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Quality and stability of topical formulation based on the extract of black locust flower (*Robiniae pseudoacaciae flos*)

Original Article

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Abstract:

Black locust flowers are the source of a significant amount of antioxidants that can be useful against free radicals formed on the skin after the environmental factors. Having in mind these facts, the ultrasound-assisted extraction of antioxidants from plant materials was previously developed. The aim of this study was to prepare a topical formulation based on the extract of black locust flowers. The extract enriched with antioxidants was prepared using the ultrasound-assisted extraction using 60% (v/v) ethanol at 60 °C and a liquid-to-solid ratio of 10 mL/g for 30 min. The two topical formulations in the form of o/w emulsion at different concentrations of the extract (1% and 2%) were prepared by a hot-hot emulsification process. The product quality was estimated and concluded that the emulsion is white in the semi-solid state. The formulations can have adequate application properties due to their present ingredients, which do not leave a greasy film on the skin. The pH value of the prepared formulations was according to the allowed range for the application to the skin. The proper choice of ingredients enabled the achievement of satisfactory formulation stability. The formulations can be microbiologically safe to use for human purposes. A better antimicrobial activity against the investigated microorganism strains showed the formulation with higher content of the extract so it can be selected as an adequate for further analysis.

Key words:
black locust flowers, extract, topical formulation, quality control, stability

Apstrakt:

Kvalitet i stabilnost topikalne formulacije na bazi ekstrakata cvetova bagrema (*Robiniae pseudoacaciae flos*)

Cvetovi bagrema su izvor značajnih količina antioksidanasa koji mogu biti korisni za hvatanje slobodnih radikala nastalih na koži nakon dejstva spoljašnjih faktora. Imajući u vidu ove činjenice, ultrazvučna ekstrakcija antioksidanasa iz biljnog materijala je prethodno razvijena. Cilj ove studije je bio da se pripremi topikalna formulacija na bazi ekstrakta cvetova bagrema. Ekstrakt obogaćen antioksidansima je pripremljen primenom ultrazvučne ekstrakcije korišćenjem 60% (v/v) etanola na 60 °C i pri odnosu tečnosti i čvrste materije 10 mL/g u toku 30 min. Dve topikalne formulacije u obliku U/V emulzije pri različitim koncentracijama ekstrakta (1% i 2%) pripremljene su toplo-toplo emulzifikacionim procesom. Kvalitet proizvoda je procenjen i zaključeno je da su emulzije bele polučvrste konzistencije. Formulacije mogu da imaju adekvatna aplikativna svojstva zbog prisutnih sastojaka, s obzirom da ne ostavljaju mastan film na koži. pH vrednost pripremljenih formulacija bio je u skladu sa dozvoljenim opsegom za primenu na koži. Pravilan izbor sastojaka omogućile su postizanje zadovoljavajuće stabilnosti formulacije. Formulacije mogu da budu mikrobiološki bezbedne za ljudsku upotrebu. Bolju antimirkobnu aktivnost na analiziranim sojevima mikroorganizmima ispoljila je formulacija sa većim sadržajem ekstrakta, tako da za dalju analizu može biti pogodna.

Ključne reči:
cvetovi bagrema, ekstrakt, topikalna formulacija, kontrola kvaliteta, stabilnost

Introduction

The use of medicinal plants and their extracts in pharmaceutical and cosmetic industries increases due to the desire for a healthier lifestyle and youthful appearance. Medicinal plants contain bioactive compounds, which have positive effects on human health (Veiga et al., 2020). Among the bioactive

compounds, polyphenols retaken a significant place. This group of compounds, also known as secondary metabolites of plants, shows antioxidant (de Lima Cherubim et al., 2020), antimicrobial, anticancer (Dzah et al., 2020), anti-inflammatory, anticollagenase, and antielastase activities (Madan & Nanda, 2018). According to these properties and the ability to pass through the stratum corneum and penetrate the epidermis and dermis, they are suitable for topical application (Zillich et al., 2015). They are topically applied for care (Guimarães et al., 2021) and photoprotection of skin (Farjadmand et al., 2021), as well as for the prevention of skin cancer (Souto et al., 2019). To demonstrate their certain biological activities, polyphenolic compounds have to release from the formulation. The formulations should be chemically, physically, and microbiologically stable. To ensure good migration of polyphenolic compounds into the skin, their precipitation in the vehicle of the formulation should not occur. Polyphenolic compounds precipitate rapidly because of their poor solubility in water (Figueroa-Robles et al., 2020). The solubilization of polyphenolic compounds can be improved and their precipitation can be prevented by the addition of surfactants, such as Labrasol and Tween (Jee et al., 2019) or block copolymers (Shin et al., 2018) in the formulation. Emulsions are the most suitable type of vehicle for topical formulations since they have adequate solubilization capacity for lipophilic and hydrophilic ingredients (Yang et al., 2020). The lower content of the oily phase in the emulsions makes it easier to release polyphenolic compounds and a higher rate of their penetration through the skin (Zillich et al., 2013). This type of emulsion has a lower viscosity, which allows better diffusion of polyphenols within the emulsions. Skin hyperhydration can be achieved using formulations with a high content of water (o/w emulsion) that cause the higher permeability of polyphenols through the skin. The addition of aqueous and oily plant extracts does not disturb the structure of the emulsion itself (Smaoui et al., 2012), but it may affect its rheological properties. Due to the dilution effect and the interaction of polyphenols with protein or polysaccharide emulsifiers (Li et al., 2019), the viscosity is increased by the addition of the extract (Leite et al., 2019). The interactions of polyphenols with nonionic or anionic surfactants, commonly used in emulsions, are rarely described. On the other hand, polyphenols have also surfactant properties (Katsouli et al., 2017). The oxidative and storage stability of emulsions can be improved in the presence of polyphenols (Choulitoudi et al., 2021).

There are dermo-cosmetics formulations with polyphenolic compounds (quercetin, resveratrol, gallic acid, rutin, curcumin, apigenin, etc.) and extracts enriched with these compounds (grape extract, liquorice root extract, Aloe vera extract, calendula extract, lilac extract, lavender extract, etc.) in the market. Due to the growing market demand for dermo-cosmetic products recognized as safe, curative, and with synergistic or multifunctional effects, there is a continual need to find new sources of polyphenolic compounds. According to our previous studies, the ethanol extract of black locust flowers is an excellent source of polyphenolic compounds with expressed antioxidant activity (Savic Gajic et al., 2019). The aim of this study was to prepare the topical formulation with the extract of black locust flowers enriched with antioxidants. Based on the properties of the topical formulation and its physico-chemical stability, the product quality was estimated.

Materials and Methods

In this study, Sabowax SX and Sabonal C1618 (cetearyl alcohol) (Sabo S.p.A., Italy), white beeswax (*Ceraalba*, extra pure, Centrohem, Srbija), triethanolamine (Care BASF, Germany), phenoxyethanol SA (Schülke & Mayr GmbH, Germany), mineral oil (Techno-Chem D.O.O., Serbia), glycerol (85%), carbomer, trichloroacetic acid (Sigma-Aldrich, Serbia), and distilled water (*Aqua purificata*) was used. Ethanol extract of black locust flowers was obtained by ultrasound-assisted extraction (Savic Gajic et al., 2019).

Preparation of topical formulation

The o/w topical formulations with and without (base) black locust flower extract were prepared according to **Tab. 1**.

The oily and water phases were prepared separately by heating in enameled paten via water bath at a temperature of 70±1 °C. The topical formulation was prepared by the hot-hot emulsification process. This process involves the addition of the oily phase into the water phase gradually with stirring using a laboratory propeller mixer at 800 rpm and constant temperature. After the emulsification process, a stirring of the phases was continued at 500 rpm until the temperature was below 40 °C. Phenoxyethanol SA was added as a preservative after cooling the sample. Triethanolamine was added to adjust the pH of the formulation. The prepared formulations were stored at room temperature (22±2 °C) in a plastic polypropylene container. The base was prepared in the same way but without the addition of the black locust flower extract.

Formulation quality assay

Organoleptic properties (appearance, color, homogeneity, and separation of phases) of the base

Table 1. Composition of topical formulations expressed in the percentage (**F1**-topical formulation with 1% of the extract; **F2**–topical formulation with 2% of the extract)

Ingredients	Base	Topical formulation	
		F1	F2
		Oily phase	
White beeswax	1	1	1
Mineral oil	6	6	6
Sabowax SX	5	5	5
Sabonal C1618 (cetearyl alcohol)	6.5	6.5	6.5
Trichloroacetic acid	7	7	7
Triethanolamine	0.7	0.7	0.7
Phenoxyethanol SA	0.2	0.2	0.2
		Water phase	
Water	68.1	67.1	66.1
Carbomer	0.5	0.5	0.5
Glycerin	5	5	5
Black locust flower extract	-	1	2

and topical formulations with black locust flower extract (F1 and F2) were assessed by visual observation (Ph. Jug. IV, 1984). Determination of emulsion type was achieved by electrical conductivity measuring (Stojiljković et al., 2013). The measurement of electrical conductivity was performed by direct immersion of the conductometer electrode (CDM 230, Radiometer, Copenhagen, Denmark) into the creams samples at room temperature (22±2 °C). Before starting the work, the measuring electrode was calibrated with 0.01 mol/LKCl solution. To measure the pH value of the samples, a potentiometric method was used (Stojiljković et al., 2013). A glass electrode of pH meter (HI 9321, Hanna instruments, Lisbon, Portugal) was directly immersed in the samples at room temperature (22±2 °C). The device was calibrated with standard pH buffers of 4.0 and 7.0. The viscosity of prepared formulations was determined using a viscosimeter (Visco basic plus, Fungilab Inc., USA) with an SP-R5 spindle, at a 12 rpm rotation speed (Maia Filho et al., 2012).

Physical stability of the formulations

Centrifugation assay was applied to assess the physical stability of the prepared samples on the gravity effect according to the previously described method with slight modification (Juttulapa et al., 2017). Precisely 4 g of the samples were weighed into a 10 mL plastic test cuvette and centrifuged twice at 3000 rpm for 15 min. A laboratory centrifuge (LC 320, Tehtnica, Železniki, Slovenia) was used. The measurements were performed at room temperature (22±2 °C) 24 h after preparation. The samples were observed visually to notice eventual changes (phase separation). Cyclic temperature stress test or accelerated aging test of formulations was realized through 6 cycles. Each cycle included the following steps: 1) the samples were placed in the freezer at -10 °C for 24 h; 2) the samples were removed from the freezer and left at room temperature (22±2 °C) for 24 h; 3) the samples were placed in thermostat oven (Sutjeska, Yugoslavia) at the temperature of 40 °C for 24 h; 4) the samples were removed from the thermostat oven and left at room temperature (22±2 °C) for 24 h. At the same time, one sample was left under the isothermal condition at room temperature (22±2 °C), in a fridge at +4 °C, and in a thermostat oven at +40 °C. After the third and sixth cycles, the pH value and electrical conductivity were measured in each sample. If the product is stable, the appearance, color, and consistency should not change. A long-term aging test or Shelf test was applied to estimate the physical stability of the prepared formulations based on the changes in organoleptic properties, pH values, and electrical conductivity. The samples were stored in primary packaging (plastic containers) at room temperature for 3 months, and the tests were performed 7, 30, 60, and 90 days after their preparation.

The chemical stability of the extract in pure form and after its incorporation into the formulations (F1 and F2) was assessed based on the change in total antioxidant content (TAC) over time. The TAC in the black locust flower extract and formulations was determined by the Folin-Ciocalteu method (Savic Gajic et al., 2019). The analyzed samples

were exposed to the influence of lower and higher temperatures (-18 °C, +4 °C, and +22 °C), as well as daylight/darkness for 3 months. Sampling was performed after 7, 30, 60, and 90 days, whereby the TAC expressed as milligrams of gallic acid equivalent per 100 g of dry weight (mg GAE/100 g) was determined for each sample. The absorbance of the samples was measured at 740 nm after incubation at room temperature (+22 °C) for 30 min. Varian Cary-100 UV-Vis spectrophotometer (Malgrave, Victoria, Australia) and 1×1 cm quartz cuvettes were applied for scanning the samples.

Microbiological safety of formulations

The method is based on the isolation and identification of bacteria, yeasts, and molds present in the sample (https://www.paragraf.rs/propisi/pravilnik-uslovima-pogledu-zdravstvene-ispravnosti-predmeta-opste-upotrebe.html).The samples were seeded on a nutrient medium and incubated under optimal conditions.

Antimicrobial activity

Antimicrobial activity of black locust flower extract, the base, and formulations was examined against Gram-positive (*Staphylococcus aureus* ATCC 6538, *Streptococcus pneumoniae* ATCC 49619) and Gram-negative bacteria (*Escherichia coli* ATCC 8739, *Proteus mirabilis* ATCC 25933, *Klebsiella pneumoniae* ATCC 10031), as well as on fungi (*Candida albicans* ATCC 10231) using disk diffusion method. The samples were dissolved in dimethyl sulfoxide (DMSO) which was used as a negative control. The antibiotic medium-1 nutrient medium was used for the growth of bacteria, while Sabouraud 4% dextrose agar nutrient medium was used for fungal growth. An adequate amount of nutrient medium was dissolved in purified water and then sterilized at 121 °C in an autoclave for 15 min. The sterilized medium was cooled to 40–45 °C and an appropriate concentration of microorganisms' suspension was added to it. The nutrient medium (15 mL) was poured into Petri dishes with a diameter of 90 mm. The paper discs with a diameter of 6 mm, soaked with 30 μL of extract (1 mg/mL) and formulations (0.1 g/mL) were arranged on a cooled and solid surface. Gentamicin was used as a positive control. Bacteria were incubated at 37 °C for 18–24 h and fungi at 25 °C for 24–48 h under anaerobic conditions. After the incubation, the inhibition zones were measured in millimeters. Based on the inhibition zones, the antimicrobial activity of the samples was estimated.

Statistical analysis

All obtained data were presented as mean value±standard deviation. All statistical analyzes were performed using the OriginPro9 software (OriginLab Corporation, Northampton, USA), with a significance level of $p<0.05$.

Results and discussion

The o/w emulsion formulations were prepared by a technological procedure in the laboratory conditions. The oily phase consisted of white beeswax, mineral oil, Sabowsax SX, cetearyl alcohol (Sabonal C1618), trichloroacetic acid (TCA), triethanolamine, and phenoxyethanol SA. White beeswax is a mixture of esters of higher fatty acid and higher fatty alcohols, free fatty acids and alcohols, carotenoids, aromatic and mineral substances. Due to its regenerative, emollient, and soothing properties, white beeswax is suitable for dry skin and winter skincare routines. Mineral oil is the odorless, colorless, and tasteless oily liquid of mineral origin. This oil is a mixture of saturated hydrocarbons, which due to their size cannot penetrate the skin and therefore do not close the skin pores. It is not carcinogenic and does not cause allergic reactions on the skin. It can form a protective layer on the skin that prevents transdermal water loss, making skin soft and smooth. White beeswax and mineral oil, in addition to achieving an occlusive effect, are also important for regulating the consistency of the developed formulation (Baumann, 2011). Sabowax SX is an anionic self-emulsifying o/w base and presents a mixture of cetearyl alcohol, fatty alcohols, and sodium alkyl sulfate. In topical formulations, it is used in concentrations of 4–7% and can improve the stability and consistency of the preparation. Cetearyl alcohol (Sabonal C1618) is a fatty alcohol used as an emulsifier in the formulations, improving the viscosity of the product. It has a soothing effect on the skin, making it smooth and soft. In the formulations, TCA can be used in different concentrations depending on the purpose. It has a significant role in smoothing fine surface wrinkles, removing surface stains, and correcting pigment problems. In the developed formulations, triethanolamine was used as a pH adjusting agent. Depending on the desired pH value, it can be used in the concentration of 0.1–1%. Due to good antimicrobial properties and low toxicity, phenoxyethanol SA was used as a preservative for the developed formulation. If the formulation contains only one preservative, the maximum permissible concentration is 0.4%, while for a mixture of preservatives, the allowable value is 0.8%.

Unlike the oily phase, the water phase consisted of demineralized water, carbomer, and glycerin. Prepared ethanol extract of black locust flower in the concentration of 1% and 2% was added to the water

phase. The extract was obtained by ultrasound-assisted extraction and characterized in our previous study (Savic Gajic et al., 2019). The TAC in the extract was 3.12 GAE/100 g d.w. Among the identified individual polyphenolic compounds, rutin was the most abundant compound (56.9 mg/100 g d.w.). The IC_{50} value of the extract was 120.5 µg/mL, which makes it suitable for the development of dermo-cosmetic formulations. The water used in the formulations was toxins and microbes free. Carbomer found wide application in the topical products as a safe substance with extremely low irritant and sensitizing potential. In the developed formulations, a carbomer was used to stabilize the emulsion. It should be in the concentration range of 0.1-0.5% to use as an emulsion stabilizer. Glycerol as humectant was added to the aqueous phase of the emulsion. It is known that glycerol forms an invisible film on the skin which binds water molecules from the air and thus hydrates the skin.

Formulation quality assay

The prepared formulations were a semi-solid consistency, homogeneous, white, and with a characteristic smell. The adequate selection of oily phase ingredients enabled the samples to be stable 24 h after preparation. Determination of electrical conductivity is one of the most reliable methods for the determination of emulsion type. According to the literature data, the electrical conductivity higher than 50 µS/cm indicates o/w emulsion, while values less than 1 µS/cm indicates w/o emulsion (Jiang et al., 2013). The electrical conductivity for the base, F1, and F2 of 78, 86, and 91 µS/cm, respectively, indicate that the prepared formulations are o/w emulsion. The physiological pH value of skin ranges from 4 to 7 (Lambers et al., 2006), while the average value is 5.5. Topical formulations should have a pH value close to this range. The pH values of the base, F1, and F2 were 6.41, 6.38, and 6.35, respectively. The addition of the extract to the base led to a slight decrease in the pH value (Akhtar et al., 2011). The viscosity of the formulation is important for handling and storage. Based on this parameter, the stability of the product is possible to evaluate. The measured values for the viscosity of base, F1, and F2 of 13.522, 13.261, and 12.789 mPa·s, respectively, indicated that the formulations were semi-solid consistent. The addition of extract to the base led to a decrease in viscosity.

Physical stability of formulations

The prepared formulations were physically stable because there was no phase separation after the action of mechanical stress (centrifugation assay). Shelf-life assessment is a bottleneck during designing a new product. Therefore, it is necessary to lead an „accelerated aging" test. Applying this test in a short time interval can provide information about the physical stability of the product. In that case, it is necessary to observe parameters (pH value, electrical conductivity, stratification rate, flocculation, etc.) with rapid changes in values under the influence of some external factors. Temperature is one of

Table 2. pH and electrical conductivity values of the formulations after the cyclic temperature stress test

Sample	Storage conditions	Parameters	
		pH	Electrical conductivity (mS/cm)
F1	Cycle III	6.39	85
	Cycle VI	6.34	86
F2	Cycle III	6.36	90
	Cycle VI	6.33	90
Base	Cycle III	6.41	77
	Cycle VI	6.39	78
Comparative F1	+4 °C	6.38	86
	room temperature	6.38	87
	+40 °C	6.22	88
Comparative F2	+4 °C	6.35	91
	room temperature	6.36	92
	+40 °C	6.19	91
Comparative base	+4 °C	6.39	76
	room temperature	6.38	78
	+40 °C	6.23	79

the most commonly used factors for accelerated testing of product stability. In the cyclic temperature stress test, the sample was alternately exposed to higher and lower temperatures, which can cause destabilizing phenomena (stratification, flocculation, and coalescence). In **Tab. 2**, the measured values of pH and electrical conductivity for the samples after III and IV cycles are presented. The comparative formulations were stored isothermally at +4 °C, room temperature (+22 °C), and +40 °C.

The base, F1, and F2 had a slightly lower pH value after cycle VI compared to cycle III. In comparative samples of the formulation was observed a slight decrease of pH value at +40 °C. The high temperature probably destabilized the formulation, but it did not affect the overall product quality because the pH value was still around 6.0. This value is acceptable since it is close to physiological pH and does not irritate the skin. The changes in electrical conductivity of samples were insignificant under all examined conditions. After using the cyclic temperature stress test, the prepared formulations were relatively stable, because the significant changes in the pH value and electrical conductivity were not noticed. Also, the changes in the organoleptic properties were not observed.

In addition to the accelerated aging tests (centrifugation assay and cyclic temperature stress test), a long-term aging test was also applied for assessing the physical stability of the prepared formulations. The samples were stored at room temperature after preparation for 3 months. The pH value and electrical conductivity were measured after 7, 30, 60, and 90 days of formulation preparation (**Tab. 3**)

Chemical stability of formulations

Polyphenols in their structure contain one or more benzene rings to which are attached at least two hydroxyl groups. These groups are highly reactive and susceptible to epimerization, autooxidation, esterification, alkylation, carboxymethylation and dealkylation reaction, chelate formation, and other reactions. These reactions result in a decrease in the stability or loss of the pharmacological activity of polyphenols (Garcia et al., 2016). Among the environmental factors, the effect of temperature and light were studied on the stability of polyphenols. To investigate the effect of temperature on stability, black locust flower extract was stored at -18 °C, +4 °C, and room temperature for 90 days. At -18 °C, a significant change in the TAC (less than 2%) was not observed. This slight decrease is most likely due to the inhibition of phenoloxidase activity at lower temperatures (Wei & Zhang, 2008). At +4 °C and room temperature, the decrease in the TAC was about 8% until day 90 which was in accordance with available results (Tsali & Goula, 2018). The effect of temperature on the stability of formulations was carried out in the same way as in the case of the extract. The smallest decrease in the TAC was noticed for the samples stored at -18 °C (less than 1%), while this decrease was about 4% at higher temperatures (+4 °C and room temperature). The formulation can be considered stable since the decrease in the TAC was less than 5% compared to the initial value (ICH Q1A (R2)). The satisfactory chemical stability of the formulations is the result of an adequate choice of ingredients, especially those in the oily phase. There is no available literature data about the stability of

Table 3. The change of pH and electrical conductivity values of formulations during storage

Sample	pH				Electrical conductivity (mS/cm)			
	day 7	day 30	day 60	day 90	day 7	day 30	day 60	day 90
F1	6.34	6.19	6.02	5.90	87	88	89	90
F2	6.31	6.16	5.96	5.86	91	93	95	96
base	6.38	6.22	6.06	5.99	79	81	83	85

The pH values of the formulations were decreased but in the range of acceptable limits. The decrease in the pH value was less than 10% for the formulations stored at room temperature for 90 days. These results indicated that the formulations are physically stable. Unlike the pH value, the electrical conductivity was increased in all samples during the time. After the storage, the samples were without visible changes in the organoleptic properties. This behavior is the result of good physic-chemical properties and long-term stability of the formulations.

black locust flower extract. Srivastava & Gupta (2009) monitored the chemical stability of aqueous and methanol chamomile flower extract at different temperatures (-20, +4, and +25 °C) based on the change in the flavonoid content and obtained similar results. At +4 °C and room temperature, the flavonoid content decreased about 10% during 120 days. The storage of prepared chamomile extracts at -20 °C was not caused a significant reduction in the flavonoid content. Certain polyphenolic compounds, such as quercetin (Golonka et al., 2020), resveratrol (Chen

et al., 2020), and catechin (Yuann et al., 2021), etc. are susceptible to photodegradation. Isomerization and polymerization are the most common reactions that occur in the extract under the light effect (Deng et al., 2018; Latva-Mäenpää et al., 2021). The chemical stability of extract and formulations stored in daylight and the dark for 90 days was estimated based on the changes in the TAC. After 90 days of storage in daylight and the dark, the change in the TAC was about 3% and 2%, respectively. Also, the reduction in the TAC was about 1.5% in daylight and 1.2% in the dark after 90 days. The formulation remained photostable after the addition of the black locust flower extract. Also, it can be concluded that there were no interactions between the extract and other ingredients.

Microbiological safety of formulations

Microbiological analysis of the samples showed that the total number of aerobic mesophilic bacteria, yeasts, and mold spores in 1 g of the sample corresponds to the permissible number of microorganisms according to regulations (https://www.paragraf.rs/propisi/pravilnik-uslovima-pogledu-zdravstvene-ispravnosti-predmeta-opste-upotrebe.html). The presence of pathogenic bacteria was not confirmed in 0.1 g of the sample. Therefore, it can be concluded that the prepared formulations were microbiologically safe.

Antimicrobial activity

The antimicrobial activity of formulations with black locust flower extract (0.1 g/mL) was tested on appropriate strains of microorganisms. The base and extract were prepared at the concentration of 1 mg/mL and considered the positive controls. The results of antimicrobial activity are shown in **Tab. 4**. Both DMSO solvent and the base did not show an inhibitory effect against the microorganism strains. Gentamicin affected only bacteria, while it did not show an effect on the analyzed fungus.

Black locust flower extract showed the strongest activity against Gram-positive bacteria (*S. aureus* ATCC 6538, *S. pneumoniae* ATCC 49619) and weaker against Gram-negative (*E. coli* ATCC 8739, *P. mirabilis* ATCC 25933, *K. pneumoniae* ATCC 10031). It did not show an inhibitory effect against the fungus *C. albicans* ATCC 10231. *S. aureus* bacterium causes skin infections, such as ulcers, carbuncles, impetigo and cellulitis. This bacterium can also cause serious infections of postoperative wounds and burns. Since the extract showed the strongest activity against these strains, it can be used in the treatment of skin infections. Rosu et al. (2012) showed that ethanol extract of black locust flower has an effect on Gram-positive bacteria at the concentration of 100 mg/mL. Black locust flower essential oil has antimicrobial activity against selected food pathogens, such as *S. aureus* KCTC 1621*, Bacillus subtilis* KCTC 3569, *Listeria monocytogenes* KCTC 3569*, E. coli* O157:H7, and *Salmonella enterica* ATCC 4731 with MIC and MBC values of 250-1000 mg/mL (Bhalla & Bajpai, 2017).

Conclusions

The topical formulations with black locust flower extract were developed in the laboratory conditions. The formulation was free of synthetic fragrances, colors, and aggressive emulsifiers. The prepared formulations were safe to use and their measured pH was about 6 corresponding to the pH value of healthy skin. The viscosity of 13.261 and 12.789 mPa·s for F1 and F2, respectively, indicated that the prepared formulations were semi-solid consistency. The formulations were stable since the significant changes in the pH value, electrical conductivity, organoleptic properties, and TAC were not noticed during storage. Satisfactory product stability was the result of an adequate choice of ingredients, especially for the oily phase. The optimal conditions

Table 4. Results of antimicrobial activity using disk diffusion method

Microorganisms	Extract	F1	F2	Base	DMSO	Gentamicin
S. aureus ATCC 6538	+++	++	+++	-	-	+++
S. pneumoniae ATCC 49619	+++	++	++	-	-	+++
E. coli ATCC 8739	++	+	++	-	-	++
P. mirabilis ATCC 25933	++	+	+	-	-	++
K. pneumoniae ATCC 10031	++			-	-	+
C. albicans ATCC 10231	-	-	-	-	-	-

No antimicrobial activity (-), inhibition zone <15 mm. Weak antimicrobial activity (+), inhibition zone of 15–16 mm. Moderate antimicrobial activity (++), inhibition zone of 17–19 mm. High antimicrobial activity (+++), inhibition zone of 20–22 mm. Strong antimicrobial activity (++++), inhibition zone of >23 mm. Standard deviation±0,5 mm.

for product storage were room temperature and a plastic polypropylene container. Due to the antioxidant and antimicrobial activity (against *S. aureus*) of incorporated black locust flower extract, the formulations can be useful against skin diseases caused by the effect of free radicals and infections. Also, they can be suitable for daily hydration and softening of the skin due to the presence of emollient (glycerin) and moisturizing ingredients. Having in mind that the formulation with higher contents of the extract enabled better antimicrobial activity against *S. aureus* and *E. coli*, it can be selected as a better one and subjected to further studies.

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