Research Article

Preparation and Evaluation of Semisolid dosage forms Containing Ferula Hermonis Extract

Khuzama Al Zougbi*, Mohammad Othman, Issa Hassan
Dept. of Pharmaceutics & Pharmaceutical Technology, Faculty of Pharmacy - Damascus University – Damascus, Syria.
***Corresponding author's E-mail:** Lavandula4@hotmail.com

Accepted on: 04-02-2014; **Finalized on:** 31-03-2014.

ABSTRACT

Ferula hermonis Boiss or ''Zalooh root'' in spite of being used for many purposes, it does not have adequate studies. Lately it has been reported as a strong antioxidant, antibacterial and antimycotic agents. The aim of this study was to develop a pharmaceutical semisolid dosage forms that contain an alcoholic extract of Ferula hermonis root and to study the release of this extract from these dosage forms by using a dissolution test apparatus II with Enhancer Cell with artificial membrane poly vinylidene fluoride PVDF 0.22 µm. It was found that cream (A2), in which Emulgade SE was used as emulsifier, released the largest amount of F. hermonis extract during the first five hours, while polyethylene glycol ointment (B3) prolonged the released of the extract. The creams were evaluated by examining the size and the shape of the droplets under a microscope immediately after manufacturing and after a month of preserving. Two samples of alcoholic roots extract of Ferula hermonis were analyzed by mass spectrometer associated with Gas chromatography GC and mass spectrometer associated with Liquid chromatography LC. Results were similar and they matched the published articles. The positive ESI-MS spectrum of ferutinin contained a signal at *m/z* 341 $[M\text{-}H_2O + H]^+$ and a fragment at *m/z* 203 [M-H2O-pOHBzOH+ H]$^+$.

Keywords: Enhancer cell, Ferula hermonis, Ferutinin, GC-MS, LC-MS, Semisolid dosage forms.

INTRODUCTION

Ferula hermonis belongs to Umbelliferae (Apiaceae) family and the genus Ferula. Anyway, there are over 170 types of this genus which are known as the source of good components and they are biologically effective. However, there are about 100 types spread out over a wide range of the Mediterranean areas and Middle Asia.[1-3]

Ferula hermonis contains vitamins such as D, C, B6, B2, B1 and vitamin A[8] and a high percentage of vitamin E (α-tocopherol).[4] It also contains irons like magnesium, selenium, and zinc[8] and great deal of terpenes family specifically sesquiterpenes like Teferin, Ferutinin and Teferidin with Estrogenic activity.[12] Ferula hermonis roots have been used in the Middle East for a long time to improve the sexual conduct and in frigidity and erectile dysfunction treatment. It was called Lebanese Viagra[3,11] for its use as a treatment for the erectile dysfunction and for dysmenorrhea as well.[5] Also, Ferula hermonis was used either for cooking or for chemical purposes.[2] On the other hand, Ferula hermonis roots contain 17 Ducane sesquiterpenoid esters.[5] Previous phytochemical studies on this plant have revealed the presence of various sesquiterpenes. The effects of anti-inflammatory go back to the two basic Ducane esters Teferin, Ferutinin which were isolated from the roots oil of F. hermonis. These Ducane esters were evaluated by the carrageenan induced oedema model in mice.[6] Obviously, the anti-inflammatory effect of both Ferutinin and Teferin was observed with the dose of 100 mg/kg, while Teferidin did not show any anti-inflammatory effect.[6] This indicated that the anti-inflammatory effect of Teferin might have involved all inflammatory mediators including PGs, histamine, 5-hydroxytryptamine, bradykinin or nitric oxide, all of which have been reported in carrageenan-induced oedema.[6] The antimicrobial activity was evaluated by determination of MIC using the microdilution method against six bacterial strains and one fungal strain[7]. The components Teferin, Ferutinin and Teferidin demonstrated potent activity against Gram positive strains (*S.aureus, B.subtilis*), as well as Mycobacterium strains M. bovis BCG and M. tuberculosis.[7] In the antioxidant study using the DPPH assay method, the highest radical scavenging activity was observed for compounds Teferin, Ferutinin and Teferidin.[7] In addition, a study about the effect of the oil isolated from Ferula hermonis roots on fungi was done. This study compared this effect to some fungicidal that are available in markets.[9] *Ferula hermonis* roots extract should be considered as a promising source of medicine for the treatment of *Staphylococcus aurous* infection in both human and live stalk.[10] Accordingly, Ferula hermonis has been widely used in the ancient times as general tonic.[8] Recently a patent which used F.hermonis in cosmetics was recorded. This patent relied on the existence of Ferutinin in F.hermonis extracts which is known as phytoestrogens. In this patent, it was discovered that ferutinin increased skin firmness and hydration.[12] In this study, a pharmaceutical semisolid dosage forms containing an alcoholic extract of Ferula hermonis roots was developed and examined *in-vitro.* Drug release was conducted using Enhancer Cell with artificial membrane poly vinylidene fluoride PVDF 0.22 µm.

MATERIALS AND METHODS

Materials

The materials used were: Ferula hermonis roots which were picked from Al Shiekh Mountain in Syria, Ethanol, distilled water, sunflower oil, Span80, Tween80, cetostearyl alcohol, stearic acid, methylparaben, propylparaben, Ascorbic acid, Emulgade SE, PEG 400, PEG 4000, pluronicF-68 (poloxamer 188) and white petroleum.

Equipment

The equipments used were: Buchi- Rotavapor R200, (Hitachi U 1800- spectrometer) detector SPD-30A prominence Ultraviolet rays, Enhancer Cell (local made), dissolution tester Aparatus-II (Germany) Erweka DT, Delicate scale, Termo orion PH meter720 (Germany), Myr Rotational Viscosity meter V2 (VISCOTECH), Electronic mixer for making emulsions (ANALIS- NAMUR-EMW), poly vinylidene fluoride PVDF Membrane hydrophilic 0.45µm (Millipore Austria), analyzed by mass spectrometer associated with Gas chromatography GC and mass spectrometer associated with Liquid chromatography LC.

Methods

Extracting Ferula hermonis root with ethanol

Powdered dried roots of Ferula hermonis (366g) were extracted exhaustively in Rotary evaporator with ethanol. The ethanol extract was evaporated at 40°C temperature to give 25.35 g of gummy residue. Then it was conserved in tightly sealed glass vials and kept in -20°C temperature until use. The extraction yield was 7%.

Defining the solubility of Ferula hermonis extract

In order to find the best solvent for the dissolution test and for calibrating the extract by spectrophotometer, solubility of Ferula hermonis extract was identified in each of the following solvents: Ethanol, distilled water, acetone, PEG 400, water and ethanol 95% mixture (30:70), water and ethanol 95% mixture (40:60), and water and ethanol 95% mixture (50:50).

Scanning the extract of ferula hermonis roots by spectrophotometer and Calibration

According to F.hermonis solubility, the solution of the extract in water /ethanol 95% mixture (50:50) at concentration 0.4 mg/ml was scanned by Spectrophotometer UV in wavelength from 200 to 400nm to find the peak of absorption in order to calibrate the extract.

Then a standard series of F.hermonis extract solutions in water /ethanol 95% mixture (50:50) was prepared from the stock solution 0.4 mg/ml and the absorptions were measured to determine the linearity of the Standard series.

Preparing semisolid dosage forms containing an alcoholic root extract of Ferula hermonis

Creams

The creams were prepared as oil in water emulsion. Emulsifiers were chosen depending on the appropriate HLB values.[13] F.hermonis extract was added to the half amount of oil while preparing.

Cream (A1): (50g)

Ferula hermonis extract	(0.25g)
cetostearyl alcohol	(2.5g)
stearic acid	(2.5g)
sunflower oil	(5.15g)
propylparaben	(0.05g)
distilled water	(50g)
Methylparapen	(0.05g)
Span80	(0.05g)
Tween80	(0.5g)

Cream(A2): (50g)

Ferula hermonis extract	(0.25g)
cetostearyl alcohol	(2.5g)
stearic acid	(2.5g)
sunflower oil	(5.15g)
distilled water	(50g)
Methylparapen	(0.05g)
Emulgade SE	(1g)

Ointments

Ointment (B1): (50g)

The extract (0.25g) was added to half quantity of white petroleum (25g) then it was mixed very well until the extract distributed homogeneously in white petroleum. After that, the second half of the white petroleum was added (25g) and again still continued mixing until the complete distribution of the extract in the whole amount of white petroleum.

Ointment (B2): (50g)

- The extract (0.25g) was dissolved in polyethylene glycol PEG 400 (25g). Then 5 ml of dis lled water containing 0.15 g of Pluronic was added to the mix. The mix was heated to 45° C temperature.
- polyethylene glycol PEG 4000(25g) was grinded and melted.
- Finally, the PEG 4000 was added to the first mix and continued mixing until it became cold.

Ointment (B3): (50g)

- The extract (0.25g) was dissolved in PEG 400 (25g). The mix was heated to 45°C temperature.
- PEG 4000(25g) was grinded and melted.
- Finally, the PEG 4000 was added to the first mix and continued mixing until it became cold.
-

Studying the creams under the optical microscope

The shape, size and spread of the droplets were studied for each cream individually in 5 different microscopic spaces immediately after manufacturing. This procedure was repeated after one month of manufacturing to study the stability of each cream.

Scanning by mass spectrometer to analyze the components of Ferula hermonis extract samples

First sample was analyzed by mass spectrometer associated with Gas chromatography (GC- MS) type Scan within a range (100-500 m/z)**.** Second sample was analyzed by mass spectrometer associated with liquid chromatography (LC- MS) type **positive ES-MS** and the range of scanning was between (100-1400m/z)**.** Retention Time and the specific mass-to-charge ratio (m/z) for each component were compared with the published international results.

Studying the release of extraction components from creams and ointments through artificial membranes

For measuring the release of drug from semisolid dosage forms and as an alternative to the Franz-type *in vitro* dissolution testing system, the Enhancer Cell was introduced commercially in the early 1990's by VanKel Industries. The system consists of a donor chamber (the cell body) for the dosage form that is covered by a synthetic membrane. The entire assembly is placed in the bottom of a standard USP Apparatus 2 dissolution vessel.[14,15]

In this study:

- The used Enhancer Cell (local made) is covered by a Hydrophilic Membrane poly vinylidene fluoride 0.22µm.Surface area of the cell is 3.14 cm².
- The number of cycles per minute is 50 rpm at temperature 32± 2˚C .
- Released media is water/ ethanol 95% mixture (50:50).
- An amount of 1.5 g from each form (cream-ointment) was put in the cell.
- The samples were taken in the following times 30, 60, 120, 180, 240, 300 minutes.
- The experiment was repeated three times. Mean values and standard deviation were calculated.

Statistical methods

The above studies were statistically analyzed by SPSS which is a computerized statistical program (version 17.0). Paired t-Test was used when comparing two means. The differences between data are considered to be significant if P<0.05 and a highly significant if P<0.01.

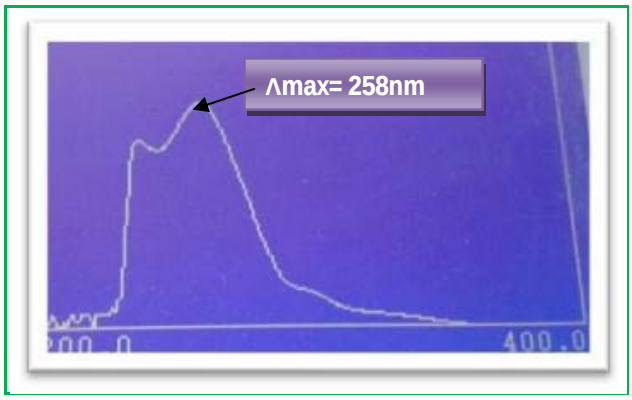
RESULTS AND DISCUSSION

Studying the Solubility of Ferula hermonis extract in different solvents

Ferula hermonis extract is freely soluble in ethanol (95%), polyethylene glycol 400 and Acetone; very slightly soluble in distilled water; Soluble in sunflower oil and water/ Ethanol (95%) (50:50) . According to this Solubility, water/ Ethanol 95% mixture (50:50) was used as a solvent in the dissolution test, PEG 400 was used in preparing ointments B2 and B3 and sunflower oil was used in preparing creams A1 and A2.

Scanning the extract of ferula hermonis roots by spectrophotometer and Calibration

One absorption peak at 258 nm wavelength was found when the solution of the extract in water/ Ethanol 95% mixture (50:50) at 0.4 mg/ml concentration was scanned by Spectrophotometer UV in wavelength from 200 to 400nm.This is similar to the results of another published research which reported that Ferutinin absorption wavelength is 256 nm.[20] Therefore, wavelength 258nmwas adopted for calibration. Figure 1 shows the absorption peak in the scanning curve of the solution.



X-axis: nm; Y-axis: Absorbance

Figure 1: Spectral scanning of the solution of F.hermonis extract in water/ Ethanol 95% (50:50) in wavelength range from 200 to 400 nm

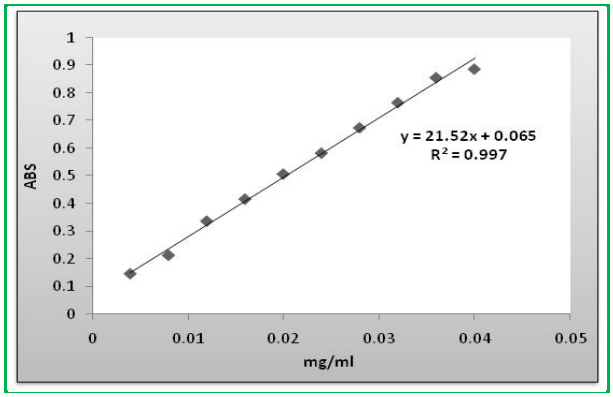


Figure 2: The standard curve of F.hermonis extract solution in water/ alcohol (50:50) by Spectrophotometer.

Figure 2 shows the straight graph line with the equation y = 21.52x + 0.065 and a correlation coefficient r^2>0.99. This indicates that the method of assay is linear.

Studying the creams under the optical microscope and monitoring the physical properties

Figure 3 shows the distribution of different sizes of the droplets in the creams. It was found that most of the droplets in cream A1 were in size15-25 and 25-35 µm, while most of the droplets in cream A2 were in size 5-10 and 10-15 µm. Figure 4 shows the examination of creams droplets under the microscope immediately after manufacturing and one month after manufacturing.

When the creams were placed in closed containers far away from light and at room temperature 25°C for 30 days, no change was found in the external form, odor or color for each formula.

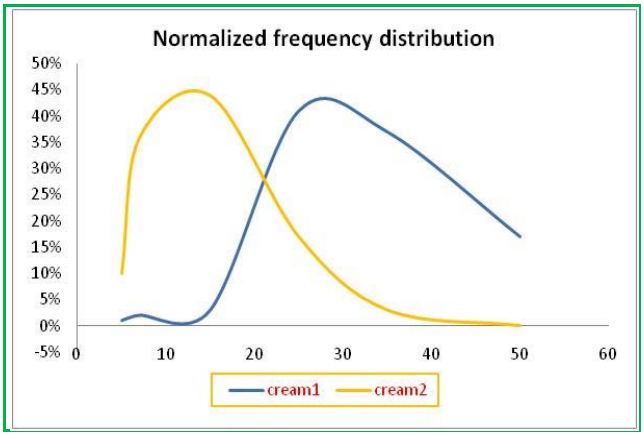
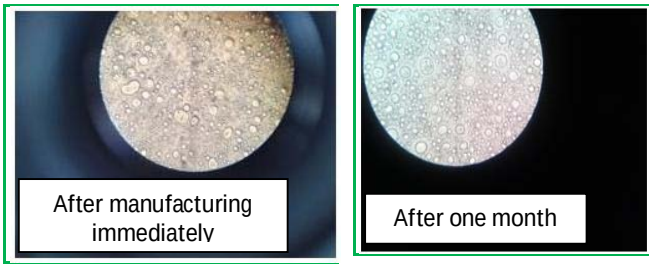


Figure 3: Normalized frequency distribution of the droplets.

Cream A1:



Cream A2:

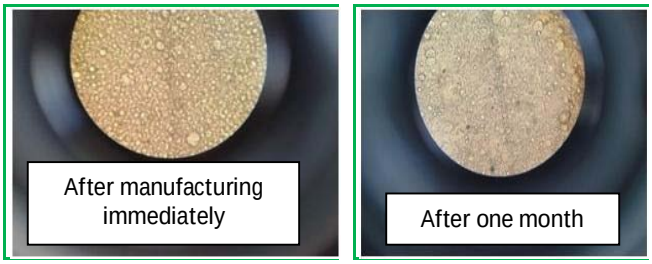


Figure 4: Images under an optical microscope of the creams (A1+A2) immediately after manufacturing and after one month. [100X power]

Studying the release of the extract from the creams and ointments through artificial membranes

When the accumulated released quantity of F.hermonis extract from semi-solid dosage forms was compared during the first five hours (Figure 5), it was concluded that cream (A2) where Emulgade SE was used as emulsifier had the best released amount of F.hermonis extract during the first five hours with the presence of significant statistical difference in terms of (p = 0.001), while the (Vaseline) ointment (B1) did not release the extract during the first five hours and ointment (B3) prolonged the released of the extract.

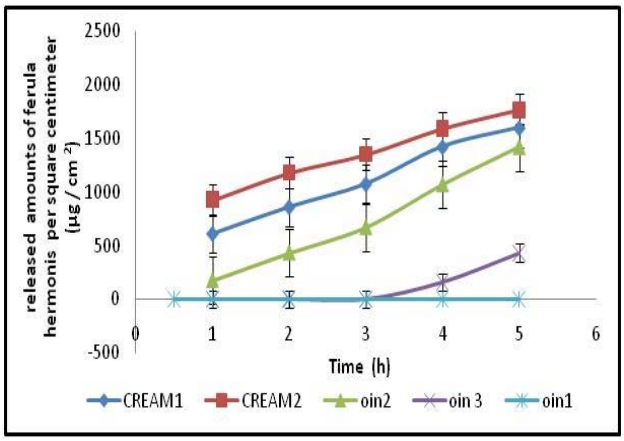


Figure 5: *In-Vitro* release Profile of F.hermonis extract creams and ointment formulations.

Drug Release Kinetics Studies

The drug release data of F.hermonis extract from creams A1, A2 and B2 were fitted to models representing zero order, first order, Higuchi's, Hixson-crowell and Korsmeyer's equation kinetics to know the release mechanisms. The changes in quality and quantity in formula may change medicine release and the performance inside the organism (16,17). The data were processed for regression analysis using MS EXCEL statistical function. It was obvious that the extract releasing from this cream A1 followed zero order kinetic with equation y = 294.5x + 241.6 and highest correlation coefficient (R^2=0.990), extract releasing from this cream A2 followed Higuchi kinetic with equation y =689.4x + 204.9 and highest correlation coefficient (R^2=0.995), and It was obvious that the extract release from ointment B2 followed zero order kinetic y=308.2x - 169.7 and highest correlation coefficient (R^2=0.992).

Scanning by mass spectrometer to analyze the components of Ferula hermonis extract samples

- Figure 6 shows the results of the first sample of Ferula hermonis extract which was examined by mass spectrometer associated with Gas Chromatography.
- Based on the approved references[18,19] twenty five components were more likely to be characterized in F.hermonis which was picked from Al Shiekh Mountain in Syria. The most prominent components in the extract were camphor, myrtenyl acetate, myrtenol, Ferutinin, limonene, D-verbenone, Teferdin and α-pinene, in addition to other compounds that may present in the F.hermonis extract in small amounts. Look at Table 1.

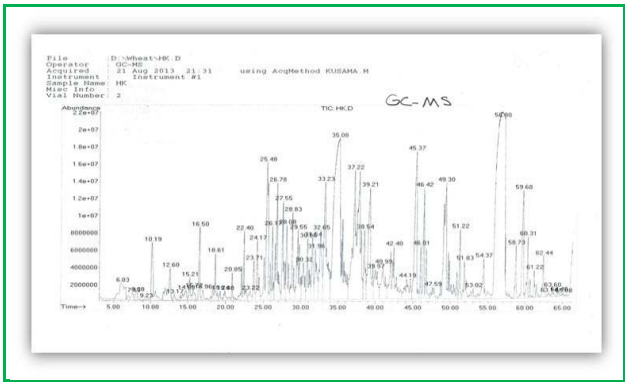


Figure 6: Results of analyzing the first sample of F.hermonis extract by GC-MS (RT)

Table 1: Comparing the detected retention time to the retention time in the published references to conclude the extract compounds.

NO	RT	RT (adopted References)	Compound accor. to published reference	Ref.
1	7.55	7.33 (±0.09)	Epoxybenz	B*
2	14.68	-	Myrtenol	-
3	12.60	12.34(±0.26) 12.11	Ferutinol vanillate sabinene	B A
4	13.17	13.95(±0.20)	Ferutinin	B
5	14.68	14.26	myrcene	A
6	15.21	-	1- Verbenone	-
7	18.61	18.22	thujol	A
8	19.14	19.56	cis-β-terpineol	A
9	22.40	22.12	nonanal	A
10	23.22	23.32	camphenol	A
12	23.71	23.93(±0.44)	Teferdin	B
13	24.17	24.24	pulegone	A
14	25.48	25.75	D-verbenone	A
15	26.78	26.67 26.98	Camphor isoborneol	A B
16	27.55	27.99	myrtenol	A
18	29.55	29.27	fenchyl acetate	A
19	10.19	11.09	α-pinene	A*
20	16.96	16.69	limonene	A
21	18.61	18.22	thujol	A
22	35.08	34.73	myrtenyl acetate	A
23	37.23	37.82	α-copaene	A
24	38.54	38.99	α-ylangene	A
25	39.21	39.60	α-copaene	A
26	39.97	39.79	γ-elemene	A
27	42.40	42.09	aromadendrene	A
28	44.19	44.00	germacrene-D	A

***(A**: Ehab A. A bourashed. et al. National Center for Natural Products Research, University of Mississippi,2000[18]; **B**:Elif odabas, Kose. et al. Journal of Medicinal Plants Research Vol. 4(17).2010[19]**)**

Figure 7 and Figure 8 show the results of the second sample of Ferula hermonis extract which was examined by mass spectrometer associated with Liquid Chromatography LC-MS (positive ES-MS). It was found that there is a signal in The positive ESI-MS spectrum of ferutinin at *m/z* 341 $[M\text{-}H_2O + H]^+$ and a fragment at *m/z* 203 [M-H2O-*p*OHBzOH+ H]$^+$ that show a very high tendency to lose H_2O.[20]

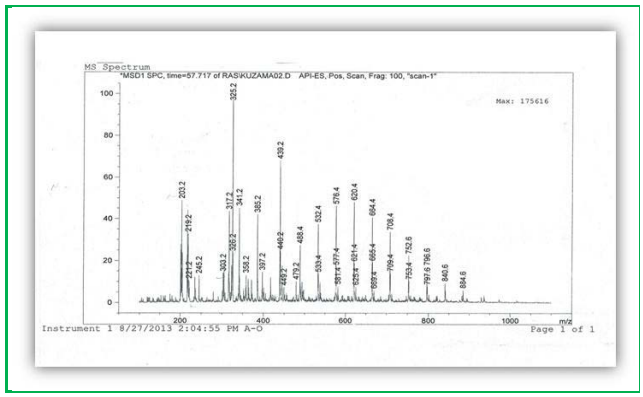


Figure 7: Results of analyzing the second sample of F.hermonis extract by LC-MS (M/Z)

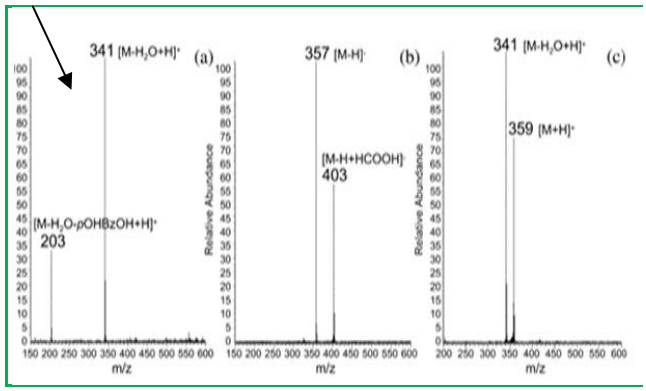


Figure 8: ESI-MS positive (a)-ESI negative (b)- APCI positive (c) of ferutinin.[20]

CONCLUSION

This study demonstrated that F.hermonis extract is soluble in oils and Organic solvents and it can be formed in pharmaceutical semisolid dosage forms by using the alcoholic extract of Ferula hermonis roots.

Cream A2, in which Emulgade SE was used as emulsifier, released the best amount of F.hermonis extract during the first five hours. Emulgade SE that is prepared from (Glyceryl Stearate / Ceteareth-20 / Ceteareth-12 / Cetearyl Alcohol / CetylPalmitate) is a nonionic emulsifying wax and it is used as an emulsifying agent in the production of oil-in-water emulsions that are unaffected by moderate concentrations of electrolytes and are stable over a wide pH range.[21] F.hermonis roots have a complex mix of components and various charges. So, using Emulgade SE as emulsifier plays a good role in increasing the solubility and the distribution of the extract in the oil phase and eases the release of the extract from the cream.

The release of the extract from ointments was slow and hard. This was because of the waxy, oily and lipophilic nature of the F.hermonis extract. Pluronic F68 (0.3%) which was used in ointment B2increased the solubility of the extract in PEG and thus eased the release of the components from the ointment. Pluronic F68 is a nonionic polyoxyethylene–polyoxypropylene copolymer which is used primarily in pharmaceutical formulations as emulsifying or solubilizing agent and may also be used as a wetting agent in ointments.[21]

In the positive ESI-MS spectrum, a signal was found at *m/z* 341 [M-H2O-pOHBzOH+ H]$^+$ and a fragment at *m/z* 203 [M-H2O-pOHBzOH+ H]$^+$ which refer toH_2Olose and thus it refers to ferutinin exhibition in the F.hermonis extract.

REFERENCES

1. Frensh DM, Biology and Chemistry of Umbelliferae. Academic Press: London, 1971, 400-401.
2. Hadidi KA, Aburjai T, Battah AK, A comparative study of Ferula hermonis root extracts and sildenafil on copulatory behavior of male rats, Fitoterapia, 74, 2003, 242–246.
3. Said O, Khalil K, Fulder S, Azaizeh M, Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region, J Ethnopharmacol, 83, 2002, 251–265.
4. Emile Aoun, Jamil Rimab, Ghiwa Chidiacb, Khalil Hanna, High-performance liquid chromatographic and spectrofluorometric determination of a-tocopherol in a natural plant: Ferula hermonis (Zalooh root), Journal Of Food Composition And Analysis, 18, 2005, 607–615.
5. Zanoli P, Benelli, ARivasi M, Vezzalini F, Baraldi C, Baraldi M, Opposite effect of acute and subchronic treatments with Ferula hermonis on copulatory behavior of male rats, Int J Impot Res, 15, 2003, 450–455.
6. Afaf Geroushi, Abdurazag A. Auzi, Abdalla Salem Elhwuegi, Fawzi Elzawam, Akram Elsherif, Lutfun Nahar, Satyajit D. Sarker, Anti-inflammatory sesquiterpenes from the root oil of Ferula hermonis Journal Phytotherapy Research, 10, 2010, 1-14.
7. Zedan Zeid Ibraheim, Wael M. Abdel-Mageed, Huanqin Dai, Hui Guo, Lixin Zhang and Marcel Jaspars. Antimicrobial Antioxidant Daucane Sesquiterpenes From Ferula Hermonis Boiss, Journal Phytotherapy Research, 26, 2012, 579–586.
8. Yousry Naguib YMA, U.S. Patent No. 6623768: Herbal compositions and methods for diabetes and Weight Loss Management, 2004, 1-8.
9. Al-Ja'fari A-H, *et al*., Composition and antifungal activity of the essential oil from the rhizome and roots of *Ferula hermonis*, Phytochemistry, 72, 2011, 1406-1413.
10. Seham S. Kassem, Emtenan M. Hanafi, A. Abd El Raouf, Madiha M. Abdel-Kader and Ghada.A. Awad, Evaluation of *Ferula Hermonis* for Treatment of Staphylococcal Infection Journal of Applied Sciences Research, 8, 2012, 3985-3991.
11. Elinoar Shavit, Lebanese Viagra – Ferula, Pleurotus, And Geopolitics. Medicin Al Mushrooms- FUNGI Volume 2:3, 2009, 52-56.
12. Ezio, Bombarrdelli, Croppellp Cairoli, Gabriele Fontana, Aldo Criston, Enrico Merealli. U.S. Patent No. 8101215: Process For Cosmetic Treatment Using Ferutinine From Ferula Genus Plants, 2012, 1-10.
13. Ankurman Shrestha, Effect Of Span 80 –Tween 80 Mixture Compositions On The Stability Of Suflower Oil-Based Emulsions, Department of Biotech & Medical Engineering National Institute of Technology- Rourkela, 769008, 2011, 1-26.
14. The United State Pharmacopia–National formulary USP 35-NF20 2ndsupplement. (Tests to appear in performance). USP chapter <1724> semi-solid medical products, 2012.
15. Wilna Liebenberg, Eileen Engelbrecht, Anita Wessels, Bharathi Devarakonda, Wenzhan Yang, Melgardt M. De Villiers. Comparative Study Of The Release Of Active Ingredients From Semisolid Cosmeceuticals Measured With Franz, Enhancer Or Flow-Through Cell Diffusion Apparatus, Journal Of Food And Drug Analysis, 12(1), 2004, 19-28.
16. Suvakanta Dash, Padala Narasimha Murthy, Lilakanta Nath, Prasanta Chowdhury, Kinetic Modeling On Drug Release From Controlled Drug Delivery Systems. Acta Poloniae Pharmaceutica-Drug Research, 67(3), 2010, 217-223.
17. Mehta Piyush, Sharma Deepak , Dashora Ashok , Sahu Deepak, Garg Rahul Kumar , Agrawal Piyush , Kapoor Deepak N. Design, Development And Evaluation Of Lipid Based Topical Formulations Of Silver Sulfadiazine For Treatment Of Burns And Wounds, Innovare Gournal Of Life Science, 1(1), 2013, 38-44.
18. Ehab A. Abourashed, Ahmed M. Galal, Farouk S. El-Feraly, Ikhlas A. Khan. Separation and Quantification of the Major Daucane Esters of Ferula hermonis by HPLC. National Center for Natural Products Research, University of Mississippi, 67, 2000, 681-682.
19. Elif odabas, Özgür Aktaş, Gökhan Deniz, Cengiz Sarikürkçü, Chemical composition, antimicrobial and antioxidant activity of essential oil of endemic Ferula lycia Boiss, Journal of Medicinal Plants Research, 4(17), 2010, 1698 -1703.
20. Lolita Arnoldi, Mauro Ballero, Nicola Fuzzati, Andrea Maxia, Enrico Mercallia, Luca Pagni, HPLC-DAD-MS Identification Of Bioactive Secondary Metabolites From *Ferula Communis* Roots, Fitoterapia, 75, 2004, 342–354.
21. Raymond C. Rowe, Paul J. Sheskey, Sia^n C. Owen, Hand book of pharmaceutical excipients-5th ed./Pharmaceutical Press and American Pharmacists Association, 2006, 536 & 816.

Source of Support: Nil, **Conflict of Interest:** None.