Georgian Scientists
ქართველი მეცნიერები
Vol. 4 Issue 5, 2022
https://doi.org/10.52340/gs.2022.04.05.17

Physicochemical characteristics of fresh and lyophilized Georgian royal jelly and Formulation bentonite-based cream

Lia Tsiklauri[1]*, Malkhaz Getia[1], Aliosha Bakuridze[2]
[1]Tbilisi State Medical University, I.Kutateladze Institute of Pharmacochemistry, 36 P. Sarajishvilist, 0159, Tbilisi, Georgia.
[2]Tbilisi State Medical University, 33, Vaja-Pshavela Ave. 0186, Tbilisi, Georgia
*Corresponding author e-mail:l.tsiklauri@tsmu.edu; 577418414; ORCID 0000-0002-7808-5329

ABSTRACT

Royal Jelly (RJ) is one of the most valued and studied bee products used in medicine and cosmetics due to its diverse pharmacological properties. Chemical composition and biological activity of RJ depends on many factors, including constitution and geographical distribution of plants, the harvest time and bee species. The objective of the present study was physico-chemical characterization and comparison of fresh and lyophilized RJ; and development dermatological formulation by incorporating RJ in Tikha Ascane base.

The evaluations of RJ and RJ loaded formulations were performed by oven-dry, titrimetric, potentiometric, FTIR methods; 10-hydroxy-2-decenoic acid (10-HDA) was determined by high-performance liquid chromatography. In addition, the physical stability of the developed formulations was investigated by examining viscosity, spreadability, thermal and colloidal stabilities, pH, homogeneity and appearance.

Lyophilized RJ displayed up to similar physico-chemical properties (solubility, pH, acidic number) as fresh RJ. The chromatographic analysis showed alike fingerprints and the existence of 10-HAD in tested samples. No spectral differences (in the range of 3600–2800 cm^{-1} and 1750–950 cm^{-1}) were observed in FTIR measurements after lyophilization.

The study shows that prepared formulations were found to be stable. The tested samples had appealing appearance with smooth texture and no signs of separation. Viscosity, homogeneity, spreadability, pH and release profile of RJ from the formulations were acceptable.

KEYWORDS: *Royal Jelly, bentonite clay –Tikha Ascane, Royal Jelly cream.*

Introduction

Bee products are actively used for medicinal purposes and in the manufacture of cosmetics. Special attention has been paid to royal jelly (RJ), which contains variety of chemical compounds exhibiting several pharmacological properties including anti-aging, anti-inflammatory, and antioxidant /1/.

The chemical composition and biological activity of RJ depends on many factors, such as, constitution and geographical distribution of plants, the harvest time and bee species /2, 3/. Comparative examination of the bee species revealed that RJ from Georgian bees had the highest biological activity /4/. Fresh RJ has a limited shelf-life at ambient temperature; to ameliorate the storability, freeze drying is the most common technological method for this purpose however, some studies indicate on the changes in RJ that may occur by reason of lyophilisation /5- 6/.

Clays and particularly bentonites are used in pharmaceutical and cosmetics industry not only as excipients (thickening and emulsifying agent, carriers and releaser of active ingredients, disintegrates, diluent and binder) but also as active components, because of their curative and cosmetic effects /7-8/. Preparation Tikha Ascane, obtained and investigated at the I. Kutateladze Institute of Pharmacochemistry, TSMU from Georgian bentonite clay, is approved by the National healthcare authorities for the medical and pharmaceutical application /9-10/. Various emulsion and suspension type semisolids and dry ointment concentrates were elaborated on the bases of Tikha Ascane /11, 12/. This bentonite compound does not cause allergy and skin sensibilization /10, 12/.

The objective of the present study was physico-chemical characterization and comparison of fresh and lyophilized RJ and development dermatological formulation by incorporating RJ in Tikha Ascane base.

Material and Methods

RJ was obtained directly from beekeepers from eastern region of Georgia. Samples of fresh royal jelly were collected from the hives with colonies of the honeybee species Apis mellifera caucasia (Georgian) from May to August 2020 located in Shida Kartli (Georgia). Lyophilized RJ was obtained after lyophilization process of the fresh one, using a lyophilizator (LyoQuest - Telstar®). Bentonite Clay - Tikha Ascane, was provided by the Direction of Pharmaceutical Technology (I. Kutateladze Institute of Pharmacochemistry, TSMU). All the other chemicals or solvents used were commercially available and of reagent grade.

Chemical Characterization of raw materials

The following parameters were determined for Fresh and lyophilized RJ: water content by lyophilization and oven-dry methods, pH values – by potentiometric (model pH 100), total acidity by titration with 0.1 N NaOH /3, 13/.

10-Hydroxy-trans-2-decenoic acid (10-HAD), also known as royal jelly acid, is the major and unique acid of RJ; it was determined by high-performance liquid chromatography (Agilent technologies 1260) at 215 nm /14/. For the separation an Eclipse Plus C18 column (4.6 mm x250 mm; 5 μm, 100˚A) was used. The mobile phase was a mixture of methanol, pure water, and formic acid (50:50:0.1, v/v/v). The mixture was eluted using an isocratic elution over a period of 25 min at a flow-rate of 1.0 mL/min. The injection volume was 10 μL. The samples were dissolved with 5mL of methanol (HPLC grade) in 25 mL volumetric flasks, diluted to volume with Milli-Q water.

Formulation of cream

The cream was formulated by incorporating RJ into base prepared from Tikha Ascane aqueous gel and glycerin. The formula of the cream with good consistency, appearance and stability was optimized and selected for further study.

Evaluation of cream

The homogeneity of all the formulations (blank and drug loaded) was tested visually for the presence of any aggregates or clumps and for appearance /15/.

The microscopic features of the optimized samples were studied under Light microscopy (ZEISS Jeneval Microscope CF250; 3,2x/0,06 GF planachromat 40x/0,65 GF Planachromat) and photomicrographs were captured on a laboratory PC /15, 16/.

Fourier transform infrared (FT-IR) spectra of the samples were recorded using a Jasco 600 FT-IR spectrometer operating in the region 4000–350 cm^{-1}, equipped with a deuterated triglycine sulphate detector (DTGS) with KBr beam splitter /6/.

Rheological experiments were conducted to measure the viscosity and rheology of the optimized formulations. Measurements were performed at room temperature using a Visco QC 300 Anton Paar rotational viscometer /15, 16/.

The spreadability of the cream was measured using two sets of glass plates (20 X 20 cm) on a scale of graph paper: 0.5 g samples were placed on a glass slide in a pre-marked circle with a diameter of 1 cm; second glass plate was placed on it forming a sandwich arrangement. Afterwards a 500 g weight was positioned on the glass slides for 5 min and the increase in diameter was measured. Using Formula (1), results were expressed in regard to applied mass and spreading area /17, 18/

$$Ei = d^2 \frac{\pi}{4} \qquad (1)$$

Where: Ei = spreadability of the sample (mm^2);

d = diameter (mm).

Spreadability factor (Sf) was calculated using Formula (2):

$$Sf = \frac{A}{W} \qquad (2)$$

Where: A = total area (mm^2); W = total weight (g).

Centrifugation test was carried out by placing samples, in the centrifuge tube and rotated for 10 min, at 3000 rpm in laboratory centrifugation machine /16/. At the end of the centrifugation period the formulations were examined for phase separation which is an indication of samples instability.

The thermal stability studies were conducted at 25° ± 2°C and at 40° ± 2°C.

The release of drug from the gel was determined using a dialysis method with a molecular porous membrane /15, 16/.

All analyses were performed in triplicate. Average values and standard deviations were calculated using Microsoft Excel 2016 (Microsoft Corp.) software.

Results and Discussion

Chemical Characterization of raw materials

Fresh RJ was whitish to yellow, thick and milky substance, partially soluble in water; the lyophilized RJ was a light-coloured, highly hygroscopic powder with an acid-specific odour. The yield of lyophilized RJ from fresh one was 39.19% (Figure 1).

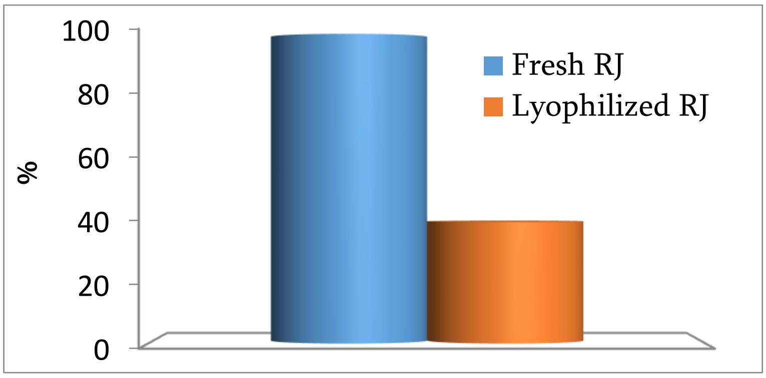

Figure 1. The yield of lyophilized RJ from fresh RJ

Moisture content is an important quality criterion of RJ and its determination is essential for each sample. Water amount in fresh RJ varies from 57 to 70% /14 /. Freeze drying extracts the water from RJ and enhance its storage stability. We evaluated the water content in samples by vacuum oven and sublimation drying methods (Table 1).

Table 1. Moisture content of fresh and lyophilized RJ

Sample		Moisture content, %
Fresh RJ	vacuum oven method	67.44
	sublimation drying method	61.09
Lyophilized RJ		3.97

Similar results on moisture content (67 and 61 %) of fresh RJ were obtained from different methods in our experiments. In freeze dried samples water content was reduced to 3.97% (Table 1). These data are in correlation with Boselli et al /19/.

Formulation and Preparation of cream

On the basis of preliminary studies combination of Tikha ascane aqueous gel and glycerin was selected as the suitable cream base for the incorporation of RJ. This base is well investigated and used for the formulation of semisolid dosage forms with various pharmacologically active substances in the Direction of Pharmaceutical Technology (I. Kutateladze Institute of Pharmacochemistry, TSMU) /9, 11/. Amount of RJ was selected on the basis of published data /6, 15/. The final concentration of RJ was 3%, Tikha Ascane - 15% and glycerin - 10% (w/w). Similarly, were prepared the cream base samples without any active components.

Evaluation of cream

Results showed that the creams were light yellowish gray, homogenous mass with no signs of phase separation, non- greasy and had a smooth feeling on application. From the results, it was concluded that the formulated cream showed good appearance and homogeneity.

Total acidity and pH of RJ depend on the storage conditions. According to the obtained data presented in Table 2, the values of total acidity for RJ samples correspond to the normal range given in the published literature: 3–6 ml 0.1 n NaOH/g. The pH value was found to be 7.53 for base and was decreased to 5.92 when the active ingredient was incorporated. The pH of the tested samples was similar to the skin's normal value (4.5 – 6), therefore, low risk of irritation, drying and alterations in skin hydration with the formulation is expected /20/.

Table 2. pH and total acidity of tested samples

Samples	RJ	Blank formulation	RJ loaded formulations
pH (mean ± SD)	3.57± 0.02	7.53 ± 0.01	5.92 ± 0.01
total acidity	3.062 ±0.85		0.51±0.05

The presence and distribution homogeneity of RJ in the formulations were investigated by microscopy. The microphotographs of the samples provided in Fig.2 indicate the homogenous and uniform distribution of RJ in cream formulation.

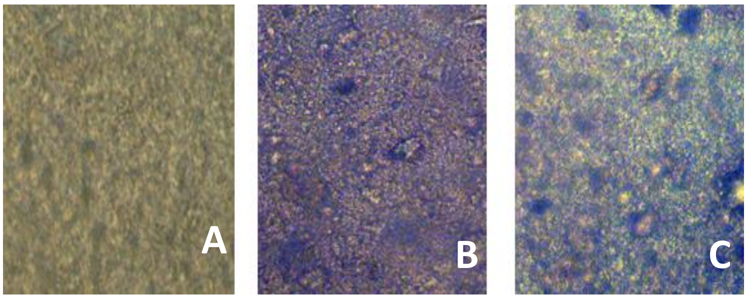

Figure 2. Light microphotographs of tested samples. A – RJ; B base; C – RJ loaded cream (3% w/w).

Infrared spectroscopy (IR) is a method for qualitative and quantitative analysis of compounds in complex matrices. In the FT-IR spectrum of composites two sharp peaks were observed in the range of 3600–2800 cm^{-1} and 1750–950 cm^{-1} (Fig. 3). Absorption spectra in 3600– 2800 cm^{-1} region indicate the presence of N – H and –CH2– groups of primary and secondary amines. The band which varied between 1750 and 950 cm^{-1} matches to N-H bending of Amide I due to protein content /6 /. Furthermore, in the IR spectra of RJ cream, the absorption areas characteristics for bentonite clay were also revealed. Thus, it can be concluded, that the incorporation of RJ in the bentonite does not cause qualitative changes of the active substance and RJ is not chemically bound with base, hence the release profile of the active substance will not be affected by Tikha Ascane.

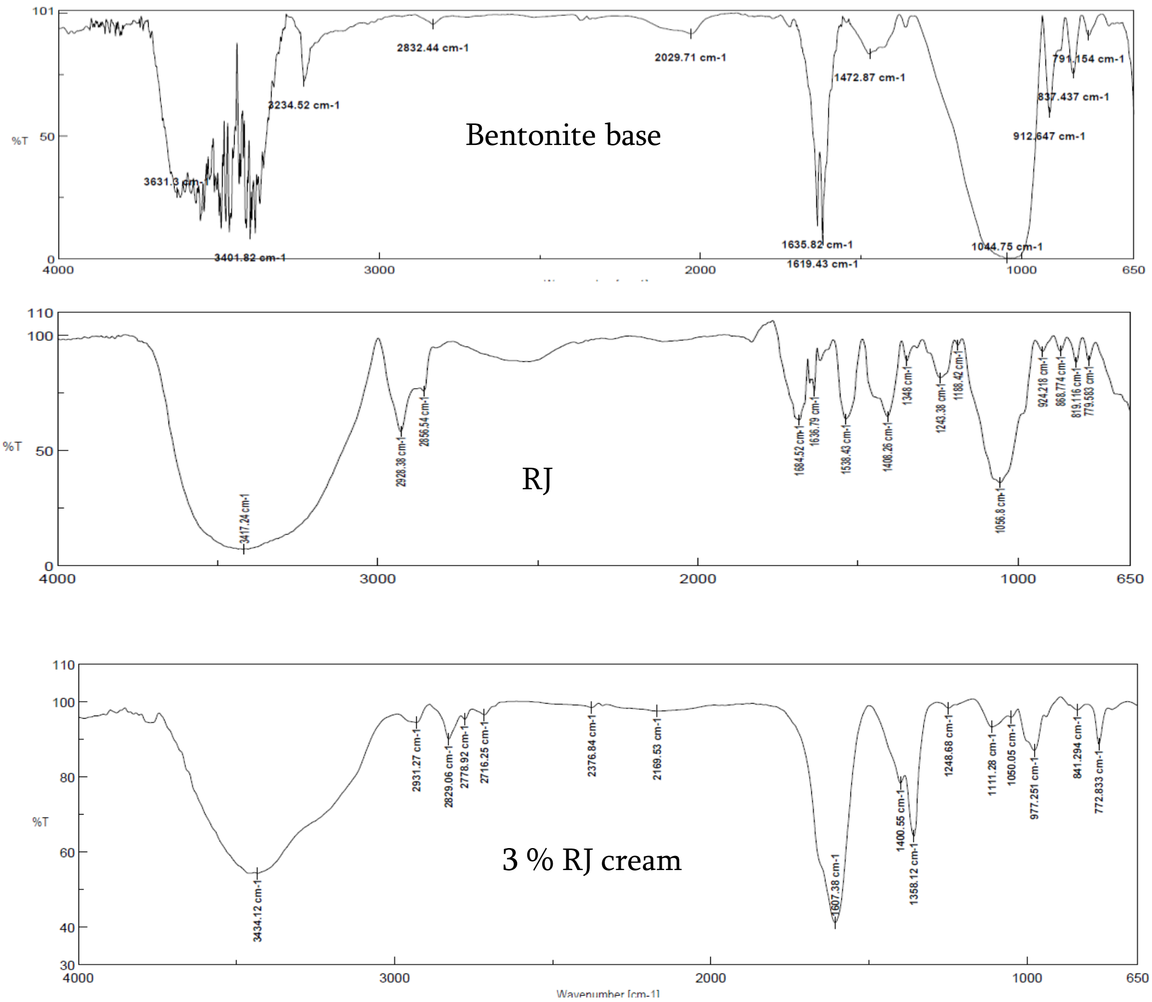

Figure 3. IR spectra of the tested samples.

The viscosity of the formulations was determined at varying shear stresses using a Brookfield viscometer and was reported in a unit of centipoise (cPs). Fig. 4 shows the apparent viscosity vs. RPM and share rate for the samples. A non-Newtonian behaviour with pseudoplastic flow was observed in the tested formulations as expected. It is undoubtedly to note from Fig. 4.A. that as the speed raise, the viscosity reduced in the samples. Also, formulation with RJ exhibited a lower viscosity than cream base. The incorporation of RJ affects on the structural viscosity of the system and decreases this value for drug-loaded formulation. The rheograms in Fig. 4B depict, that formulations show the thixotropic behaviours, since the down curve shifted to the above of the upcurve when viscosity was plotted against share rate. Thus, tested samples are shear thinning - rheopectic systems.

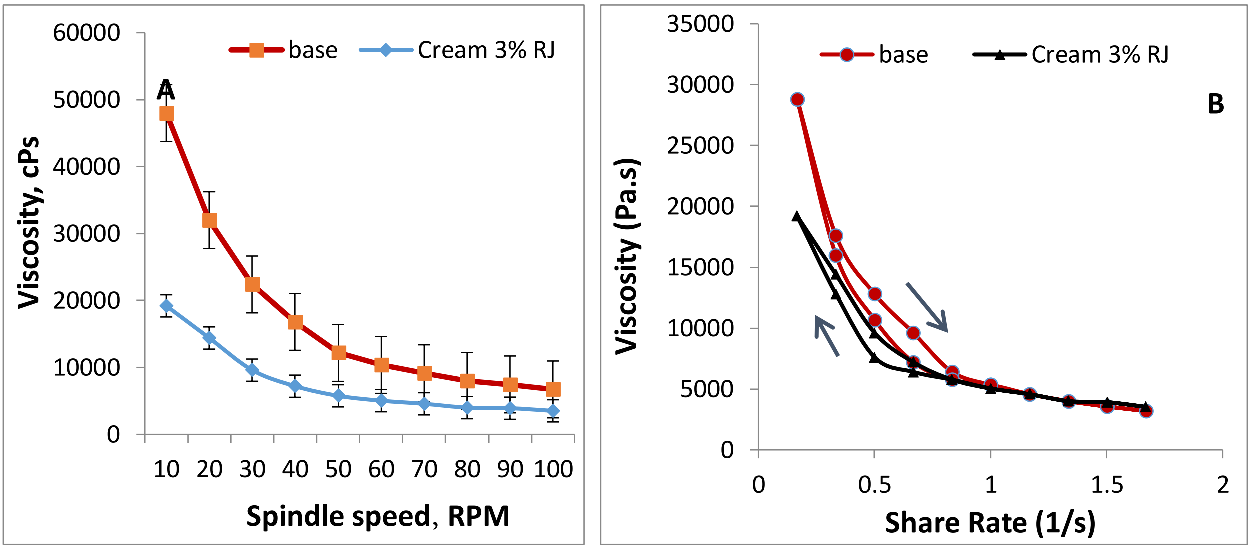

Figure 4. A - Viscosity data versus RPM and B - Viscosity data versus share rate of formulations with and without RJ.

Spreadability is a major characteristic of semisolid preparations for consistent drug delivery and patient compliance. It defines the appropriate distribution and uniform application of the topical formulation on the target area /18, 21/. According to the obtained results (Fig. 5), formulation with RJ is characterized by good spreadability: the average diameter of the spread is 5.67 ± 0.55 cm, and the spreadability factor is 5.41 ± 0.04 mm^2/g. It should be noted as well that incorporation RJ into Tikha Ascane base increases the spreadability factor by 1.5 times (Fig. 5).

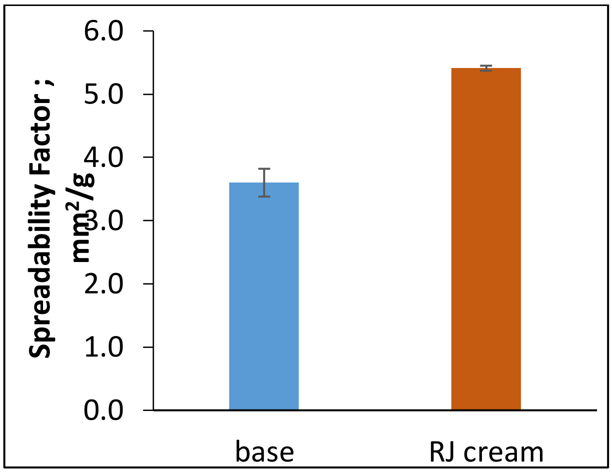

Figure 5. A - Spreadability data of formulations with and without RJ

10H2DA represents the principal lipid component in royal jelly and is considered as a biomarker of this product. In these studies, the chromatographic profile of the tested samples displayed identical fingerprints and the existence of 10H2DA in all samples (fresh RJ, lyophilized RJ and RJ cream), indicating that the lyophilization process and its incorporation into the bentonite base did not alter the fatty acid (Figure 6).

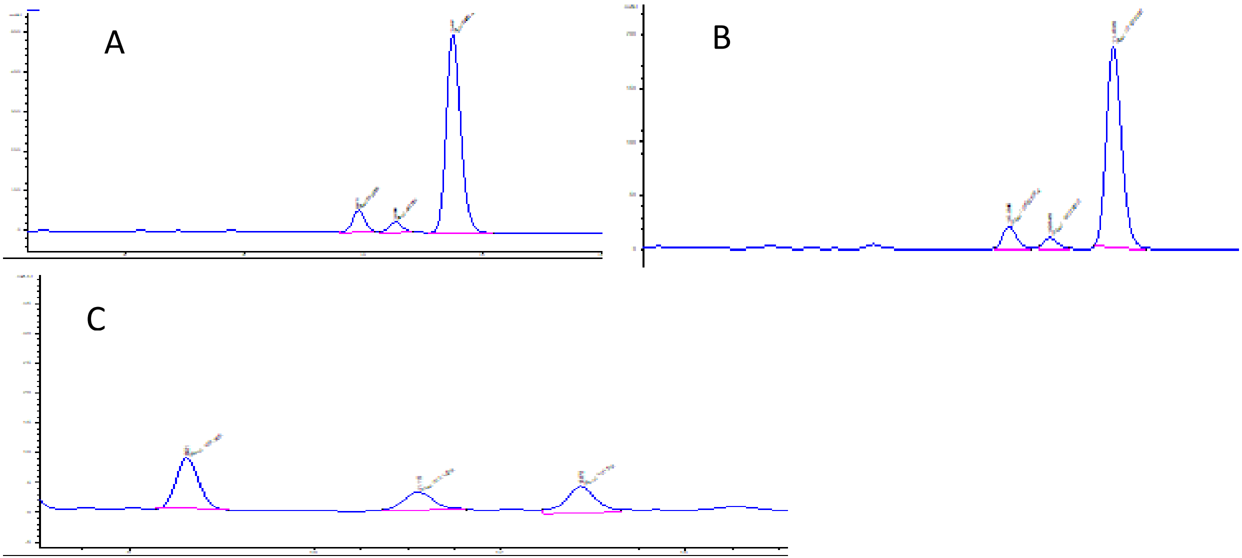

Figure 6. Chromatographic profile of 10H2DA in the tested samples: A – fresh; B - lyophilized RJ; C- RJ cream.

We determined the RJ content in the cream, which was in the range of 2.93-3.15% (Fig. 6).

The stability of the samples was evaluated according to the methods reported in section Materials and Methods. All samples showed good mechanical properties and passed the centrifugation test. No observable changes in colour and stability (physical appearance, phase separation, texture, homogeneity) were detected for creams.

The release of the active substance from tested samples was investigated by in vitro method. The cream (1 g, exact weight) placed in the dialysis membrane was incubated in purified water at 37 ° C with stirring at 20 ± 2 rpm. Samples were taken after 0, 0.5, 1.5 and 3 hours and the area was filled with the same volume of water. The active substance was determined by a HPLC method. According to the results obtained, 82.2% of the active substance is released within 3 h.

Conclusion

Main physico-chemical properties were evaluated for Georgian RJ and concluded that this characteristics are not changed after lyophilization process; Tikha Ascane-glycerine base was deemed optimal for incorporation RJ; the formulated cream fulfilled quality requirements in terms of texture, appearance, stability, pH values, technological properties and drug release profile.

References

1. Ahmad S, Campos MG, Fratini F, Altaye SZ, Li J. New Insights into the Biological and Pharmaceutical Properties of Royal Jelly. Int J Mol Sci. 2020 ; 8;21(2):382.
2. Khazaei M, Ansarian A, Ghanbari E. New Findings on Biological Actions and Clinical Applications of Royal Jelly: A Review. J Diet Suppl. 2018; 15(5):757-775.
3. Kausar SH, More VR. Royal Jelly: Organoleptic characteristics and physicochemical properties. Lipids. 2019; 6(2):20-4.
4. Kirkitadze G, Japoshvili G. Renewed checklist of bees (Hymenoptera, Andrenidae) from Georgia. Annals of agrarian science. 2015; 13: 20-32.
5. Maghsoudlou A, Mahoonak AS, Mohebodini H, Toldra F. Royal Jelly: Chemistry, Storage and Bioactivities, Journal of Apicultural Science. 2019; 63 (1):17-40.
6. Lazarevska S, Makreski P. Insights into the infrared and Raman spectra of fresh and lyophilized royal jelly and protein degradation IR spectroscopy study during heating, Macedonian Journal of Chemistry and Chemical Engineering. 2015; 34(1): 87–93.
7. Babahoum, N., Ould Hamou, M. Characterization and purification of Algerian natural bentonite for pharmaceutical and cosmetic applications. BMC Chemistry 15, 50 (2021). https://doi.org/10.1186/s13065-021-00776-9
8. Cervini-Silva J, Antonio-Nieto-Camacho, Kaufhold S, Ufer K, de Jesús ER. (The anti-inflammatory activity of bentonites. Applied Clay Science. 2015; 118:56–60. https://doi.org/10.1016/j.clay.2015.08.039
9. Tsagareishvili GV. Some results of research and application of bentonites of Georgia in pharmacy and medicine" - Tbilisi. "Metsniereba". 1974. 130 (in Russian).
10. Aladishvili V.A. The use of bentonite of the Askane deposit in medicine. Tbilisi. 1969. 24-27 (in Russian) .
11. Tsiklauri L, Dadeshidze I, Tsagareishvili G. Study of the Stability and Specific Activity of the Emulsion Containing Sea-Buckthorn (Hippophae rhamnoides L.) Oil and Ticha - Ascanae. Bulletin of the Georgian Academy of Sciences. 1998; 157(2):251-253.

12. Tsiklauri L, Tsagareishvili G. Gastroenterological and dermatological soft drug formulations containing Georgian bentonite clay preparation – Thicha-ascanae. Tbilisi. “Universal”. 2011, 89, (ISBN 978-9941-17-458-2) (in Georgian).
13. Kanelis D, Tananaki C, Liolios V, et al. A suggestion for royal jelly specifications. Arh Hig Rada Toksikol. 2015; 66(4):275-284. doi:10.1515/aiht-2015-66-2651
14. Balkanska R. Determination of Trans-10-Hydroxy-2-Decenoic Acid in Royal Jelly by High Performance Liquid Chromatography after Different Bee Feeding. Int. J. Curr. Microbiol. App.Sci. 2018; 7(4): 3738-3743
15. Bocho-Janiszewska A, Sikora A, Rajewski J, Łobodzin P. Application of royall jelly in moisturizing creams. Pol. J. Cosmetol. 2013; 16: 314–320
16. Chen M X, Alexander K., Baki G. Formulation and Evaluation of Antibacterial Creams and Gels Containing Metal Ions for Topical Application. Journal of Pharmaceutics, 2016; 1–10. doi:10.1155/2016/5754349
17. Knorst M.T., Desenvolvimento tecnológico de forma farmacêutica plástica contendo extrato concentrado de Achyrocline satureioides. (Lam). DC. Compositae. (Marcela), M. Sc. thesis, Universidade Federal do Rio Grande do Sul, Porto Alegre, 1991, p. 257
18. Khan MFA, Ur.Rehman A, Howari H, Alhodaib A, Ullah F, Mustafa Zu, Elaissari A, Ahmed N. Hydrogel Containing Solid Lipid Nanoparticles Loaded with Argan Oil and Simvastatin: Preparation, In Vitro and Ex Vivo Assessment. Gels. 2022; 8(5):277. https://doi.org/10.3390/gels8050277
19. Boselli E, Caboni MF, Sabatini AG, Marcazzan GL, Lercker G. Determination and changes of free amino acids in royal jelly during storage, Apidologie. 2003; 34: 129 – 137
20. Ali, S.M. and G. Yosipovitch, Skin pH: from basic science to basic skin care. Acta dermato-venereologica, 2013; 93(3): 261-269
21. Pattanayek S, Puranik S. Formulation and Evaluation of Ketoprofen Loaded Nanoparticulate Gel for Topical Delivery. IJPPR. 2018; 11(3):1–11.

ქართული ფუტკრის ნედლი და ლიოფილიზებული რძის და ბენტონიტურ ფუძეზე მომზადებული მისი შემცველი კრემის ფიზიკურ-ქიმიური მახასიათებლები

ლია წიკლაური[1]*, მალხაზ გეთია[1], ალიოშა ბაკურიძე[2]

[1]თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ი.ქუთათელაძის ფარმაკოქიმიის ინსტიტუტი, პ.სარაჯიშვილის 36, 0159, თბილისი, საქართველო. [2] თბილისის სახელმწიფო სამედიცინო უნივერსიტეტი, ვაჟა-ფშაველას გამზ. 33, 0186, თბილისი, საქართველო

ფუტკრის რძე (ფრ) ერთ-ერთი ყველაზე ღირებული და კარგად შესწავლილი პროდუქტია, რომელიც ფართოდ გამოიყენება მედიცინასა და კოსმეტოლოგიაში მისი მრავალფეროვანი ფარმაკოლოგიური თვისებების გამო. ფრ-ის ქიმიური შემადგენლობა და ბიოლოგიური

აქტივობა დამოკიდებულია მრავალ ფაქტორზე, მათ შორის მცენარეების შემადგენლობასა და გეოგრაფიულ გავრცელებაზე, შეგროვების დროსა და ფუტკრის სახეობებზე. წარმოდგენილი კვლევის მიზანი იყო ნედლი და ლიოფილიზებული ფრ-ის ფიზიკურ-ქიმიური თვისებების შესწავლა და შედარება; აგრეთვე მისი შემცველი დერმატოლოგიური კრემის ოპტიმალური რეცეპტურისა და მომზადების ტექნოლოგიის შემუშავება თიხა ასკანის ფუძეზე.

ფუტკრის რძის და მისი შემცველი ნიმუშების თვისობრივი და რაოდენობრივი შეფასება ჩატარდა ტიტრიმეტრიული, პოტენციომეტრული, მიკროსკოპული, ინფრაწითელი სპექტროსკოპული და მაღალეფექტური სითხური ქრომატოგრაფიული მეთოდებით; შესწავლილ იქნა შემუშავებული კრემის ტექნოლოგიური მახასიათებლები: სიბლანტე, რეოლოგია, წაცხებადობის უნარი, ერთგვაროვნება, თერმული და კოლოიდური სტაბილურობა.

დადგინდა, რომ ნედლი და ლიოფილიზირებული ფუტკრის რძის ფიზიკურ-ქიმიური თვისებები (ხსნადობა, pH, მჟავური რიცხვი) და ინფრაწითელი სპექტრები თითქმის იდენტურია 600-2800 სმ$^{-1}$ და 1750-950 სმ$^{-1}$ დიაპაზონში; ქრომატოგრაფიულმა ანალიზმა აჩვენა საკვლევი ობიექტების მსგავსი პროფილი და 10 ოსქიდეცენის მჟავის (10H2DA) არსებობა ნიმუშებში.

მიღებული შედეგების მიხედვით შემუშავებული ნიმუშები გამოირჩევიან ჰომოგენურობით და მაღალი სტაბილურობით; სიბლანტე, pH და ფრ-ის გამოთავისუფლების პროფილი შესაბამისობაშია რბილი წამლის ფორმებისადმი წაყენებულ მოთხოვნებთან.

საკვანძო სიტყვები: *ფუტკრის რძე, ბენტონიტური თიხა –თიხა-ასკანე, ფუტკრის რძის კრემი.*