Journal of Pharmaceutical Research International

23(1): 1-10, 2018; Article no.JPRI.42143
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Formulation and Characterization of Dill Seed Vaginal Cream Based on a Traditional Medicine

Zohreh Sarhadinejad[1,2], Fariba sharififar[3], Zarrin Sarhadynejad[1,2], Zohreh Salari[4], Haleh Tajadini[5,2], Farzaneh Mohamadi[6], Amir Asadi-Khanouki[1] and Mehdi Ansari[6*]

[1]Herbal and Traditional Medicines Research Center, Kerman University of Medical Sciences, Kerman, Iran.
[2]Department of Persian Medicine, Faculty of Persian Medicine, Kerman University of Medical Sciences, Kerman, Iran.
[3]Department of Pharmacognosy, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran.
[4]Obstetrics and Gynecology Center, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran.
[5]Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran.
[6]Department of Drug and Food Control, Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran, Haft-Bagh Blvd, Kerman, P.O.Box 7616911319, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MA, Z. Sarhadinejad and FS designed the study, performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors Z. Sarhadynejad, Z. Salari and HT managed the analyses of the study. Authors FM and AAK managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2018/42143
Editor(s):
(1) Dr. Rafik Karaman, Professor, Bioorganic Chemistry, College of Pharmacy, Al-Quds University, USA.
Reviewers:
(1) Kelly Mari Pires de Oliveira, Universidade Federal da Grande Dourados, Brasil.
(2) Alok Nahata, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history/25485

Original Research Article

Received 26th April 2018
Accepted 5th July 2018
Published 10th July 2018

ABSTRACT

Background: Dill seed has been used as a vaginal discharge remedy according to traditional origins, but its application by traditional methods is difficult and time-consuming.
Aims: The main aim of this study was preparing a vaginal dosage form with acceptable

**Corresponding author: E-mail: mansari@kmu.ac.ir, mansari1345@yahoo.com;*

physicochemical properties from dill seed extract-essential oil combination to be easily applied, and the dosage can be precisely determined.
Study Design: This is an experimental study and statically designed according to the approved protocol for drug assessments.
Place and Duration of Study: Pharmacognosy Department, Kerman University of Medical Sciences, Kerman, Iran, between May 2016 and June 2017.
Methodology: Dill seed was purchased and authenticated. Some phytochemical properties like volatile contents, total flavonoid, and phenolic content, extractable matter were determined. In addition, gas chromatography/ mass spectrophotometer (GC/MS) analysis and thin layer chromatography fingerprints were done. Physical properties, pH, chemical stability, microbial contamination, and release study of the vaginal cream were carried out.
Results: GC-MS analysis showed that the main components of the essential oil are dl- Limonene and carvone. The presence of rutin in the extract was confirmed by TLC fingerprints. Total flavonoid content was expressed as rutin equivalent to 22.5±1.6 µg per mg of the extract. The total phenolic content was expressed as gallic acid equivalent to 201.6±1.0 µg per mg of the methanolic extract. The released percentage, on the basis of total extract from the cream after 24 hours was about 36.9±8.4. Chemical stability showed that major components of DSVC including carvone and limonene remained about 102.8 and 106.9%, respectively after 24 months. Microbial control of the final products showed no pathogenic contamination.
Conclusion: The results from phytochemical of the dill seed and physicochemical of the vaginal cream were in an acceptable range which supports its stability and applicability in clinical trials.

Keywords: Anethum graveolens L.; Antifungal drug development; Physicochemical characterization; Traditional Medicine; Vaginal cream.

ABBREVIATIONS

DSVC: Dill seed vaginal cream; TEE: Combination of dill seed total extract and essential oil cream; EO: Dill seed essential oil cream; TPM: Traditional Persian medicine; GAE: Gallic acid equivalents; TFC: Total flavonoid content; TPC: Total phenolic contents; GC/MS: Gas chromatography/ Mass spectrophotometry; TLC: Thin layer chromatography; CFU: Colony count unit; HPMC: Hydroxy propyl methyl cellulose.

1. INTRODUCTION

Vaginitis is one of the most common conditions in gynecologic clinics. After bacterial vaginitis, vaginal candidiasis is the second cause of vaginitis, and approximately 75% of all women suffered this infection once in their lifetime. Which half of them experience recurrent candidiasis probably because of drug resistance. The most important cause of the disease is the imbalance in the bacterial and fungal flora of the vagina [1]. Furthermore, the prevalence of invasive fungal infections has been increased [2]. Fungal infections like vaginal candidiasis can be treated by a variety of anti-fungal agents including topical and systemic anti-fungal azole medications. But theses few anti-fungal medicines have limitations such as drug resistances, side effects, and costs [3]. According to World Health Organization (WHO) reports, the percentage of using complementary and alternative medicine among world population is increased and estimated about 65 -80% [4]. In addition, pharmaceutical companies are paying more attention to research and development of new pharmaceutical dosage forms for drugs of traditional origins [5]. For these reasons, introducing a novel effective medicine with a traditional origin, a matter of interest today, is pleasant. *Anethum graveolens* L. (family: Umbelliferae; common name: dill) is an annual aromatic herb originated from the Mediterranean and central and southern Asia. However, in addition to its original growing places, it has been cultivated approximately all over the world because of its wide consumption in food and pharmaceutical industries. Dill seed which is commonly used as a medicine has an oval, compressed, winged shape, and about one-tenth inch wide, with three longitudinal ridges on the back [6]. In traditional Persian medicine (TPM), this seed has been used as an anti-emetic, anti-cramp [7], and vaginal discharge treatment [8]. There are a lot of reports regarding the pharmacological effects of dill seeds such as anti-fungal, anti-microbial, anti-

inflammatory, analgesic, and mucosal protective effects [9].

Recent studies indicate that essential oil of dill seed contains carvone and dihydrocarvone (30 - 60%), limonene (33%), α –phellandrene (21%), as major components, and carvacrol, terpinene, dillapiol as minor constituents [7]. Moreover, the results of phytochemical analysis on dill seeds have shown the presence of alkaloid, terpenoid, steroid, tannin, and flavonoid. [10] Other studies have reported that phenolics present in dill seed possess in vitro and in vivo anti-candidia activities [11]. Moreover, in vitro and in vivo anti-fungal effects of dill seed essential oil were also demonstrated [12]. TPM recommend several remedies in the dosage form of douching with aqueous extract, consuming as a decoction, or smoking of the seeds to be applied locally [8,13], whose applications are troublesome and time-consuming. In TPM, the effects of a medicine are often thought to be related to the whole suggested herbal part more than to a pure substance; therefore, a modern dosage form based on TPM is better to be prepared by its all components as possible. Therefore, preparation of a vaginal cream containing the extract and essences, which cover almost all volatile and nonvolatile components of the dill seed and this method would be very close to the traditional form.

The main aim of this study was preparation and formulation of vaginal cream based on extract and essential oil of dill seed for vaginal candidiasis, and evaluation of phytochemical and physicochemical properties, microbial contamination, stability and release of the dill seed vaginal cream (DSVC). Formulation of vaginal cream based on TPM which can be easily applied, reliably adjust the dosage, and is stable for a long period of time can be very useful in vaginal candidiasis treatment.

2. MATERIALS AND METHODS

2.1 Chemicals

Gallic acid and rutin were obtained from Carl Roth Co., Germany. Vazelin, and Hydroxy propyl methyl cellulose (HPMC) were purchased from Aburaihan pharmaceutical Co., Iran. Carbomer 940 p, Methyl paraben (MP), Propylparaben (PP), Cetyl Alcohol, Polyethylene glycol 300 (PEG 300), Tween® 80, Aerosil®, and paraffin were purchased from local market made by Sigma Aldrich co, Germany.

2.2 Plant Materials

In this experiment, dill was gathered from Mahan city, Kerman province (30.3°N, and 57.0°E), Iran. The date of collecting was from May to June 2016. The herbal sample was identified by an herbalist and a voucher specimen (KF 1137) kept in the Herbarium of Pharmacognosy Department, Kerman University of Medical Sciences, Kerman, Iran. In this study dill seeds were used.

2.3 Phytochemical Characterization

2.3.1 Ash values and water and volatile content

Ash values (total ash, and acid-insoluble ash), and water and volatile content of dill seeds were determined in accordance with WHO recommended procedures [14]. The results were expressed as mean ± SD in Table 2.

2.3.2 Hydroalcoholic extract

Methanol (80%) was used for hydroalcoholic extraction of the dill seeds. The entire procedure was exactly in accordance with a recent study. [15] Finally, the concentrated extracts were dried at less than 40°C for 48 h. The percentage of the extractable amount was presented as mean ± SD.

2.3.3 Thin layer chromatography fingerprints

Three different standards (quercetin, kaempferol, and rutin, 100 µg/ ml) were spotted on chromatogram GF254 and let to go ahead in a mobile phase of chloroform, ethyl acetate, methanol, and distilled water (3:2:1:0.5 v/v/v/v). The plate was observed at 254 and 366 nm after drying to detect constituents in the dill extract.

2.3.4 Total flavonoid content (TFC)

Total flavonoid content of the dill extract was determined as described by Dowd [16] with some modifications. At first, a stock solution (50 ppm) was prepared from rutin as the major flavonoid. Then, a volume of 2 ml of aluminium trichloride (AlCl3, 2% in methanol) was added to the equal volume of rutin. After 30 min incubation, λ max was determined by a spectrophotometer (Synergy HTX, USA) in the range of 200-400 nm. The same procedure was carried out to determine absorbance of different concentrations of rutin (50, 100, 200, 500 µg per ml) and at 275 nm and 270 nm for rutin and plant extract (100 ppm) versus a blank sample at λmax. The total

flavonoid content was expressed as rutin equivalent (RE) mg per g of extract.

2.3.5 Total phenolic content (TPC)

Folin-Ciocalteu method was used to measure the dill seed total phenolic content [15]. In this method, a spectrophotometer (Synergy HTX, USA) was used to measure the absorbance of the blue color at 765 nm versus a methanol blank. The results were calibrated with a standard curve of gallic acid solution in methanol (50, 100, 200, 300, 400, 500, 1000 µg/ml). Finally, the quantity of the dill seed total phenolic content was calculated mg gallic acid equivalents (GAE)/ 100gr of the dried samples.

2.3.6 Gas chromatography/ mass spectrophotometry (GC/MS) analysis of essential oil

The essential oil content of dill seed was analyzed using GC/MS (Agilent Technologies 7890A; coupled with a 5975 MSD and equipped with a DB-1MS capillary column (30 m (L.)× 0.250 mm (i.d.), film thickness: 0.25 µm)). A quantity of 1 µl of the sample was injected splitless. Other conditions were as follows. Gas carrier: helium, flow rate of 0.81ml/ min; column temperature: 60-275°C at 5°C/min; injector temperatures: 280°C; detector temperatures: 250°C; and Aux Heater 250°C ionization potential: 70 even. Retention indices relative to C8-C24 n-alkenes were utilized to identify the essential oil components. The results were matched in accordance with the Wiley 7n.l mass spectral library of the GC/MS data system [17].

2.4 Herbal Processing of Formulation

This formulation was originated from traditional sources with some innovation [8,13]. For preparing the herbal vaginal cream, collecting of essential oil and extraction were carried out as follow: The Hydro-distillation method by a Clevenger apparatus was used to collect the essential oil (2% v/w). The obtained oil was stored at -20°C in a tight closed container until use. The aqueous residue from hydro distillation was filtered, concentrated for 30 min and was dried in room temperature for 48 h.

2.5 Preparation of DSVC

The ingredients of the different vaginal creams have been summarized in Table 1.

Table 1 demonstrates all the components for preparation of DSVC. These oil in water emulsions were prepared according to Mali et al [18] with a slight modification. For preparing 500 g of TEE (F1) cream, 90 g of dill seed total extract (dried aqueous extract) was dissolved in 100 g distilled water; then this solution was added to the aqueous phase (Table 1). The components of oil phase were also dispersed and heated up to 70° C, and then the aqueous phase was added to the oil phase. Finally, at ambient temperature, 10 g of the essential oil was added to the formulation. As it can be seen in Table 1, in preparing EO cream the formulation base was as the same as TEE cream, but the total extract was omitted. F2 was prepared the same as F1 except in which HPMC was deleted.

Table 1. Different vaginal cream formulations

No.	Ingredient	DSVC Formulations (%)			
		TEE (F1)	EO	Placebo	F2
1	HPMC	2	2	2	0
2	Carbomer 940	0.2	0.2	0.2	0.2
3	PEG 300	3	3	3	3
4	Propylene Glycol	2	2	2	2
5	Tween 80	6	6	6	6
6	MP	0.5	0.5	0.5	0.5
7	PP	0.1	0.1	0.1	0.1
8	Cetyl alcohol	8.5	8.5	8.5	8.5
9	Aerosil	1.5	1.5	1.5	1.5
10	paraffin	6	6	6	6
11	Vaseline	12.1	12.1	12.1	12.1
12	Dried aqueous extract	18	0	0	18
13	Essence	2	2	0	2
14	Water	38.1	56.1	58.1	40.1

**HPMC: Hydroxy propyl methyl cellulose; MP: Methyl paraben; PP: Propylparaben; PEG: Polyethylene glycol; EO: Essential Oil; F1: Combination of dill seed total extract and essential oil vaginal cream; F2: F1 formulation without HPMC*

2.6 Physicochemical Characterization of the Creams

2.6.1 Physical properties

Some descriptive properties such as colour, homogeneity and consistency of the prepared formulations were inspected visually at different temperatures (2-8°C, 25 ± 2°C, and 40± 2°C) at the beginning of the experiment, 2, 4, 12, and 24 weeks after the formulation preparation. The pH of TEE and EO creams were determined by a pH meter at ambient temperature. Samples were prepared by dilution of two grams from each formulation with 100 ml of ethanol: water (50:50) combination [17].

2.6.2 Release study

In vitro release study of TEE, cream was carried out using modified Franz diffusion cell (receptor volume: 15 ml) [19]. Cellophane membrane was soaked in distilled water for 24 h before use. To measure the amount of total phenolic compounds released, a calibration curve was prepared based on the different concentration of total extract (10, 50, 100, 200, and 500 mg/L) in water was used. The release measurements were performed on the TEE cream (combination of total extract and essential oil) by placing 0.5 g TEE cream gently spread on the cellophane membrane. Distilled water: ethanol (50:50) was used as the receptor media. The entire procedure was done at 37 ± 2ºC with magnetic stirring at 250 rpm. At different time intervals, up to 24 h 1ml of aliquots were withdrawn from the receiver compartment in each interval. Also, for maintaining the sink conditions, 1 ml of receptor compartment was replaced. A UV spectrophotometry method was used to calculate the amount of in vitro drug release at a wavelength of 247 nm. All the measurements were carried out at least three times, and the results were expressed as mean± SD. The release study was performed for two different formulations, and each release percentage was plotted against time. All the data were fitted on Peppas equation (Eq.1) [20].

$$(Mt/ M\infty = kt^n) \quad (1)$$

In which Mt is the amount release until time t, M∞ is the released amount until the infinite time, n=diffusional exponent; t= time; k= constant (characteristics of macromolecule specifically geometry).

In addition, to find any similarity between the two release profiles, F2 test (Similarity factor) using the following equation was used (Eq.2).

$$f2=50*\log [(1+ (1/n) \Sigma nt=1 (Rt-Tt) 2)-0.5*100] \quad (2)$$

Where N= number of time points, Rt and Tt= dissolution of reference and test products at a definite time.

If f2 is greater than 50 it is considered that two products share similar drug release behaviours and it will be more if the result gets near 100 [21].

2.6.3 Microbial contamination

Microbiological quality control of the raw herbal sample and of vaginal creams (TEE, and EO) was performed in accordance with the United States Pharmacopeia (USP) [17] by using the pour-plate method to determine the total viable counts for fungi and bacteria. A colony count unit (CFU)/g of the samples was used to express the total aerobic microbial count. Furthermore, TEE and EO creams were examined for the presence of specific pathogens.

2.6.4 Chemical Properties

The major components of dill seed essential oil in the formulation were analyzed by GC-MS to determine their residual ingredients and compared with the measured amounts of major components of essential oil [17]. The sample was prepared by dispersing 0.5 g of the cream in isopropyl alcohol to break the cream structure and diluting by methanol to 100 mL, the solution was centrifuged and then filtered supernatant was injected into GC-MS (Agilent Technologies 7890A). The sample was prepared from the cream at the end of storage in the refrigerator for 24 months.

2.7 Data Analysis

The Microsoft Office Excel 2016 was used for drawing the graphs and perform statistical analyses. Each treatment was carried out in triplicates and presented as mean± SD.

3. RESULTS

3.1 Phytochemical characterization

3.1.1 Ash values, loss on drying, and extractable amount

Table 2 demonstrates the results of the ash values, water content, and extractable amount.

Table 2. The results of ash values, water content, and extractable amount

	Ash value (%)			Extractable value	
	Total ash	Acid-insoluble ash	Loss on drying (%)	Extractable amount (%)	Air dried extract (%)
Anethum gravelones L.	10.90± 0.29	0.14± 0.01	5.10± 0.54	9.58± 0.34	18± 1.2
Standard amounts*	10 ± 0.0	3 ± 0.0			

Standard amounts in Iranian Herbal Pharmacopoeia [6]*

3.1.2 TLC fingerprints

Rutin was selected as the major component of phenolic ingredients in the extract. The presence of rutin was confirmed by TLC fingerprints at 254 nm.

3.1.3 Total Flavonoid and total phenolic content

The amount of RE as a representative for flavonoid compounds have been determined by using standard calibration curve of rutin in water measured by spectrophotometry with a calibration curve of y=0.154x+0.0084; R2 = 0.996. Results showed that 22.5± 1.6 mg of RE per 100 mg. TPC was expressed as gallic acid equivalent to 201.6 ± 1.0 µg per mg of the methanolic extract with an equation on a chart Y= 0.0007x– 0.0319; R2= 0.999.

3.1.4 GC-MS analysis of essential oil

Table 3 shows the major components identified by GC-MS library, their retention indices (RI, KI) and percentages accounting for 100% of total oil.

3.2 Physicochemical Characterization of Creams

3.2.1 Physical properties

The appearance of the cream formulations was pale brown and no colour change was observed during storage in the different temperatures. Also, in all the conditions, the texture of the creams was smooth for more than two years storage in the refrigerator. Moreover, the creams showed no particulate materials or coarse disparity. According to the phase ratio and type of surfactant used, the creams were O/W emulsion in which no phase separation and physical instability were seen. These evaluations were carried out for 6 months.

3.2.2 Determination of pH

The pH values of TEE and EO were determined 5.8 ±0.1, and 5.3 ± 0.1, respectively.

3.2.3 Release study

The drug release parameters calculated by Peppas equation for the 2 formulations of TEE cream (F1, F2) are presented in Table 4.

According to the calibration curve constructed by UV absorbance, measurement of different concentrations of total extract in water measured at 247 nm. And this λ max was used for released amount calculation. In vitro release profiles of the 2 formulations have been shown in Fig. 1. The results showed that the released % of the extract for F1 and F2 were 15.5±5.6% and 12.8±3.9%, respectively, after 8 h. After 24 h, it reached 36.9 ±8.4%, and 42.2%±4.3, respectively. The similarity factor (F2) was calculated as 70. It means that two profiles are not significantly different, and this result shows that the presence of HPMC has no significant ($p<0.05$) effect on the release rate.

Table 3. Characterizations of dill seed essential oil

No.	KI	RI	Compound	Percent (%)	Identification	Identification
1	10.28	10.73	dl- limonene	31.3	MS, RI	GC/MS, KI
2	12.04	15.71	Cis and trans Dihydrocarvone	4.7	MS, RI	GC/MS, KI
3	12.49	16.85	Carvone	55.1	MS, RI	GC/MS, KI
4	12.71	26.63	Dillapiole	8.8	MS, RI	GC/MS, KI
			Total identified	100		

**RI: Retention indices, KI: Koats indices*

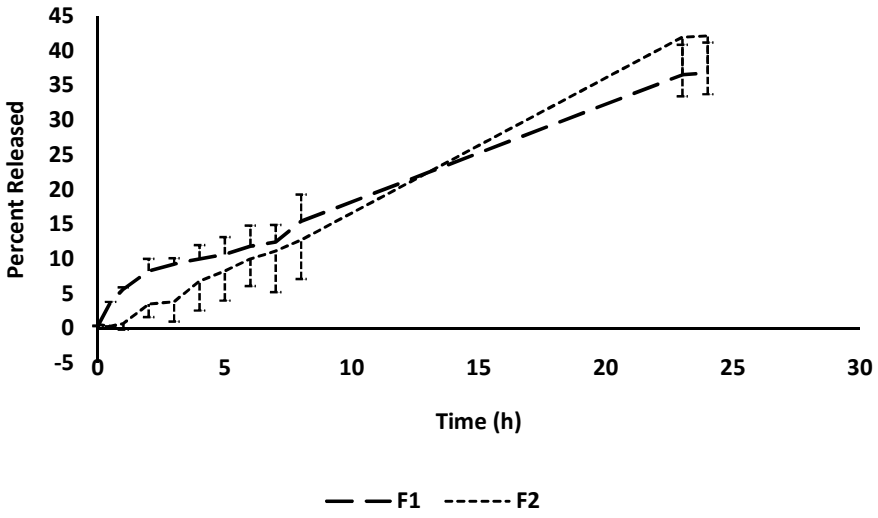

Fig. 1. Release profiles of two formulations with HPMC (F1) and without HPMC (F2)

Table 4. Peppas equation coefficients calculated for the two formulations

Formulation	Diffusional exponent (n)	Release rate constant (k)	Correlation coefficient (r^2)
F1	0.5	0.85	0.95
F2	1.2	-1.63	0.98

**F1: Combination of dill seed total extract and essential oil vaginal cream; F2: F1 formulation without HPMC*

Table 5. A measured amount of limonen and carvone in DSVC and pure dill seed essential oil

No.	RI	Compound	AUC [a]	AUC [b]	Remained percent (%)
1	10.728	limonen	31050	29034	102.8
2	16.851	carvone	52411	50980	106.9

**RI: Retention Indices; AUC [a] Area under the curve of DSVC; AUC [b] Area under the curve of the equivalent amount of essential oil; DSVC: Dill seed vaginal cream*

3.2.4 Microbial contamination

Results demonstrated no microbial and fungal contamination in the vaginal creams (TEE, and EO). In addition, Pathogenic bacteria and fungus including *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Candida albicans* were not seen in the vaginal creams.

3.2.5 Estimation of stability of essential oil in the formulations

Table 5. Presents the area under the curve of limonen and carvone as principal components of the essential oils measured in DSVC and essential oil.

4. DISCUSSION

Over the past decades, some studies have been designed and performed to evaluate quality control, standardize, and modify the traditional products for the purpose of preparing novel, safe and efficient formulations [5] So, in the current research, a traditional preparation recommended for vaginal discharge from traditional Persian medicine was selected and improved for comfortable application as a vaginal cream dosage form. Furthermore, the standardization of the final product was carried out to have access to reproducible and diagnostic characteristics for therapeutic and research purposes.

In the present study, the percentage of the acid-insoluble ash was very low indicating a few mineral impurities in the sample with sand and silica. The results of another study have shown that high phenolic and flavonoid content of dill seed extract can be very useful in treating fungal diseases and also demonstrated that phenolics could be used as anti-fungal agents because of advantages such as natural source, easy availability, less drug resistance and fewer side effects [22]. Moreover, the obtained results demonstrated that the use of polyphenols such as rutin and quercetin in combination with other anti-fungal agents or alone reduces anti-fungal MIC [22,23].

In the present study, Carvone and dl limonene, identified as major constituents, made about 86% of the total volatile oil. It is remarkable that these compounds have been recommended as potential anti-fungal [9,11]. Fungal mycelia of *candida albicans* easily absorb dill seed essential oil because of the lipophilic property of the essential oil. The antifungal mechanism of limonene and carvone alone is affecting the plasma membrane and mitochondria of *candida albicans* and also, they have synergistic effect when combined together [9]. Concerning ecological conditions, various chemical compositions for dill seed like dill apiole, Haxadecanol, Limonene, n-octane, α-Phellandren, D-Carvone and Cyclo-hexasiloxane have been reported [24].

Our obtained results of the physical evaluation showed that characteristics such as odour, colour, texture and appearance were stable for 6 months. Therefore, it could be concluded that the creams could be stable in a normal storage condition for more than 2 years. The pH value of the prepared creams was around 5.5 which was compatible with the pH of the normal healthy vagina [25].

According to Peppas equation, to find the most probable release mechanism, diffusional exponent (n) was calculated as 0.5 for F1 cream. This value demonstrated square root of time dependent and Fickian release mechanism. Moreover, n was estimated more than 1 for F2, indicating non Fickian super case 2, erosion of polymeric chain, stresses and state transition in hydrophilic glassy polymers which swell in water. Low per cent release even after 24 h. may be related to some factors including low solubility of the extracted constituent or essential oil components in the receiver compartment of diffusion cell, the non-significant difference between solubility and concentration at each time, low permeability of the used membrane and inappropriate receiving media [20]. Based on similarity factor, the calculated amount for both formulations was 70. It means that the two profile is not significantly different, and it shows the presence of HPMC has no effect on the release rate [21]. Our results also showed that using HPMC couldn't have an effect on the release of extract, however, HPMC can affect adhesiveness of the formulation to the vaginal mucosa [19]. In response to the goal of this study, the results showed a physically stable vaginal cream with proper release properties and a standard amount of dill seed extract and essential oil (0.02 g essential oil (0.01 g carvone)/g of vaginal cream) at the end of the shelf life (2 years).

Evaluation of chemical stability demonstrated that the remained major components of DSVC, limonene and carvone, in comparison with these pure essential oil's components [17], were 102.8 and 106.9%, respectively. This performance was carried out after 24 months storage of the samples at 4°C. This means that the formulation may have a shelf life of at least two years.

4. CONCLUSION

The ancient dosage form of dill seed that has been recommended for vaginal discharge in TPM was douching (abzan), but this dosage form has some limitations like dis-comfortable use and non-standardized and reproducible characteristics. Furthermore, a few conventional antifungal agents are available for vaginal candidiasis treatment. In this study, for the first time, a modified dosage form (O/W emulsion) was designed and its phytochemical and physicochemical properties were evaluated as an antifungal drug development. Results depicted dill seed vaginal cream (DSVC) seems to have appropriate physicochemical characterizations, release properties and stability which may be used as an effective, appropriate, stable and reproducible herbal product. Also, the results of a clinical trial were performed by the authors of this study showed the efficacy and safety of this herbal product in human (Sarhadinejad, Z., Kerman University of Medical Sciences, Iran, Unpublished results).

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

ACKNOWLEDGEMENTS

We would like to thank Deputy of Research, Kerman University of Medical Sciences (Grant no: 95/170 to M. Ansari) for supporting this research. It was a part of Ph. D thesis to Zohreh Sarhadinejad.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Brek J, Adash E, Hilard P. Novak's Gynecology Disease. 14th ed. Tehran: Golban; 2012.
[PMid:23014157 PMCid:PMC3527184].
2. Pianalto KM, Andrew Alspaugh J. New Horizons in Antifungal Therapy. J Fungi. 2016;2(26).
3. Gavanji S, Zaker SR, Nejad ZG, Bakhtari A, Bidabadi ES, Larkia B. Comparative efficacy of herbal essences with amphotricin B and ketoconazole on Candida albicans in the in vitro condition. Integr Med Res. 2015;4: 112-118
Available:https://doi.org/10.1016/j.imr.2015.01.003
[PMid:28664116 PMCid:PMC5481788].
4. Fidel P and Sobel J. Immunopathogenesis of recurrent vulvovaginal candidiasis. Clin Microbiol Rev. 1996;9:335-348.
[PMid:8809464 PMCid:PMC172897].
5. Sarhadynejad Z, Sharififar F, Pardakhty A, Nematollahi M, Sattaie-Mokhtari S, and Mandegary A. Pharmacological safety evaluation of a traditional herbal medicine "Zereshk-e-Saghir" and assessment of its hepatoprotective effects on carbon tetrachloride induced hepatic damage in rats. J Ethnopharmacol. 2016;190:387-395.
Available:https://doi.org/10.1016/j.jep.2016.07.043
[PMid:27426508].
6. Iranian Herbal Pharmacopoeia committee. Iranian Herbal Pharmacopoeia. Tehran (in persian): Ministry of health, treatment and medical education, Food and drug department. 2002;506-510.
7. Al-snafi AE. The pharmacological importance of *Anethum graveolens*. A review. Int J Pharm Pharm Sci. 2014;6:11-13.
8. Avecina. The canon of medicine; 1991. Tehran (in persian): Soroush.
9. Chahal KK, Monika, Kumar A, Bhardwaj U, Kaur R. Chemistry and biological activities of *Anethum graveolens* L. (dill) essential oil: A review. J Pharmacogn Phytochem. 2017;6:295-306.
10. Ishikawa T, Kudo M, Kitajima J. Water-soluble constituents of dill. Chem Pharm Bull. 2002;50:501-507.
Available:https://doi.org/10.1248/cpb.50.501
Available:https://doi.org/10.1248/cpb.50.1471
[PMid: 11963998].
11. Zabka M, Pavela R. Antifungal efficacy of some natural phenolic compounds against significant pathogenic and toxinogenic filamentous fungi. Chemosphere. 2013;93: 1051-1056.
Available:https://doi.org/10.1016/j.chemosphere.2013.05.076
[PMid: 23800587].
12. Zeng H, Tian J, Zheng Y, Ban X, Zeng J, Mao Y, et al. *In vitro* and *in vivo* activities of essential oil from the seed of *Anethum graveolens* L. against candida spp. Evid Based Complement Alternat Med. 2011; 2011:1-8.
Available:https://doi.org/10.1155/2011/659704
[PMid: 21716714, PMCid: PMC3119046].
13. Aghili khorasani Alavi Shirazi M. Makhzan-ol-Adviyah. Tehran: Sabzarang; 2009.
14. World Health Organization. Quality control methods for herbal materials. Geneva; 2011.
15. Sarhadynejad Z, Pardakhty A, Mandegary A, Afsharypuor S, Sharififar F. Physicochemical characterization, standardization and *In vitro* determination of radical scavenging activity of Zereshk-e-Saghir, A Traditional Preparation, and Its Ingredients. J Young Pharm. 2017;9:224-229.
Available:https://doi.org/10.5530/jyp.2017.9.44
16. Kaska A, Çiçek M, Deniz N, Mammadov R. Investigation of phenolic content, antioxidant capacities, anthelmintic and cytotoxic activities of *Thymus zygioides*

Griseb. J Pharm Res Int. 2018;21(1):1-13. Available:https://doi.org/10.9734/JPRI/2018/39688

17. USP. United States Pharmacopeia, USA: United States Pharmacopoeial Convention; 2016.
18. Mali AS, Karekar P, Yadav AV. Formulation and evaluation of multipurpose herbal cream. International Journal of Science and Research. 2015;4: 1495-1498.
Paper ID: NOV151515.
19. Rençber S, Yaprak Karavana S, Ay Şenyiğit Z, Eraç B, Hoşgör M, Baloğlu L, et al. Mucoadhesive in situ gel formulation for vaginal delivery of clotrimazole: Formulation, preparation, and in vitro/in vivo evaluation. Pharm Dev Technol. 2017;22:551-561.
Available:https://doi.org/10.3109/10837450.2016.1163385
[PMid: 27055376].
20. Siepmanna J, Peppasb N. Modeling of drug release from delivery systems based on hydroxypropyl methylcellulose (HPMC). Adv Drug Deliv Rev. 2001;48:139–157.
Available:https://doi.org/10.1016/S0169-409X(01)00112-0
21. Deveswaran R, Abraham S, Bharath S, Basavaraj BV, Furtado S, Madhavan V. Design and characterization of diclofenac sodium tablets containing Tamarind seed polysaccharide as release retardant. International Journal of Pharm Tech Research. 2009;1:191-195.
22. Teodoro GR, Kassapa E, ChamindaJ S, Koga-Ito S, Koga-Ito CY. Potential use of phenolic acids as anti-candida agents: A Review. Front Microbiol. 2015;6:1-11.
Available:https://doi.org/10.3389/fmicb.2015.01420
[PMid:26733965 PMCid:PMC4685070].
23. Oliveira VM, Carraro E, Auler ME, Khalil NM. Quercetin and rutin as potential agents antifungal against Cryptococcus spp. Braz J Biol. 2016;76:1029-1034.
Available:https://doi.org/10.1590/1519-6984.07415
[PMid: 27166572].
24. Larijani K, Shakouri MJ, Pishbin N, Mansourbahmani S, Heidari AA. The essential oils of Dill (*Anethum graveblens* L.) collected from Gilan, Iran. Ann Biol Sci. 2012;3:1010-1013.
Available:www.scholarsresearchlibrary.com
25. Kumar N, Behera B, Sagiri SS, Pal K, Ray SS, Roy S. Bacterial vaginosis: Etiology and modalities of treatment—A brief note. J Pharm Bioallied Sci. 2011;3:496-503.
Available:https://doi.org/10.4103/0975-7406.90102
[PMid: 22219582 PMCid: PMC3249696].

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/25485