



Preparation of a Traditional Poly Herbal Gel for Abnormal Vaginal Discharge

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Abstract

Background and objectives: Plants are usually used for treatment of different diseases according to their traditional application. The use of the plant based medications is increasingly becoming popular all over the world. Traditional herbal medicines should be transformed into new forms to make better acceptance and more comfortable use by patients. The aim of the present study was to formulate a poly herbal gel based on Iranian traditional medicine (ITM), and determine its physicochemical characteristics. **Methods:** The gel formulation consisted of *Boswellia* spp., *Carum carvi* L., *Punica granatum* L. and *Myrtus communis* L. extracts. The product was evaluated for the physicochemical parameters. The laboratory stability tests, microbiological tests and rheological behavior were performed on the gel. Additionally, total tannin content of the gel was measured as pyrogallol using Folin-Ciocalteu reagent and hide powder. **Results:** Stability and physicochemical evaluations as well as microbiological tests showed that the prepared formulation was stable towards physical changes with no growth of pathogenic microorganisms. Moreover, it represented plastic behavior which is in favor of a topical product and also total tannins contents were determined to be 0.27 % in the gel. **Conclusion:** The product was acceptable due to its physical characteristics and so appropriate for topical use. Regarding the antibacterial, antifungal and anti-inflammatory properties of the medicinal herbs in the gel and also, the role of tannins as astringent agents, this formulation could be a suitable candidate for abnormal vaginal discharge with regards to its traditional application in ITM.

Keywords: *Boswellia*; *Carum carvi*; Iranian traditional medicine; *Myrtus communis*; *Punica granatum*

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Introduction

The history of herbal products mainly began with our ancestors who learned that using certain herbs resulted in healing of some diseases [1]. Traditional medicine is known as a useful source of potentially valuable compounds for prevention and treatment of wide range of diseases [2]. About 60-80% of the world trust in herbal drugs [3]. World Health Organization (WHO) remarks that 74% of the plant based medicines are used in modern medicine that their modern usage correlates with their traditional application as

herbal drugs [4]. In the United States, usage of complementary and alternative medicine (CAM) has developed as well [5].

Abnormal vaginal discharge is a common gynecological complaint among women in reproductive ages. Persistent vaginal discharge can cause considerable distress to many women and it can be one of the major reasons for women to visit gynecologist. Physiological reasons for increased vaginal discharge are high estrogen levels during mid-cycle, pregnancy and sexual

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arousal [6,7]. The pathological causes can be divided into those that are infective or non-infective. The most common pathological causes of vaginal discharge are trichomoniasis, bacterial and fungal infections and inflammation, which vaginitis is one of its subsets [6-8]. Differentiating the normal and abnormal vaginal discharge is essential in early detection of pathological discharge in order to prevent the possible complications of delayed treatment for reproductive tract infections such as infertility, ectopic pregnancy, increased risk of HIV transmission and infant death [7,9]. In Iranian traditional medicine (ITM), also known as Persian medicine, herbal medicines have been recommended for prevention and treatment of different diseases. Various prescriptions were used for abnormal uterine discharge presented in different dosage forms including topical formulations [10-12]. Among them, a mixture of frankincense (*Boswellia* spp.), caraway (*Carum carvi* L.), pomegranate (*Punica granatum* L.) and myrtle (*Myrtus communis* L.) (equal amounts) has been used as a topical preparation traditionally named "Zemad" [10]. Since traditional forms should be converted to modern dosage forms for better acceptance by patients and due to faster release and better percutaneous absorption of the drug in the gel-based formulation compared to other topical forms [13,14], in the present investigation, a topical poly herbal gel, containing the above mentioned herbs which was retrieved from ITM, has been formulated and its physicochemical characteristics was determined.

Material and methods

Ethical considerations

Shahid Beheshti University of Medical Sciences approved this research with the code of IR.SBMU.RETECH.REC.1395.330

Chemicals

Propylene glycol (Merck), triethanolamine (Fluka), ethanol (Merck), Folin-Ciocalteu (Merck) and Hide powder (Sigma-Aldrich) were used in the experiment. Other solvents and chemicals were of analytical grade.

Plant material

Oleo gum resin of *Boswellia* spp., fruit peels of *Punica granatum* L., fruits of *Carum carvi* L. and leaves of *Myrtus communis* L. were purchased

from Tehran herbal market. The samples were identified in the Herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. Herbal Market Samples (HMS) of *Boswellia* spp (No. 454), *Punica granatum* L. (No. 452), *Carum carvi* L. (No. 453) and *Myrtus communis* L. (No. 455) have been stored at the Herbarium of TMRC. The specimens were kept separately in well-closed containers.

Plant material analysis

Quality control tests were performed on the herbal samples according to their monographs in pharmacopoeies [15,16].

Extraction

All dried samples were powdered. Then, aqueous extracts of *P. granatum* fruit peels and *M. communis* leaves, ethanol extract of *Boswellia* spp. oleo gum resin and hydro-alcoholic (ethanol 70%) extract of *C. carvi* fruits were prepared using decoction and maceration methods for aqueous and alcoholic extracts, respectively (plant: solvent, 1:10 w/v). The extracts were concentrated under vacuum pressure up to ten times of their primary volume

Formulation of the topical herbal gel

Poly herbal gel was made by using 5% of the each mentioned plants. Gel base was prepared by applying carbopol in water (0.5, 1 and 2%) and the most suitable formulation was selected. *Punica granatum*, *M. communis* and *C. carvi* extracts were added to the gel base, respectively and were mixed well. Then various percentages of propylene glycol were added to frankincense extract (to increase solubility) and mixed with the gel. In the final step, triethanolamine was added to the mixture to form the gel.

Evaluation of the topical gel

Physical examination

Physical properties of the product such as appearance, homogeneity, odor and color were examined.

Mechanical stability

In this experiment, samples of the product were centrifuged for 15 min at 3750 rpm. Then physical changes, including phase separation were evaluated in the gel [17].

Determination of pH

pH amount of the final product, diluted 1:10 in distilled water, was determined using a pH meter (Mettler-Toledo AG, Seven easy model, Switzerland). The tests were done three times at room temperature and their average was recorded.

Viscosity determination and assessment of rheological features of the gel

Viscosity of the final formulation was measured using Brookfield Rheometer (Brookfield, DV2 RV model, USA) with spindle SC4-29 at room temperature. Various shear stresses and shear rates were used on the sample and the resulting rheogram was plotted to highlight the viscosity and rheological behavior of the herbal gel.

Temperature stability

In temperature stability tests, six samples of the gel were selected. Three samples were placed in refrigerator at 4° C and the rest were put in incubator at 40° C. After two weeks, the samples were replaced. Subsequent to 28-days cycle, the samples were evaluated for apparent changes such as homogeneity, uniformity, odor and color [17].

Total tannins content of the gel

Total tannins content was determined in the gel as a marker using Folin-Ciocalteu reagent and hide powder in accordance with British pharmacopoeia with some modifications [15]. Briefly, the proper dilution of the gel sample was oxidized with Folin-Ciocalteu reagent and the reaction was neutralized with aqueous solution of sodium carbonate (29%, w/v). The sample was placed in dark for 30 min. The absorbance of the resulting blue color was determined at 760 nm using water as compensation liquid to get the amounts of total phenolics. Measurement of tannins was conducted in continuation of the above mentioned method by mixing the same dilution of sample with hide powder to separate tannins from other polyphenols. Subsequent to shaking vigorously for 60 min, the compound was filtrated and the above colorimetric procedure was conducted on the filtrates to measure the amounts of polyphenols which were not adsorbed by hide powder. Tannins content of the solutions was determined according to the following equation:

$$\text{Content of tannins} = [\text{Total phenolics content} - \text{non-adsorbed polyphenols content}]$$

Quantification was done on the basis of the standard curve of pyrogallol. Results were mentioned as gram of pyrogallol equivalent per 100 gram of the gel. All measurements were performed at room temperature in triplicate.

Microbiological tests

Microbial tests were performed in accordance with WHO instructions [18].

Results and Discussion

In this research, a poly herbal gel suggested (for abnormal vaginal discharge according to ITM prescriptions) was prepared. The results of the analysis of the plants contained in the product have been shown in table 1. The results were within the acceptable range of pharmacopoeias [15,16].

One of the main ingredients of the formulation is the gelling agent. The concentration of gel forming agent is very important because lower concentrations will result in a mixture with very low consistency, while high concentrations can lead to the formation of a gel with high viscosity causing a non-uniform distribution of drug in the formulation and the lack of proper dispersion on the skin [19]. In this study, various percentages of gelling agent were tested to choose the most suitable concentration for the formulation. The gel base including 0.5 % of carbomer 940 was a diluted gel whose liquefaction was observed 24 h later and it was inappropriate for gel formulations. With increasing the carbomer concentration to 1.0 %, the uniform and smooth gel base was observed. The gel base containing 2.0 % of carbomer 940 was thick and sticky with lack of proper distribution; therefore, 1.0% of carbomer 940 was determined as the most appropriate concentration for gel forming. The ingredients of the final gel formulation along with their quantity have been presented in table 2. The prepared gel showed acceptable physicochemical properties including appearance, homogeneity, uniformity as well as smooth and soft feeling after using on the skin, without being sticky or feeling of dryness. The pH of the polyherbal gel was measured to be sure that the formulated could be used without the risk of irritancy and sensitivity to the skin. The pH was within the appropriate range for the skin products (pH= 4-6) [20]. No signs of phase separation and physical changes were observed during centrifugation and temperature stability tests.

Table 1. Analysis of *Boswellia* spp, *Carum carvi*, *Punica granatum*, *Myrtus communis*

Plant materials	Total ash%	Acid insoluble ash%	Water soluble ash%	Sulfated ash%	Loss on drying%	Essential oil%	Total polyphenolics %
<i>Boswellia</i> spp.	5.55± 0.1	-	-	-	6.4± 0.5	5.0± 0.5	-
<i>Carum carvi</i>	4.66± 0.2	-	-	-	8.45± 0.7	5.0± 0.4	-
<i>Punica granatum</i>	5.04± 0.2	-	-	-	-	-	16.5± 0.6
<i>Myrtus communis</i>	-	2.83± 0.1	2.04± 0.1	4.06± 0.2	-	1.5± 0.1	-

Table 2. Composition of the ingredients in the herbal gel

Ingredients	Quantity (%)
Plant extracts	5% from each one (based on dried plant)
Carbopol 940	1%
Propylene glycol	7%
Triethanolamine	0.5%
Distilled water	Up to 100%

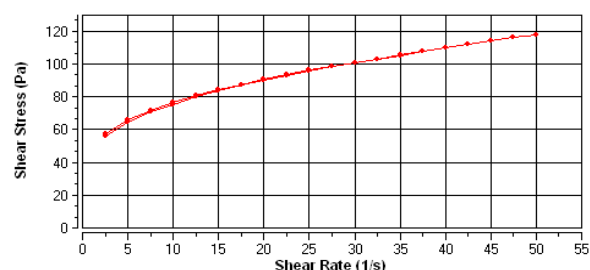
Table 3. Physicochemical characteristics of the poly herbal gel

Parameters	Results
Color	yellow-brown
Odor	Characteristic with dominant odor of frankincense
Homogeneity	Good
pH	5.35 ± 0.04
Tannins %	0.27±0.01

Moreover, the results of microbiological tests were in agreement with requirements [18] (table 3). The product was satisfactory with regards to its physical parameters and so suitable for topical application with no need for any preservatives. The flow properties of semi solid products influence each step of the pharmaceutical development process, such as filling, mixing, packing and removal from the container before the application on the action site. The time-dependent change in viscosity provides pharmaceutical formulations with the flexible rheological manifestation, which subsequently affects the release profiles of loaded drugs. Therefore, it is essential for researchers to outline the flow properties and influencing factors, and their effects on the pharmacological efficacy of thixotropic formulations, particularly gels, ointments, emulsions and colloids [21]. Rheological behaviors consist of plastic, viscous and elastic features and a mixture of these, viscoelasticity, is one of the most important parameters in the evaluation of topical formulations [17,22,23] that ultimately leads to a suitable combination with favorable viscosity, stability and durability on the surface of the skin [24,25]. The rheogram of the formulated gel was measured using stainless steel Brookfield rheometer (figure 1). According to the figure, the rheogram was non-linear indicating non-Newtonian behavior. Since the curve did not start

from the origin of the coordinates, it can be deduced that the formulation exhibited plastic (Bingham) rheological behavior and also the curve was transformed to a line that represented a typical feature of plastic behavior that was predictable for semisolid compounds. In order to make non-linear region as linear as possible and determine the y-intercept more accurately, the log values of shear stress were drawn against the log values of shear rate (figure 2). By calculating the antilog of y-intercept of the equation ($y = 0.253x + 1.631$) corresponding to the linear plot, the yield value was defined (42.75 Pa). The calculated plastic viscosity and Bingham yield stress of the gel were 2.19 Pa.s and 94.35 Pa, respectively, obtained from the slope and y-intercept of the equation ($y = 2.190x + 94.35$) corresponding to the linear part of the rheogram (figure 3).

Abnormal vaginal discharge is a common gynaecological complaint among women in reproductive age. Due to the role of bacterial and fungal infections as well as inflammation as pathological causes [6-8], it seems that antimicrobial, antifungal and anti-inflammatory agents could improve the problem.

**Figure 1.** Rheogram of the poly herbal gel, representing the presence of a plastic behavior

Natural remedies are more acceptable regarding that they are safer with less side effects compared to synthetic ones.

Myrtus communis L. is an evergreen perennial shrub belonging to the Myrtaceae family [26].

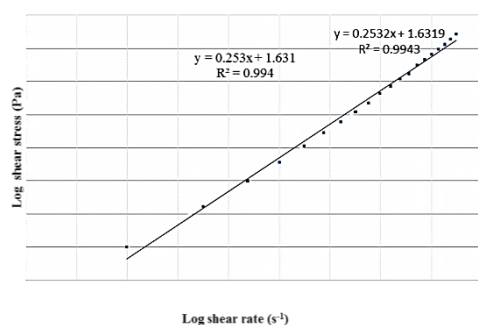


Figure 2. The linear plot of log shear stress-log shear rate for the poly herbal gel

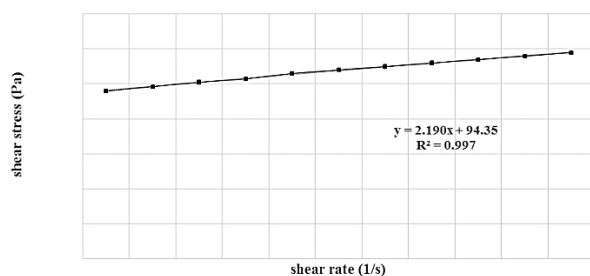


Figure 3. The linear plot of the poly herbal gel rheogram

The extracts of the plant have been investigated for their polyphenolics and tannins previously [27]. The antibacterial activity of methanol, ethanol, and ethyl acetate extracts of *M. communis* leaves has been revealed [28]. *Myrtus communis* has shown antibacterial activity against *Bacillus subtilis* and *E. coli* as well as *Staphylococcus aureus*, *Proteus mirabilis* and *Klebsiella pneumonia* [27,29,30]. Eslami et al. proved that the plant methanol extract showed a strong antibacterial activity on regular and IMP-producing *P. aeruginosa* strains [31]. Furthermore, *M. communis* L. essential oil (EO) has shown to be effective against *Candida* species [32,33]. Mehrabani et al. showed that ethyl acetate and total methanol extracts of *M. communis* leaves demonstrated the most considerable antifungal effects among other extracts of the plant [26]. In another study, the aqueous leaf extract of the plant showed toxic activity against *Trichomoniasis vaginalis* at pH 4.65 [34]. Anti-inflammatory activity of *n*-hexane and acetone extracts, as well as the essential oils obtained from the berries and leaves of *M. communis* were determined based on the inhibition of the increase in vascular permeability [35]. Also, the anti-inflammatory effects of Myrtucommulone and semimyrtucommulone found in the leaves of myrtle, were demonstrated

through in vivo model [36-38].

About 50% of the total fruit weight of *Punica granatum* Linne (pomegranate) corresponds to the peel, which is an important source of bioactive compounds such as phenolics, flavonoids, ellagitannins (ETs), and proanthocyanidin compounds [39]. Pomegranate peel is rich in hydrolyzable tannins, mainly punicalin, pedunculagin, and punicalagin [40]. Gullon et al. demonstrated antibacterial activity of peel of *P. granatum* and determined its total phenolics and tannins contents [41]. Colak proved the antifungal and antibacterial activities of pomegranate skin extract and it was concluded that pomegranate skin extract could be used as a preservative and coloring material [42]. The anti-*Trichomoniasis vaginalis* effect of *P. granatum* extract (in-vitro and in-vivo) showed considerable results [43,44]. The results of another study revealed that hydrolysable tannins of pomegranate inhibited NO production and iNOS expression in RAW 264.7 cells and could be used as a standard marker for the anti-inflammatory activity of *P. granatum* [45]. In a study performed in 2017, Houston et al. demonstrated that pomegranate rind extract down regulated expression of COX-2 more than total pomegranate tannins verifying that the rind extract penetrated the skin and modulated COX-2 regulation in the viable epidermis [46]. Ben Saad et al. showed that ellagic acid, gallic acid and punicalagin A&B obtained from *P. granatum* potentially inhibited LPS-induced NO, PGE-2 and IL-6 production and could be considered as the compounds responsible for anti-inflammatory potential of the plant [47].

Marichali et al. demonstrated that the leaves extracts of *Carum carvi* L. (Umbelliferae) were rich in total flavonoids and phenols [48]. Also, Thies showed that the caraway possess tannins [49]. Antibacterial activity of the essential oil extracted from fruits of *C. carvi* was determined against Gram-positive and Gram-negative bacterial species and also it has been revealed that aqueous extract of the plant can be used as a potent antibacterial agent for human pathogens [50-53]. In a study, it was found that caraway had a high inhibitory effect against *Aspergillus* species [54]. It has been revealed that *C. carvi* aqueous extract inhibited the production of inflammatory mediators such as iNOS, COX-2, IL-6 and TNF- α . Moreover, *C. carvi* exhibited anti-neuroinflammatory effects via regulation of

NF-kappa B signaling [55].

The chemical analysis of oleo-gum resin water extract of *Boswellia dalzielii* (Burseraceae) demonstrated the presence of total phenols, flavonoids, anthocyanins and tannins in the herb [56]. Afsar et al. showed that the methanol fraction of *B. serrata* leaves contained high amounts of total phenolics, flavonoids and tannins and exhibited strong in vitro anti-inflammatory activity [57]. Methanol extract of the resin of *B. carterii* exhibited marked anti-inflammatory activity for their inhibitory effect against 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice [58]. In addition, anti-inflammatory effect of *B. elongate* Balf. F has been established as well [59]. Moreover, *B. carterii* oil has demonstrated antimicrobial activity [60]. Adelakun et al. investigated that methanol and aqueous extracts of *B. dalzielii* stem bark exhibited antibacterial and antifungal activity [61].

Quantitative assay in our research demonstrated the presence of tannins in the prepared gel due to the presence of plants with high content of tannins in the gel. Tannins have astringent properties, resulting in faster healing of wounds and inflamed mucous membranes [62]. Also, tannin-rich fruits have been proven to have anti-inflammatory properties [63]. Moreover, the antimicrobial activities of tannins are well documented. Furthermore, it has been revealed that the growth of many fungi, yeasts, bacteria, and viruses was inhibited by tannins [64]. Due to antimicrobial, antifungal and anti-inflammatory effects of phytochemical components of the gel ingredient, the formulated herbal gel might be effective in abnormal vaginal discharge with respect to its traditional use.

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Author contributions

Homa Hajimehdipoor and Shirin Fahimi supervised the project; Maedeh Rezghi and Sara Zakerin performed the experimental parts; Maedeh Rezghi prepared the manuscript.

Declaration of interest

The authors declare that there is no conflict of

interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

ITM: Iranian traditional medicine; HMS: Herbal Market Samples