of these traditional dosage forms, hamul is the most commonly used vaginal dosage form. In *hamul* dosage form, a powder of drug substance is tied in a piece of muslin cloth and inserted deep into the vagina. *Hamul* as drug delivery vehicles have existed for hundreds of years with a long history of use. Reference to hamul is available in the Hebrew Scriptures and their use is also documented in Egyptian papyruses. Hippocrates in his treatise documented the use of numerous acorn-based medicines deliv-

**Citation:** Firdous K. F, Rahman K, Sultana A, Khan S. **Development, Characterization, and *In-Vitro* Antimicrobial Activity of *Lawsonia inermis* L. Leaves Hydroalcoholic Extract-Based Vaginal Suppositories.** Trad Integr Med 2023;8(1):16-25.

*Corresponding Author: Khaleequr Rahman
Department of Ilmul Saidla (Pharmacy), National Institute of Unani Medicine, Kottigepalya, Magadi Main Road, Bengaluru, India
Email: r.khaleeq@yahoo.com

ered vaginally and rectally for local pharmacological properties [2].

The drawbacks associated with this traditional vaginal dosage form are a lengthy and manual process of preparation, traditional dispensing, bulky volume, discomfort during the application, poor adhesiveness, less stability and sterility, chances of slipping off the medicaments due to body movements and gravitational force, and inaccurate dose delivery. Sometimes application of traditional vaginal dosage form even leads to irritation, pain, injury, and secondary infection to the vaginal mucosa if proper care is not taken. Therefore, though they are effective, they are neither compatible with the patients' needs nor with the present-day available technology.

One of the major reasons for women to visit a gynaecologist is acute and recurrent female genital infections such as bacterial vaginosis, candidiasis, vaginitis, cervicitis, and pelvic inflammatory diseases. Women suffering from these diseases present symptoms such as lower abdominal pain, pruritis, and excessive vaginal discharge [3,4].

In conventional medicine, the treatment for female genital infections is antibacterial, antifungal, analgesic, and anti-inflammatory drugs available in various dosage forms either through the systemic route as an oral solid dosage form, intramuscular/intravenous injections, or through a topical application such as vaginal cream, tablets, pessaries, and suppositories. However, because of emerging microbial resistance and adverse drug reactions to these drugs, researchers are searching for a better alternative [5].

There is a wide range of options available in traditional medicine to treat these gynaecological diseases. But as mentioned above due to various drawbacks associated with drugs delivered in traditional vaginal dosage form, they are not in mainstream use. These traditional dosage forms may be modified into vaginal suppositories, pessaries, gels, creams, capsules, or tablets for the treatment of vaginal and pelvic inflammatory infections. These dosage forms are more sophisticated, easy to carry and apply, have content uniformity, and do not irritate vaginal mucosa due to proper lubricants used. Further, a large volume of dissolution fluid is not required for the release of the active ingredient in these dosage forms [6].

As per Unani literature, henna leaves powder in the form of hamul or any topical vaginal application is very useful in treating vulvovaginitis, cervicitis and (pelvic inflammatory disease) PID since ancient times as it possesses *muhallil auram* (anti-inflammatory), *mujaffif* (desiccant), *mundamil-i-quruh* (promote wound healing) and *daf-i-taffun* (antimicrobial) properties [7]. In vitro and in vivo studies have proven that henna leaves have strong antimicrobial [8] and anti-inflammatory properties [9].

Hence, the present study was aimed to design, develop, and optimize water-based conventional vaginal suppository using hydroalcoholic extract of henna leaves and suitable excipients. Further, its physicochemical parameters and in vitro antimicrobial activity against selected pathogens were also determined.

## Materials and Methods

### *Procurement of drugs and chemicals*

Fresh henna leaves were harvested from April to May 2019, from the Herbal Garden, National Institute of Unani Medicine, Bengaluru. Leaves were authenticated by Pharmacognosist Prof. Noorunnisa Begum, Dept. of Pharmacognosy, Centre for Repository of Medical Resources (CRMR), Trans-Disciplinary University (TDU) under FRLHT, Bengaluru (Accession no: 5229 for *Lawsonia inermis* Linn). Fresh henna leaves were cleaned manually for impurities, rinsed with tap water and dried in the shed. Dried leaves were coarsely powdered in an electric grinder and passed through 40no. sieve. The powder was stored in an airtight glass jar with silica gel. All excipients, chemicals and reagents such as polyethylene glycol (PEG) 4000, PEG 1500, PEG 400, Tween 80, Span 60, glycerin, gelatin, methylparaben and propylparaben, ethanol, mucin (Porcine stomach mucosa, CAS Number: 84082-64-4, obtained from Merck India), petroleum ether, and chloroform, used in this study were of analytical grades and purchased from open markets of Bengaluru.

### *Extraction of henna leaves*

The cold extraction method was used for the extraction of henna leaves. The coarsely powdered henna leaves were macerated with 50% hydro-alcohol in a ratio of 1:20 (w/v) in a closed conical flask for 24 h, then it was placed in a shaker for six hours and later it was allowed to stand for eighteen hours. Thereafter, the liquid was again rigorously shaken a few times and filtered through Whatman paper no.1 in a stainless-steel tray and dried in a water bath at 60-80 °C utill a thick viscous mass was developed. Viscous mass was further dried for removal of residual solvent in a hot air oven at 60 °C for about 60 min. The dried hydroalcoholic extract was scraped off the tray and stored in airtight amber color glass containers in the refrigerator with silica gel desiccant for further use.

### *Preparation of suppositories*

Pour moulding method, also known as the hot fusion method, was used for the preparation of suppository using stainless steel mould having 12 stations and a volume of 2 mL each. The formulated suppository bases were first melted in a water bath and then extract was added to the bases with constant stirring,

to form a homogeneous mixture. The mould cavities were greased with liquid paraffin wax then the hot liquid mixture was poured into the mould cavities and allowed to cool down at room temperature. Later, the suppositories were removed from the mould and packed in aluminium foil individually and stored in airtight containers at room temperature.

Multiple batches of the suppository formulation with different concentrations of extract and excipients were prepared on a trial and error basis. The selection of the optimized batch was carried out in two stages. In the first stage, out of these multiple batches, three batches were selected based on subjective criteria such as homogenization and uniformity of extract dispersion in the finished product, elegance, consistency, stability at room temperature in the view of softening, melting, and sweating. Those three batches that passed the first stage criteria were further subjected to second stage selection criteria i.e., mucoadhesive test, disintegration test and melting point analysis to select the final/ optimised batch.

## *Parameters for optimization*

*In vitro* mucoadhesive study: Mucin was dissolved in buffer 6.0 for making a 2.5% solution. The glass slides were washed, cleaned and dried. Each glass slide was marked with a horizontal line 0.5 cm from an end for suppository placement. Prepared mucin solution (0.5 mL) was placed on a glass slide and a suppository was placed on it, waited for 30 seconds to allow the binding of the slide with mucin and excipients of a suppository which was responsible for mucoadhesion. After 30 seconds, the glass slide of the suppository was lifted from the suppository end with steady motion keeping the opposite end of the slide fixed at the table. The height/angle of the elevating end of the slide from the working table was recorded at the moment where the suppository started slipping down with the increasing inclination. The highest height of movement was taken as the highest mucoadhesive strength [10,11].

Melting point: The melting point was determined by placing a 1 mm diameter wire into the mould containing the suppository mixture before the form solidifies. The suppository was held by the wire and immersed in water. The temperature of the water was raised slowly (about 1 °C every 2-3 min) until the suppository slipped off from the wire into water. The temperature at which the suppository slipped off from the wire into the water was noted as the melting point of the suppository [12].

Disintegration test: The disintegration apparatus of the six tubes basket rack assembly was used to assess the disintegration time of the suppository. The basket rack was positioned in a one-litre beaker filled with distilled water and set at 37±2 °C temperature. Each tube of the basket was loaded with a suppository and basket assembly was reciprocated up and down through a distance of 5-6 cm at a frequency of 28 to 32 cycles/min. The period is taken for all particles to pass through the mesh screen and if any residue remains, it must be a soft mass, was taken as the disintegration time [13].

The adopted acceptance criteria for the optimization were minimum disintegration time, maximum mucoadhesiveness and melting point temperature at or nearest to the human body temperature. The batch which qualified the above sets of parameters was selected as an optimized batch and was further evaluated on various physicochemical parameters for standardization and antimicrobial activity.

## *Physicochemical parameters*

Organoleptic characters: Formulated suppository were evaluated for colour, odour, surface characteristics and appearance.

Weight variation: Randomly selected suppository (n=20) were weighed individually and the average weight was calculated. No more than 2 suppositories should fall outside the permissible percentage difference range, which was taken as pass criteria as given by USP [14].

pH Value: The pH value of the suppository was noted in 1% and 10% aqueous solutions.

Test for qualitative and quantitative analysis: Qualitative tests were carried out to check the presence of secondary plant metabolites such as starch, carbohydrates, proteins, tannins, phenols, flavonoids, alkaloids, and resins, fixed oils, steroids, and saponins [15,16].

Further quantitative analyses were carried out for total carbohydrate [17], reducing sugar [18], total tannins, total phenols, total flavonoids and total alkaloids in suppository and henna leaves powder [19].

Gas Chromatography-Mass Spectrometry (GC-MS): The GC-MS was performed by using Shimadzu QP2010Ultra Model, Turbo mass ver. 5.5 software and fused silica column packed with Elite -5MS. The oven temperature was set at 50 ºC and increased 8 ºC per minute up to 220 ºC for 5 minutes and 7 ºC per minute to 280 ºC for 15 minutes. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/ minute. An aliquot of 10 µL of the sample was injected into the column at 280 ºC with the injector with the split ratio of 10:1. The ionizing energy of 70eV was used and the electron ionization was involved. The mass range was kept between 40-600 amu. The inlet line temperature was 200 ºC and the source temperature was 150 ºC. The total GC running time taken was 60 minutes. The graphs were determined by interpretation and also by matching the spectra with reference spectra (NIST) and further cross-checked

over PubChem [20].
The same procedure was performed for both, powdered henna leaves and suppository.
Heavy metals contamination analysis: Cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) were estimated in the suppository using ICP-OES spectrometer (Optima 2000 DV (ICP-OES, PerkinElmer Corporation, USA) [21].

### *Microbial contamination analysis*

The pour plate method was used for the total bacterial and fungal count. Tests for the contamination of specific pathogens i.e. *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Salmonella* were carried out by conventional culturing and colony counting method [22].

### *Antimicrobial study*

Test microorganisms: Antimicrobial activity of suppository was evaluated against four bacterial strains and one fungal strain i.e. *Escherichia coli* (MG1655), *Staphylococcus aureus* (MTCC9886), *Streptococcus pyogenes* (clinical isolate), *Pseudomonas aeruginosa* (clinical isolate) and *Candida albicans* (MTCC227). Bacterial cultures were grown on 2 mL of Luria-Bertani broth and *Candida albicans* was grown on Sabouraud dextrose broth with chloramphenicol. Microbes were preserved at Dextrose Technologies Pvt. Ltd. Bengaluru. Bacteria and yeast were further sub-cultured on Luria-Bertani broth and Sabouraud's Dextrose broth respectively 24 h before the study.

A. Zone of inhibition: The test sample was dissolved in distilled water and a concentration of 1 mg, 2.5 mg, 5 mg, 20 mg, 30 mg, 40 mg and 50 mg per mL were prepared. The zone of inhibition was tested by the agar well diffusion method. Wells of 6mm diameter were punched on specific agar media. About 200 µL of pre-cultured test organisms were spread on the agar plates. Samples of various concentrations were loaded into the wells. Tetracycline and clotrimazole were used as a positive control for bacteria and yeast respectively. Bacterial plates were incubated at 37 ºC and yeast plates were incubated at room temperature for 24 h. Thereafter, the diameter of the zone of inhibition for the sample was measured [23].

B. Minimum inhibitory concentration (MIC): The MIC of the test drug was determined using sterile 2 mL 96-well plates. Following the initial incubation of 24 h, organisms were suspended in 10mL of physiological saline solution and optical density readings were assessed. For the MIC determination of the test sample, bacteria and fungus solutions of $1x10^7$ colony-forming units (CFU) per ml were employed. 100µl of bacteria and fungus solutions was inoculated to the samples of varying concentrations (10 µg/mL, 15 µg/mL, 20 µg/mL, 25 µg/mL, 30 µg/mL, 35 µg/mL, 40 µg/mL, 45 µg/mL and 50 µg/mL) of test sample taken in the tube and incubated at room temperature for 24 h. For bacteria, tetracycline 20 µg/mL and for *C. albicans*, clotrimazole 10 µg/mL were taken as standard antimicrobial agent. The resulting turbidity was observed after 24 h. The MIC was determined to be where growth was no longer visible by assessment of turbidity using optical density readings at 600 nm [24].

## Results

### *Optimization of vaginal suppository*

Eleven batches of the suppository with different concentrations of extract and excipients were prepared for optimization. Based on homogeneity, consistency, elegance, appearance and behaviour at room temperature, three batches F3, F8 and F11 were selected for further optimization tests and selection of the final batch. (Table 1)
Among them, batch F8 performed better on disintegration, mucoadhesive and melting point tests when compared with the other two batches. Therefore, F8 was selected as the optimized batch (Table 2).

### *Physicochemical parameters*

Organoleptic characters, pH and weight variation: The formulated suppositories of the final batch were solid, soft, glossy, bullet-shaped, dark brown (Pantone. 4975), having a pleasant aroma of henna. The mean weight of 20 suppositories was 1.568±0.0323gm with minimum and maximum percentage of weight variation of 0.12% and 4.33% respectively (Table 3). They showed pH of 4.66 and 4.57 in 1% and 10% concentration in distilled water respectively.
Qualitative tests: Qualitative analysis of henna leaves and suppository showed the presence of carbohydrates, tannins, phenols, flavonoids, alkaloids, resins, fixed oils and steroids. Whereas, starch proteins and saponins were absent.
Quantitative tests: The mean values of plant metabolites in dry henna leaves and extract-based suppositories were, total sugar 9.2 and 11.1 g/100 g, reducing sugar 1.89 and 2.60 g/100 g, tannins 5.38 and 6.19 g TAE/100 g, total phenolic content 20.6 and 24.75 g GAE/100 g, total flavonoids 7.40 and 8.69 g QE/100 g; and total alkaloids 57.3 and 67.7 mg CE/g respectively. (Table 4)
GCMS analysis of henna leaves and suppositories: The gas chromatography of leaves powder showed six major peaks and mass spectroscopic analysis of peaks showed 59 compounds. Whereas, the suppository showed, seventeen peaks and 77 compounds on mass spectroscopic analysis. (Figures 1 and 2)
Heavy metals: In the present study henna suppository

**Table 1.** Optimization of vaginal suppositories prepared with the extract of henna leaves

| Batch | Ingredients (%) | | | | | | | | | | | Remark | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ext. | PEG 4000 | PEG 1500 | PEG 400 | Gly | Gel | MP | PP | S60 | T80 | DW | | |
| F1 | 6 | 20 | 74 | - | - | - | - | - | - | - | - | Clumping of extract | R |
| F2 | 24.4 | - | - | - | 29.26 | 15.29 | - | - | 0.5 | 0.5 | 30.05 | Easily breakable, rubbery in consistency | R |
| F3* | 25 | - | - | - | 24 | 20 | - | - | 0.5 | 0.5 | 30 | Rubbery in consistency, well-formed | A |
| F4 | 30.40 | 43.47 | - | 25.13 | - | - | - | - | 0.5 | 0.5 | - | Extract was not completely soluble | R |
| F5 | 12 | 75.2 | - | - | - | - | 1.4 | 1.4 | - | 10 | - | Extract was not completely soluble | R |
| F6 | 25.65 | 37.934 | - | 18.964 | - | - | - | - | 4.363 | 4.363 | 8.726 | Easily compressible, sticky to touch | R |
| F7 | 29.90 | 43.47 | - | 25.13 | - | - | - | - | 0.5 | 0.5 | 0.5 | Extract was not completely soluble | R |
| F8* | 30 | 37.38 | - | 19 | - | - | - | - | 4.535 | 4.535 | 4.55 | Well-shaped, neither too soft nor easily compressible | A |
| F9 | 12 | 65.2 | - | - | - | - | 1.4 | 1.4 | - | 10 | 10 | Soft, sticky easily losing their shape | R |
| F10 | 12 | 72.2 | - | - | - | - | 1.4 | 1.4 | - | 10 | 3 | Easily compressible | R |
| F11* | 37.5 | 46.875 | - | - | - | - | 1.4 | 1.4 | - | 9.825 | 3 | Well-formed, good consistency | A |

*Batch selected for optimization; Ext.-Extract; PEG-Polyethyleneglycol; Gly-Glycerine; Gel-Gelatin; MP-Methylparaben; PP-Propylparaben; S60-Span60; T80-Tween 80; A-Accepted; R-Rejected; DW-Distilled water

**Table 2.** Disintegration, mucoadhesive and melting point test of vaginal suppositories of henna leaves to select the optimum batch

| S. No | Batch | Disintegration test (Min: Sec) | Muco-adhesive test | Melting point (ºC) |
|---|---|---|---|---|
| 1. | F3 | 30 | <45º | 40.0 |
| 2. | F8* | 11:45 | 90º | 37.6 |
| 3. | F11 | 13 | 90º | 31.7 |

*Batch selected as ideal/final batch.

showed lead 0.03 ppm, arsenic <0.1 ppm, cadmium 0.02 ppm and mercury <0.1ppm. (Table 3)

Microbial contamination: The total bacterial and fungal count in the suppository was <10 Cfu/g. Specific pathogens (i.e., *Staphylococcus aureus, Pseudomonas aeuginosa, Escherichia coli* and *Salmonella*) were absent in both test samples. (Table 3)

### *Antimicrobial activity*

Henna suppository showed a significant zone of inhibition (ZoI) against all microorganisms at 50 mg/mL concentration [*E. coli* (19.6±1.14 mm), *S. aureus* (17.8±1.30 mm), *S. pyogenes* (21.0±1.22 mm), *P. aeruginosa* (16.2±0.44 mm) and *C. albicans* (19.4±2.07 mm)]. Whereas, the tetracycline and clotrimazole exhibited a significant zone of inhibition in the bacterial culture at 20 mg/mL and C. albicans culture at 5mg/ml (Table 5).

Suppository exhibited MIC of 20 µg/mL, 10 µg/mL, 25 µg/mL, 25 µg/mL and 45 µg/mL against *E. coli*, *S. aureus*, *S. pyogenes*, *P. aeruginosa* and *C. albicans* respectively. Whereas tetracycline was used as standard showed MIC at 20 µg/mL concentration and clotrimazole at 10 µg/mL (Table 5 and 6).

## Discussion

The present study reveals that powdered henna leaves used as hamul can be easily formulated and developed into hydroalcoholic whole extract-based conventional vaginal suppositories with preserving its plant metabolites and antimicrobial activity. The incidence of female genital infections such as bacterial vaginosis, vaginitis, cervicitis, and PID are up surging due to the resistance of a microorganism to antibiotics and the

**Table 3.** Physicochemical parameters of vaginal suppository prepared with the extract of henna leaves

| S. No | Test applied | Result |
|---|---|---|
| 1. | Organoleptic characters | |
| | ● Appearance | Solid, soft, glossy, slippery |
| | ● Shape | Bullet-shaped (Cone on top of the cylinder) |
| | ● Colour | Dark brown (Pantone. 4975) |
| | ● Odour | Mild, pleasant, aromatic, sweet odour like *Hina* |
| 2. | Weight variation (%) | 1.629± 1.1805 |
| | pH | |
| 3. | ● 1% Solution | 4.66 |
| | ● 10% Solution | 4.57 |
| 4. | Heavy metals contamination (ppm) | |
| | ● Cadmium | 0.02 |
| | ● Lead | 0.03 |
| | ● Arsenic | <0.1 |
| | ● Mercury | <0.1 |
| | Microbial contamination | |
| | ● Total bacterial count (1.0 x $10^5$ CFU/g) | < 10 |
| 5. | ● Total fungal, yeast and mould count (1.0 x $10^3$ CFU/g | < 10 |
| | ● Test for specific pathogen | |
| | ● *Escherichia coli* | Absent |
| | ● *Salmonella* spp. | Absent |
| | ● *Staphylococcus aureus* | Absent |
| | ● *Pseudomonas aeruginosa* | Absent |

recurrence of infections in the present scenario [3,4]. Hence, it is the need of the hour to develop natural plant-derived antimicrobial drugs that can be used in topical dosage forms. Henna leaves have significant anti-inflammatory and antimicrobial activity against various microorganisms [8,9,25,26,27,28]. In the view of the present study, henna leaves extract suppositories might be suggested as a better substitute for various antimicrobial conventional vaginal products available in the market.

The extractive value of powdered henna leaves in 50% ethanol: water was found to be 25%. To develop suppositories, extract and excipient were taken in the ratio of 30:70. While designing the suppository it was noted that during the heating process some amount of liquefied excipient mixture was evaporated. Thus, 600 mg of extract when added to the 1400 mg of excipient only a suppository of 1.5 g was prepared.

The unit dosage of henna leaves powder in hamul form is 5 g, the extractive value of powdered henna leaves was 25% and a suppository weighing 1.5 g was containing 600 mg of extract. Thus, the bulk dosage of hamul was reduced from 5 g in hamul form to 3 g (two units of suppository) in the extract-based suppository. Gelatin suppository took more time for disintegration followed by F11 (higher quantity of PEG 4000), and F8 (lesser quantity of PEG 4000). F11 had a 31.7 ºC melting point as it has tween-80 9.825%, F8 had 37.6 ºC melting point with Tween-80 4.535%. F3 had melting point 40 ºC as it has Tween-80 0.535% [29].

In the present study, generated data of organoleptic and various physicochemical parameters of henna leaves suppository can be used for future reference. The finished product showed optimum disintegration time, mucoadhesiveness and melting point (i.e., approximate to human body temperature).

Molecules identified in the powder of henna leaves and formulated suppository on GC-MS analysis were similar. It confirms that the pharmacologically active components of henna leaves were preserved in their extract-based suppository dosage form. Active molecules of henna leaves such as $C_{15}H_{14}O_3$, $C_{14}H_{12}O_3$, $C_{13}H_{10}O_3$, $C_{16}H_{11}NO_2$ [30], $C_{10}H_6O_2$, $C_{10}H_6O_5$, $C_{15}H_{14}O_3$, $C_{10}H_6O_4$, $C_{11}H_8O_2$ [31], $C_{10}H_6O_4$, $C_{10}H_7NO_3$ [32], $C_{18}H_{14}O_4$, $C_{19}H_{16}O_5$, $C_{15}H_{13}NO_4$ [33] are proven for their pharmacological activity. Hence, the findings in the present study were in accordance to the previous studies related to the identification of molecules in henna leaves using GC-MS. These molecules were reported to have antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, antitumor activity against human gastric SGC-7901 cell line, hepatopro-

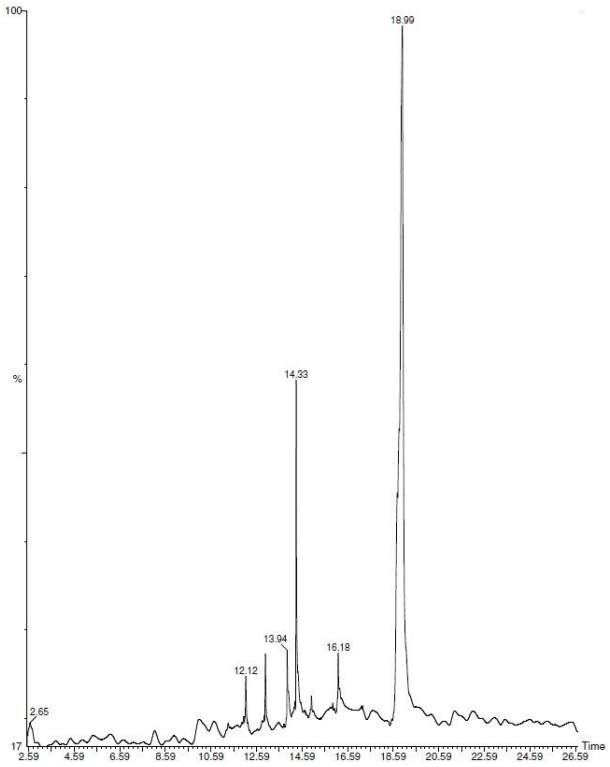


**Figure 1.** Gas chromatography-mass spectrometry of henna powder 171x215 mm (96 x 96 DPI)

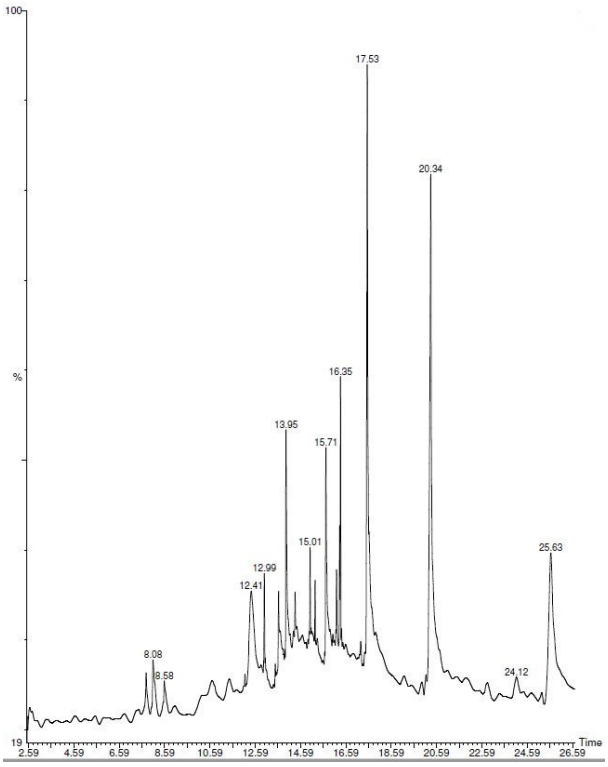


**Figure 2.** Gas chromatography-mass spectrometry of suppository prepared with henna powder 171x216mm (96 x 96 DPI)

**Table 4.** Quantitative analysis of plant metabolites in henna powder and suppositories

| Sample | Plant metabolites | Henna powder | Suppositories |
|---|---|---|---|
| 1. | Total sugar (g/100 g of sample) | 9.2 | 11.1 |
| 2. | Reducing sugar (g/100 g of sample) | 1.89 | 2.6 |
| 3. | Total tannins (g TAE/100 g) of sample) | 5.38 | 6.19 |
| 4. | Total phenolic (g GAE/100 g of sample) | 20.6 | 24.75 |
| 5. | Total flavonoid (g QE/100 g of sample) | 7.40 | 8.69 |
| 6. | Total alkaloid (mg CE/g of sample) | 57.3 | 67.7 |

G TAE-Gram of tannic acid equivalent; g GAE-Gram of gallic acid equivalent;
G QE-Gram of quercetin equivalent; mg CE- Gram of caffeine equivalent.

tective, insecticide, pesticide and antipyretic activities [20].

Dghaim et al., (2015) stated that analysis of the presence of heavy metals in any food or drug substance and the finished product is mandatory for quality and safety purposes [34]. Heavy metals and microbial load in the developed vaginal suppositories were within the permissible limit as per the WHO guidelines, hence it was safe to use. However, pre-clinical trials for toxicology studies are recommended.

The zone of inhibition and MIC of the suppository was found to be very encouraging when compared with the tetracycline used as a standard against bacteria and clotrimazole against C. albicans. The findings of the present study were also in accordance to the previous study carried out on different extracts and fractions of henna leaves [35].

Researchers are optimistic that hydroalcoholic extract-based vaginal suppositories of henna leaves design and development, its physicochemical parameters and in vitro antimicrobial activities were rationally determined. Further, the development of this conventional vaginal dosage form was able to successfully address the aforementioned drawbacks of the traditional vaginal dosage form mentioned in the introduction section.

## Conclusion

It is concluded that the newly formulated and devel-

**Table 5.** Zone of inhibition (ZoI) shown by Henna vaginal suppository against selected organisms

| Concentration of test sample (mg/ml) | Diameter of zone of inhibition (mm) | | | | |
|---|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *S. pyogenes* | *P. aeruginosa* | *C. albicans* |
| 1 | 0 | 0 | 0 | 0 | 0 |
| 2.5 | 0 | 0 | 0 | 0.2±0.44 | 0 |
| 5 | 0.4±0.54 | 10.2±1.78 | 0.4±054 | 0.2±0.44 | 0 |
| 20 | 11.6±0.54 | 11.8±1.48 | 13.2±1.92 | 14.8±1.30 | 0 |
| 30 | 12.8±0.83 | 12.2±1.64 | 16.8±1.48* | 15.8±0.83* | 0.2±0.44 |
| 40 | 13.6±1.14 | 15.0±0.70* | 20.0±1.00* | 16±0.70* | 0.4±0.54 |
| 50 | 19.6±1.14* | 17.8±1.30* | 21.0±1.22* | 16.2±0.44* | 19.4±2.07* |
| Positive control (5 mg) | 0 | 0 | 0 | 9.8±1.78 | 40±2.50* |
| Positive control (20 mg) | 25.2±1.48* | 24.4±1.81* | 35.4±2.30* | 18±1.58* | 40±1.87* |
| Positive control (50 mg) | 34.6±1.14* | 28.0±0.70* | 40.2±2.49* | 24.8±1.48* | 45.2±1.92* |

Data presented: Mean±SD; Positive control: For bacteria tetracycline and clotrimazole for C. albicans were positive control;
* ZoI >15 mm

**Table 6.** Minimum inhibitory concentration of Henna vaginal suppository against selected organisms

| Concentration (µg/mL) | Absorbance to assess minimal inhibitory concentration (MIC) at OD600nm | | | | |
|---|---|---|---|---|---|
| | *E. coli* | *S. aureus* | *S. pyogenes* | *P. aeruginosa* | *C. albicans* |
| 10 | 0.081 | 0.000* | 0.056 | 0.113 | 0.890 |
| 15 | 0.030 | 0.000 | 0.045 | 0.089 | 0.832 |
| 20 | 0.000 | 0.000* | 0.005 | 0.007 | 0.821 |
| 25 | 0.000 | 0.000 | 0.000* | 0.000* | 0.823 |
| 30 | 0.000 | 0.000 | 0.000 | 0.000 | 0.780 |
| 35 | 0.000 | 0.000 | 0.000 | 0.000 | 0.203 |
| 40 | 0.000 | 0.000 | 0.000 | 0.000 | 0.104 |
| 45 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000* |
| 50 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| Positive control (only organism) | 1.056 | 0.832 | 0.965 | 0.992 | 0.915 |
| Positive control For bacteria: Tetracycline 20 µg/mL; For *C. albicans*: Clotrimazole 10 µg/mL | 0.001 | 0.012 | 0.004 | 0.004 | 0.001 |
| Negative control (only media) | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

*Concentration of Henna vaginal suppository at which UV absorbance to assess minimal inhibitory concentration (MIC) noted as zero

oped vaginal suppository of henna leaves would be recommended for the treatment of gynaecological polymicrobial infections as it inhibits the studied pathogens. It is easy to dispense and store and convenient to use. Subsequently, approval of its efficacy in future clinical studies is also recommended. This would make a great contribution to the women of our society by developing traditional vaginal dosage forms into contemporary dosage forms.

## Research Funding
This work was supported by the contingency amount for postgraduate studies provided by the Department of Ilmul Saidla, National Institute of Unani Medicine, Bengaluru. This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sector.

## Ethical Approval
Not applicable.

## Conflict of Interests
The authors declare that they have no conflict of interest.

## Acknowledgement
Authors are thankful to the Director, National Institute of Unani Medicine, Bengaluru and the Ministry of AYUSH, Govt. of India for providing facilities for the study.

## References
[1] Sina I. Al Qanoon fit Tib (Urdu translation by Kantoori GH). Idara Kitabul Shifa. New Delhi 2010.

[2] Ham AS, Buckheit RW Jr. Designing and developing suppository formulations for anti-HIV drug delivery. Ther Deliv 2017;8:805-817.

[3] Jacob L, John M, Kalder M, Kostev K. Prevalence of vulvovaginal candidiasis in gynecological practices in Germany: A retrospective study of 954,186 patients. Curr Med Mycol 2018;4:6-11.

[4] Savaris RF, Fuhrich DG, Duarte RV, Franik S, Ross J. Antibiotic therapy for pelvic inflammatory disease. Cochrane Database Syst Rev 2017;4:CD010285.

[5] Terreni M, Taccani M, Pregnolato M. New antibiotics for multidrug-resistant bacterial strains: latest research developments and future perspectives. Molecules 2021;26:2671.

[6] Pashayan MM. Formulation and investigation of vaginal double layer suppositories containing lactobacilli and herbal extracts. New Armen Med J 2011;5:54-59.

[7] Khan A. Qarabadeen Azam. Ejaz Publishing House. New Delhi 1996.

[8] Usman RA, Rabiu U. Antimicrobial activity of Lawsonia inermis (henna) extracts. Bayero J Pure Appl Sci 2018;11:167-171.

[9] Hadisi Z, Nourmohammadi J, Nassiri SM. The antibacterial and anti-inflammatory investigation of Lawsonia Inermis-gelatin-starch nano-fibrous dressing in burn wound. Int J Biol Macromol 2018;107(Pt B):2008-2019.

[10] Caramella CM, Rossi S, Ferrari F, Bonferoni MC, Sandri G. Mucoadhesive and thermogelling systems for vaginal drug delivery. Adv Drug Deliv Rev 2015;92:39-52.

[11] Sandri G, Bonferoni MC, Ferrari F, Rossi S, Del Fante C, et al. An in situ gelling buccal spray containing platelet lysate for the treatment of oral mucositis. Curr Drug Discov Technol 2011;8:277-285.

[12] Allen LV, Loyd V. Suppositories. Quality control of suppositories. Pharmaceutical Press. UK 2007.

[13] Goupale D, Nayak S, Mhaske S. Preparation and in vitro evaluation of suppositories containing ethanolic extract of Curcuma longa Linn. J Pharm Res 2012;5:5257-5259.

[14] Khazaeli P, Mehrabani M, Mosadegh A, Bios S, Zareshahi R, et al. Formulation, physiochemical, and microbial assay of henna oil vaginal suppository formulated with polyethylene glycol bases. Iran J Med Sci 2020;45:207-213.

[15] Gautam SS, Kumar S. The antibacterial and phytochemical aspects of Viola odorata Linn. extracts against respiratory tract pathogens. Proc Natl Acad Sci India Sec B Sciences 2012;82:567-572.

[16] Physicochemical Standardization of Unani Formulations. Part IV. Central Council for Research in Unani Medicine. New Delhi 2006.

[17] Jain VM, Karibasappa GN, Dodamani AS, Mali GV. Estimating the carbohydrate content of various forms of tobacco by phenol-sulfuric acid method. J Educ Health Promot 2017;6:90.

[18] Miller Gl. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959;31:426-428.

[19] Nithya TG, Jayanthi J, Ragunathan MG. Antioxidant activity, total phenol, flavonoid, alkaloid, tannin, and saponin contents of leaf extracts of Salvinia molesta DS Mitchell (1972). Asian J Pharm Clin Res 2016;9:200-203.

[20] Sharma RK, Goel A. Identification of phytoconstituents in Lawsonia inermis Linn. leaves extract by GC-MS and their antibacterial potential. Pharmacog J 2018;10:1101-1108.

[21] da Silva CS, Pinheiro FC, do Amaral CD, Nóbrega JA. Determination of As, Cd, Hg and Pb in continuous use drugs and excipients by plasma-based techniques in compliance with the United States Pharmacopeia requirements. Spectrochim Acta B 2017;138:14-17.

[22] Quality control methods for herbal materials: Updated edition of Quality control methods for medicinal plant materials. World Health Organization. Geneva 2011.

[23] Rashid MMO, Akhter KN, Chowdhury JA, Hossen F, Hussain MS, et al. Characterization of phytoconstituents and evaluation of antimicrobial activity of silver-extract nanoparticles synthesized from Momordica charantia fruit extract. BMC Complement Altern Med 2017;17:336.

[24] Bussmann RW, Malca-García G, Glenn A, Sharon D, Chait G, et al. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. J Ethnopharmacol 2010;132:101-108.

[25] Raja W, Ovais M, Dubey A. Phytochemical screening and antibacterial activity of Lawsonia inermis leaf extract. Int J Mi-

crobiol Res 2013;4:33-36.

[26] Rao NB, Kumari SO, Goud RG. Phytochemical Analysis and antimicrobial activity of Lawsonia inermis (Henna). J Plant Sci Res 2016;3:158.

[27] Jothiprakasam V, Ramesh S, Rajasekharan SK. Preliminary phytochemical screening and antibacterial activity of Lawsonia inermis (henna) leaf extracts against reference bacterial strains and clinically important ampc βeta-lactamases producing proteus mirabilis. Int J Pharm Pharm Sci 2013;5:219-222.

[28] Gull I, Sohail M, Aslam MS, Athar MA, Phytochemical, toxicological and antimicrobial evaluation of Lawsonia inermis extracts against clinical isolates of pathogenic bacteria. Ann Clin Microbiol Antimicrob 2013;12:36.

[29] Adegboye TA, Itiola OA. Formulation effects on the mechanical and release properties of metronidazole suppositories. Afr J Med Med Sci 2003;32:247-251.

[30] Halicki PCB, Ferreira LA, De Moura KCG, Carneiro PF, Del Rio KP, et al. Naphthoquinone derivatives as scaffold to develop new drugs for tuberculosis treatment. Front Microbiol 2018;9:673.

[31] Babula P, Adam V, Havel L, Kizek R. Noteworthy secondary metabolites naphthoquinones-their occurrence, pharmacological properties and analysis. Curr Pharm Anal 2009;5:47-68.

[32] Riffel A, Medina LF, Stefani V, Santos RC, Bizani D, et al. In vitro antimicrobial activity of a new series of 1,4-naphthoquinones. Braz J Med Biol Res 2002;35:811-818.

[33] Janeczko M, Demchuk OM, Strzelecka D, Kubiński K, Masłyk M. New family of antimicrobial agents derived from 1,4-naphthoquinone. Eur J Med Chem 2016; 124:1019-1025.

[34] Dghaim R, Al Khatib S, Rasool H, Khan MA. Determination of heavy metals
concentration in traditional herbs commonly consumed in the United Arab Emirates. J Environ Public Health 2015;2015.

[35] Badoni Semwal R, Semwal DK, Combrinck S, Cartwright-Jones C, Viljoen A. Lawsonia inermis L. (henna): ethnobotanical, phytochemical and pharmacological aspects. J Ehnopharmacol 2014;155:80-103.