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Study on *Ajuga reptans* extracts as potential cosmetic raw materials

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In the study, the possibility of an application of *Ajuga reptans* leaf and root extracts in antipollution cosmetics was investigated. The influence of *Ajuga* extracts on the skin condition was also evaluated. Both leaf and root *Ajuga* ethanolic extracts were obtained and added to the developed cosmetic formulations. Two types of emulsion W/O and O/W, washing gels and eye serum, containing as an active substance *Ajuga* extracts were prepared. For the stable formulations physicochemical and user properties were studied. The obtained results show that cosmetic products, containing the *Ajuga reptans* extracts, positively affect the skin condition: causing an improvement in the degree of skin hydration and elasticity, reducing the skin pores size and skin hyperpigmentation, and reducing the wrinkles depth.

Keywords: *Ajuga reptans* root extract, *Ajuga reptans* leaf extract, antipollution cosmetic, face cream, washing gels, eye serum.

INTRODUCTION

Ajuga reptans (bugle) is an evergreen to semi-evergreen, herbaceous in the *Lamiaceae* family. The leaves of the plant, depending on the species and location, are characterized by a color ranging from green to red. Its compact mat has beautiful small clusters of purple flowers. They are aggregated in an inflorescence at the top of the stem and grow from opposite axils of green, hairy bracts. The plant has a short main root from which lateral roots branch out[1, 2].

Air pollution is a longstanding environmental problem, and consumer awareness of its effects on the skin is growing significantly. More than 80% of people interviewed worldwide believe that pollutants from the air penetrate deep into the skin, and dirt, cigarette smoke, chemicals from exhaust emissions, and factories are considered as some of the biggest causes of skin problems[3]. Protecting the skin from environmental pollutants should be characterized by proper cleansing of the skin to remove chemicals deposited during the day. Next isolation of the epidermis by creating an impermeable film on its surface to prevent direct skin contact with air pollutants, as well as the use of antioxidants to protect against free radicals or ingredients capable of increasing the antioxidant defense of epidermal cells[3]. Extracts from plants rich in bioactive compounds are now an important ingredients in cosmetic formulations. These assist the active ingredients of cosmetics in their action, among many other things, in the control of negative skin changes such as photoaging[4] or acne changes[5]. *Ajuga* is a rich source of active compounds, such as phenylpropanoids, ecdysteroids, di- and triterpenes, sterols, iridoids, and flavonoids[1, 6–8]. Because of such composition, *Ajuga* extracts provide antioxidant[4], antimicrobial, and anti-inflammatory activity[6, 9] and have been used in cosmetic products, both for skin and hair care[10]. Moreover, from the presence of phenylpropanoids, *Ajuga reptans* extracts exhibit activity to protect the skin against UVA and UVB radiation while the presence of iridoids contributes to anticancer activity[6]. An application of bugle extracts as active ingredients of anti-aging and skin moisturizing cosmetics was described in many patent applications[4, 11–12]. Products containing *Ajuga reptans* extracts also were presented as the formulations promoting hair growth and stimulating hair roots, particularly suitable for the prevention and treatment of androgenetic alopecia, both in the form of oral and topical administration[13].

It should be emphasized, however, that literature reports describing the use of *Ajuga* extracts as a multifunctional active ingredient of cosmetic and pharmaceutic products refer to the extracts from the leaves of this plant. In our study, the possibility of the application both of leaf and root *Ajuga reptans* extracts as raw materials in antipollution cosmetics was investigated.

The methods for extracts obtaining and the characterization of their antioxidant, anti-aging, and antipollution properties have been described in patent applications[14–17].

MATERIALS AND METHODS

The dry *A. reptans* ethanolic extracts were obtained and characterized in the laboratory of the Department of Organic Chemistry and Technology, at Cracow University of Technology[14–17] supplied by Gospodarstwo ogrodnicze "Świat roślin liściastych" (Poland). Glycerin, sodium benzoate was purchased in Chempur (Poland). Palmitic/ Stearic Triglyceride, Steareth-100 (and) Steareth-2 (and) Mannan (and) Xanthan Gum (Versaflex™ V-150), Sucrose Palmitate (and) Glyceryl Stearate (and) Glyceryl Stearate Citrate (and) Sucrose (and) Mannan (and) Xanthan Gum (Versaflex™ V-175), Aqua (and) Sodium Lauroyl Sarcosinate (Crodasinic™ LS30), PEG-60 Almond Glycerides (Crovol™ A70), Polyamide-8 (OleoCraft LP-20) and Ricinus Communis Seed Oil (Castor oil) were delivered by Croda (Poland). Raw materials such as Olive Oil Polyglyceryl-6 Esters (and) Polyglyceryl-6 Pentaoleate (Olivatis™ 12C), Cera Alba, Butyrospermum Parkii Butter (Shea Butter), Sodium Hyaluronate (Sodium hyaluronate 1.5–2.0 m.d.a.), Helianthus Annuus (Sunflower) Seed Wax and Tocopherol (Vitamin E) were acquired from the company Alfa.

Sagittarius (Poland). Magnesium Sulfate was delivered by RonaCare (Germany), Isopropyl Myristate and Cetyl Palmitate were delivered by CarlRoth (Germany). Materials like Xanthan Gum was delived by Brenntag Polska Sp. z o.o., Oenothera Biennis Oil by OlVita (Poland), Caprylyl Glycol (and) Phenoxyethanol by SpecChem (Poland), and Cocamidopropyl Betaine by Evonik (Germany).

Four groups of formulations containing *A. reptans* root or leaf extract were prepared: emulsions (O/W and W/O face creams), cleansing gels, and eye serums. The quality of the stable products was verified in physicochemical, dermatological, microbiological, and consumer tests. Tables 1 and 2 show the composition of the prepared formulations.

Preparation of emulsions

Table 1 shows the composition of the prepared emulsions.

Both of the emulsions (O/W and W/O) were obtained according to the same procedure. Components of the aqueous phase were mixed with the oil phase, at 60 °C, after an addition of the internal phase to the external one. The combined phases were stirred at 60 °C, with the aim of homogenizing the consistency, using a Velp ES-type mechanical stirrer, at v_1 = 700 rpm for 15 minutes. Then fragrance was added. Next, the emulsions were stirred until their temperature was reduced to room temperature. Finally, the obtained products were homogenized using a CAT Unidrive X 1000 high-speed homogenizer (Ingenieurbüro CAT), at a speed of v_2 = 13 000 rpm.

Preparation of cleansing gel and eye serum

In the case of the facial cleansing gel sodium hyaluronate was used as a rheology modifier and moisturizing agent. Table 2 shows the composition of the prepared cleansing gel and eye serum.

The cleansing gel was obtained by mixing glycerin, dry extract of *Ajuga reptans* L., and sodium hyaluronate. In the next step, the rest of the ingredients were added and mixed thoroughly until the extract and the rest of the ingredients were completely dissolved.

In the first stage of the serum preparation, the extract was dissolved in glycerin. Polyamide-8 and castor oil were heated up to 75 °C and mixed together until the ingredients were completely dissolved. Then the oil solutions were combined with evening primrose oil (*Oenothera Biennis Oil*) and mixed oils added in the first step glycerin and extract, after the whole mixture was stirred. In the next step vitamin E, preservative, and fragrance were added and stirred until all ingredients were dissolved and a clear solution was obtained.

The obtained formulations (emulsions, gels, and eye serums) were tested with the purpose of determining their stability, physicochemical, and user properties.

Emulsions stability tests

The stability of the prepared formulations was determined using centrifuge and heat shock tests. In the case of centrifuge test, HETTICH's EBA 20 apparatus was used, set at 3 500 rpm, and centrifuged over 10 minutes. During the heat shock tests the samples were stored at different temperatures (40 °C and –20 °C), alternately in 24-hour cycles. Such a cycle was repeated three times. Before and after the test, rheological measurements were performed to determine the samples stability.

Table 1. Emulsion formulas containing the extract of the *Ajuga reptans*[14–15]

Phase	Emulsion W/O		Emulsion O/W	
	INCI name of compounds	[%]	INCI name of compounds	[%]
Water	Olive Oil *Polyglyceryl*-6 Esters, *Polyglyceryl*-6 *Pentaoleate*	77.5	*Cetyl Palmitate*	92
	Magnesium Stearate		*Versaflex* V-175	
	Sodium Benzoate		*Sodium Benzoate*	
	Xanthan Gum		*Xanthan* Gum	
	Ajuga reptans Roots Extract/ *Ajuga reptans* Leaf Extract		*Ajuga reptans* Roots Extract/ *Ajuga reptans* Leaf Extract	
	Aqua		*Aqua*	
	Glycerin		*Glycerin*	
Oil	*Palmitic/ Stearic Triglyceride*	22.5	*Oenothera Biennis* Oil	8
	Cera Alba			
	Isopropyl Myristate			
	Shea Butter		*Cera Alba*	
	Oenothera Biennis Oil			
Fragrance composition	Parfum	q.s	Parfum	q.s

Table 2. Gel and serum formulation containing extract of *Ajuga reptans*[16, 17]

Phase	Skin cleansing gel		Phase	Serum	
	INCI name of compounds	[%]		INCI name of compounds	[%]
Water	*Caprylyl Glycol, Phenoxyeethanol*	86	Water	*Caprylyl Glycol, Phenoxyeethanol*	10.7
	PEG-60 *Almond Glycerides*			*Glycerin*	
	Glycerin			*Ajuga reptans* Roots Extract/ *Ajuga reptans* Leaf Extract	
	Aqua			Polyamide-8	
	Sodium Hyaluronate		Oil	*Ricinus Communis* Seed Oil	89.3
	Ajuga reptans Roots Extract/ *Ajuga reptans* Leaf Extract			*Oenothera Biennis* Oil	
Surfactant system	*Cocamidopropyl Betaine*	14		*Tocopherol Acetate*	
	Aqua (and) Sodium Lauroyl Sarcosinate				
Fragrance composition	Parfum	q.s	Fragrance composition	Parfum	q.s

Verification of the emulsion types

In order to confirm the type of the emulsions conductivity tests were performed. The conductivity of the resulting emulsions was tested using an S47 (SevenMulti™) conductivity meter. Also, emulsions were tested using the dilution technique. A small sample of each emulsion was placed on two watch glasses. A small amount of water was added to one slide and on the other evening primrose oil and mixed with the emulsions. It was observed with which medium the emulsion mixed to form a homogeneous system.

Measurement of the formulations pH values

In order to check the pH values of the cosmetics, 1g of the tested emulsion was dissolved in 50 cm^3 of deionized water. Then the pH of each solution was measured, using a pHmeter S47 (SevenMulti™) for this purpose.

Measurement of rheological properties

The rheological properties of the obtained formulations were examined using a Brookfield rheometer, equipped with a cone-plate system, at 25 °C, using a compatible control thermostat from Huber. The tests were performed using the Rheo3000 computer program. Three measurements were performed for each of the samples.

Microbiological tests

The obtained cosmetics were subjected to microbiological tests using Easicult Combi (Aidian) disposable tests. Easicult Combi is a two-medium dipslide. TTC agar allows almost all aerobic bacteria to grow, while Rose Bengal agar supports the growth of yeasts and moulds. Easicult Combi is intended for simultaneous estimation of total bacterial counts, yeasts and moulds. These tests aimed to estimate the total amount of bacteria derived from reference strains*: Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 10231, *Aspergillus brasiliensis* ATCC16404 , molds, and yeasts: *Rhizopus nigrican, Mucor plumbeus, Rhodotorula mucilaginosa, Saccharomyces cerevisiae, Paecilomyces variotii, Penicillium chrysogenum, Aspergillus fumigatus* in the samples. Prepared formulations were applied evenly to a plate coated with TTC agar which is designed to support bacterial growth and to a Rose-Bengal agar plate which supports fungal and mold growth. After applying the products, the samples were sealed in special tubes and incubated. The test was carried out for 72 hours and the temperature in the incubator was 30 °C.

Dermatological tests

In the case of the dermatological test, the semi-open patch test was applied. Patch tests according to Jadassohn-Bloch (as modified by Rudzki) were performed under the supervision of a dermatologist. The evaluation of sensitising and irritant properties of the product was made based on a sample of 20 healthy volunteers (19 women and 1 man, aged 18–69) without a history of allergy. The study was conducted using Finn Chamber patches with filter paper onto which the tested product was applied; the whole patch was then applied to the volunteer's skin (arm or interscapular region). The patches were removed after 48 hours. The first reading was done 15 minutes after patch removal, and subsequently after 72 hours. The reading was based on a generally acknowledged scale for dermatological studies, and subsequently evaluated by a dermatologist.

Microbiological and conservation tests

Microbiological tests of the products were carried out using Easicult Combi disposable tests, placed in an incubator at 30 °C for 72 hours. An estimation of the total number of bacteria was made after 24 hours of incubation. The number of molds and yeast was estimated after 72 hours of testing.

Additionally, the evaluation of the antimicrobial efficacy of the cosmetic product was also performed according to PN-EN ISO 11930:2019, using reference strains: *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Candida albicans, Aspergillus brasiliensis*. TSA, Sabouraud and PDA agar media were used in the study. The samples were incubated for 28 days at 22.5±2.5 °C. After confirming the microbiological purity of the test samples, 20 g of product was weighed into sterile containers. After that, 0.2 ml of a suspension of a given strain of known density was introduced into each container and distributed throughout the sample volume.

Dry mass content, turbidity, and solubility of gels

The prepared gels were tested to determine the dry content of the formulation. Using an instrument weighing machine MB25 from Ohaus. 1g of the formulation was metered into containers and sealed in the weighing-drying machine. The drying process of the material was carried out at 110 °C. The turbidity of the resulting gels was examined using a Nanocolor UV/VIS spectrophotometer. Three measurements were made for each type of product at 860 nm using the «Turbidity» function. The solubility of gels in water was tested using tap water and distilled water. To a beaker containing 100 ml of water in which a magnetic stirrer was stirred at v_1 = 300 rpm, 1 g of the test gel preparation was added. Observed the time it took for the product to dissolve completely in the water.

Consumer tests

The study aimed to determine the degree of hydration, and skin elasticity, as well as the visibility of pores, reduction of hyperpigmentation, and depth of wrinkles. The selected final products were tested for their organoleptic properties in the laboratory, using consumer tests. Each of the prepared samples, depending on the type of extract, was designed for use on a selected part of the right and left hand (wrist, forearm, arm). The study included 10 testers who tested the formulations for a period of 14 days. The probands applied the cosmetics three times a day. The effects of the products on the condition of the skin were tested using the AramoTS (Aramo) skin analizer. The skin of the testers was examined twice - before the cosmetic application and after 14 days of their use. Additionally, the panelists were asked to determination of the organoleptic properties of the cosmetics such as smell, color, consistency, spreading, adherence, feeling initially after application, stickiness, absorption, oily, greasiness, and smoothing.

RESULTS AND DISCUSSION

Eight different formulations: O/W and W/O emulsions, gels, and serums contained as active ingredients *Ajuga reptants* extracts from leaves and roots were obtained. The physicochemical and user properties of the stable products were studied to determine the usability of the formulations as cosmetic products.

Emulsions stability tests

Both of the stability testing methods, centrifuge, and heat shock test, indicate the stability of the obtained products. No signs of the formulations instability were observed (Table 3). The addition of *Ajuga* extracts didn't affect the product stability.

Verification of the emulsion types

Contrary to O/W emulsion, the value of electrical conductivity in the case of W/O emulsion is much lower[18, 19]. W/O type emulsions, due to the poor conductivity of oil which is the external phase, have a current conductivity close to zero (Table 3). The emulsions were also examined by the dilution method. The emulsions are easily combined with external phase[19]. Samples of O/W emulsions are mixed with water while they don't combine with oil. The opposite phenomenon occurred in the case of W/O sample. The emulsion was mixed with evening primrose oil while mixing did not occur with water.

Measurement of the formulations pH values

The pH value of cosmetic products should range between 4.5 and 6.0 pH. The results of measurements (Table 3) confirmed that the pH values of the prepared cosmetics are close to pH of human skin (4.0–7.0)[20]. The formulations can be safely applied to the skin.

Measurement of the formulations rheological properties

The viscosity curves of obtained emulsions confirm that the formulations are non-Newtonian, shear-thinning fluids. In order to confirm the stability of the samples, the first measurement was made before the thermal shock stability test (Fig. 1) and the validation one, after the variable temperature test (Fig. 2). The results of gels and serums rheological measurement are presented in Fig. 3.

Table 3. Physicochemical test results for cosmetics with extracts of *A. reptans*

Type of formulation	Emulsion stability		Type of emulsion	Average measured pH value
	Centrifuge test	Variable temperature method	Conductivity value [μs/cm]	
Emulsion W/O	Stable	Stable	0.005 ± 0.002	5.700 ± 0.050
Emulsion O/W	Stable	Stable	23.600 ± 0.896	5.770 ± 0.0173
Gel	N/A	N/A	N/A	5.470 ± 0.196
Serum	N/A	N/A	N/A	N/A

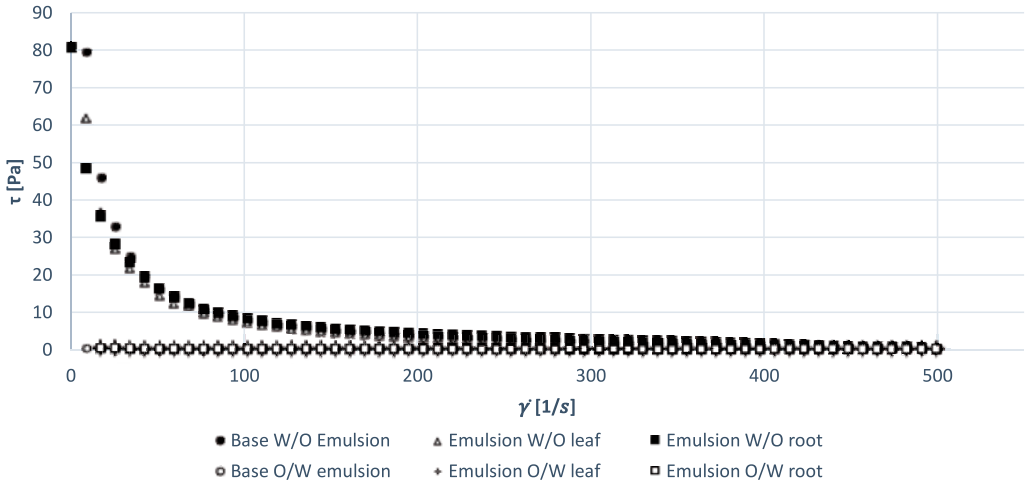

Figure 1. Viscosity curves of prepared emulsions before variable temperature tests, T = 25 °C, (n = 3)

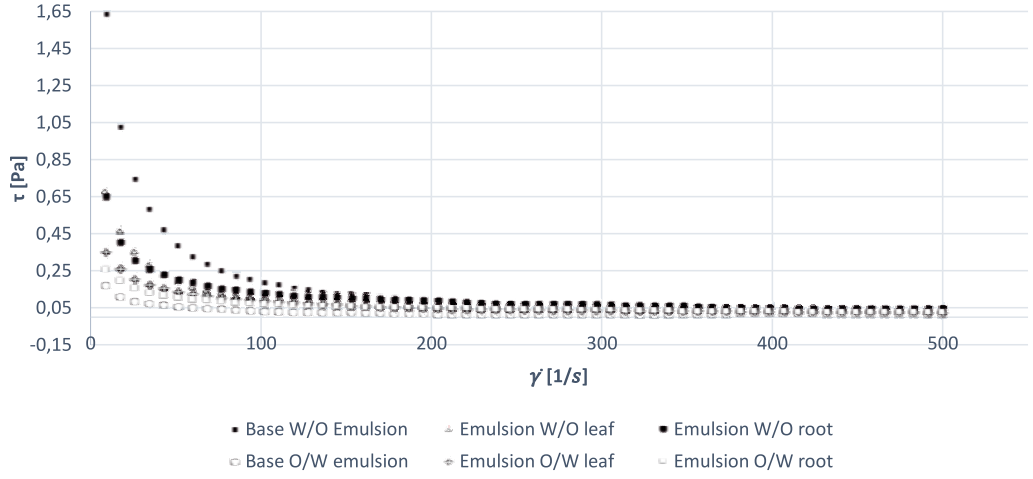

Figure 2. Viscosity curves of prepared emulsions after variable temperature tests, T = 25 °C, (n = 3)

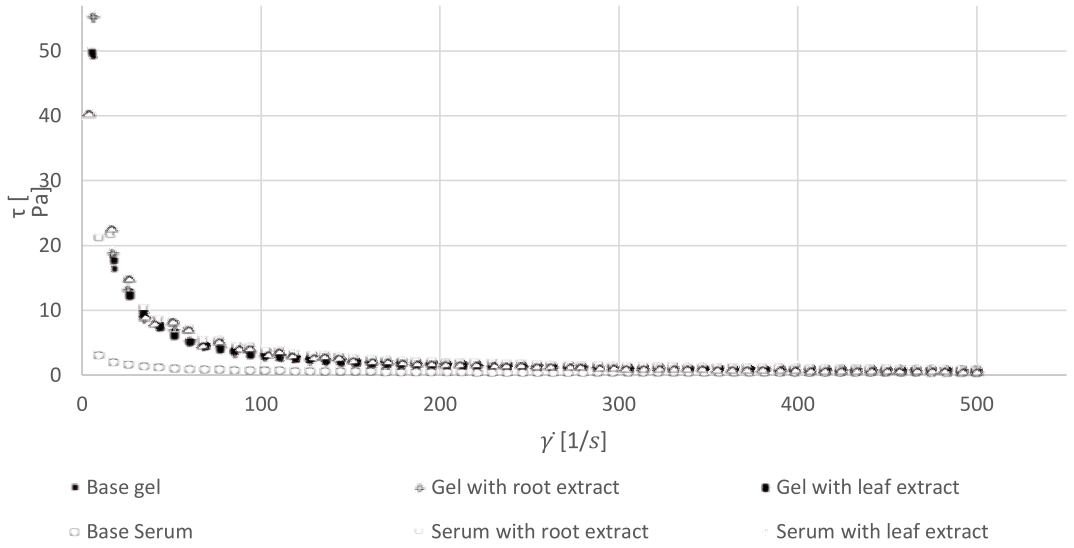

Figure 3. Viscosity curves of gels and serums, T = 25 °C, (n = 3)

Dermatological tests

The results of the dermatological tests conducted for the products containing *Ajuga reptans* extracts are recommendations of the International Contact Dermatitis Research Group (ICDRG) and show no erythematous reaction and no swelling. The mean irritation index (mean) is calculated from the skin reaction readings of 25 test subjects. The product is then classified according to the average irritation index (mean). On the basis of the obtained results, it was found that the tested products did not cause any side effect symptoms, irritation, or sensitization in the test group. The patch tests for all subjects gave a negative result (no reaction), therefore neither skin irritation nor sensitization was observed as a result of the application of the formulation.

Microbiological and conservation tests

Evaluation of the samples using Easicult Combi disposable tests showed that none of the samples tested showed the presence of bacteria, yeast, and mold (Table 4). The results of microbiological studies confirmed that the samples are not contaminated with bacteria, yeast, and mold and meet the purity criteria for cosmetic products (Table 4).

Moreover, the challenge testing ensured that the used preservatives protect the cosmetics from microbial growth and secondary infection during use[21].

Dry mass content, turbidity, and solubility of gels

The results of the dry mass content in the gels are presented in Table 5. The obtained values are consistent with the presence of dry extracts in the gels affecting the dry weight ratio of the gels containing extracts compared to the base gel without the presence of any extracts.

The turbidity measurement results of the gels (Table 5) show that the tested samples have high turbidity values. It should be emphasized that the turbidity does not affect the functional properties of the formulations.

The results of the water solubility test indicated that the gels dissolve very well and quickly in water, positively translating into their usability, dirt removal properties, and its faster rinsing from the skin surface. Comparing the solubility values of the tested gels to the literature values (Table 6), the obtained products can be awarded points from 5-4.

Table 6. Rules for classifying the dissolvability of shower gels and bath liquid

Points	Description
5	Solubility of the product up to 12 s (very fast, very good)
4	Solubility of the preparation up to 18 s (good)
3	Solubility of the preparation up to 24 s (sufficient)
2	Solubility of the preparation up to 30 s (slow)
1	Solubility of the preparation > 30 s (the preparation shows low solubility)

Table 4. Microbiological purity of prepared cosmetic products

Tested characteristic	Requirements	Emulsion W/O	Emulsion O/W	Gel	Serum
		Test result [in 1 ml]			
Total number of aerobic mesophilic microorganisms	≤1 x 10^3 in 1 ml	<10^1	<10^1	<10^1	<10^1
Total number of yeasts and molds	≤1 x 10^3 in 1 ml	<10^2	<10^2	<10^2	<10^2
Presence of *Pseudomonas aeruginosa*	Absent in 1ml	None growth	None growth	None growth	None growth
Presence of *Staphylococcus aureus*	Absent in 1ml	None growth	None growth	None growth	None growth
Presence of *Escherichia coli*	Absent in 1ml	None growth	None growth	None growth	None growth
Presence of *Candida albicans*	Absent in 1ml	None growth	None growth	None growth	None growth
Presence of *Aspergillus brasiliensis*	Absent in 1ml	None growth	None growth	None growth	None growth

Table 5. Summary of gels test results

Tested gel	Drycontent of the gel [%]	Mean turbidity value [NTU/FNU]	Time for complete dissolution of gel in tap water [s]	Time for complete dissolution of gel in distilled water [s]
Gel with root extract	23.54	74.33 ± 4.93	14	18
Gel with leaf extract	26.58	50.67 ± 1.15	12	13
Base gel	22.58	90.10 ± 5.51	10	12

Consumer tests

The results of the user properties study, in relation to the base formulations, are presented in Fig. 4.

Describing the condition of the skin when using formulations containing extracts of *A. reptans* in the formulation to those without the active ingredient a significant increase in skin hydration and elasticity is noted. There was also a reduction in the amount of hyperpigmentation and pore size as well as a reduction in the depth of wrinkles on the skin. The highest increases in skin moisture (8.3%) and elasticity (9.0%) were observed with formulations containing extracts of *Ajuga reptans* leaf. The highest increase in skin smoothness (6.2%) was observed after using cosmetics with extracts from the plant's root. Also, these products had the greatest effect on reducing the number of pores (21.6%), hyperpigmentation (9.8%), and reduced the degree of wrinkle depth (7.0%).

Each panelist additionally completed a questionnaire to assess the organoleptic properties of the products. The results, in the form of sensory profiles, are shown in diagrams 6–10.

The W/O emulsions were rated best in terms of color, smoothness, initial feeling after applying the products to the skin, as well as scent and absorption (Fig. 5). The worst-rated products were spreading. The O/W emulsions were rated best in terms of color, smoothness, and initial feeling after the products were applied to the skin (Fig. 6). Traits such as stickiness and greasiness of the products fared worse. Emulsions with extracts scored similarly.

The serums tested by the probands were rated best in their feeling when applied to the skin, color, and smoothness (Fig. 7). Their oily properties were rated the worst. The tested gels were rated best in terms of consistency, spreading and initial feeling after applying the products to the skin (Fig. 8). Due to the lack of an oil phase in the formulation of these products, oiliness and greasiness scored the worst.

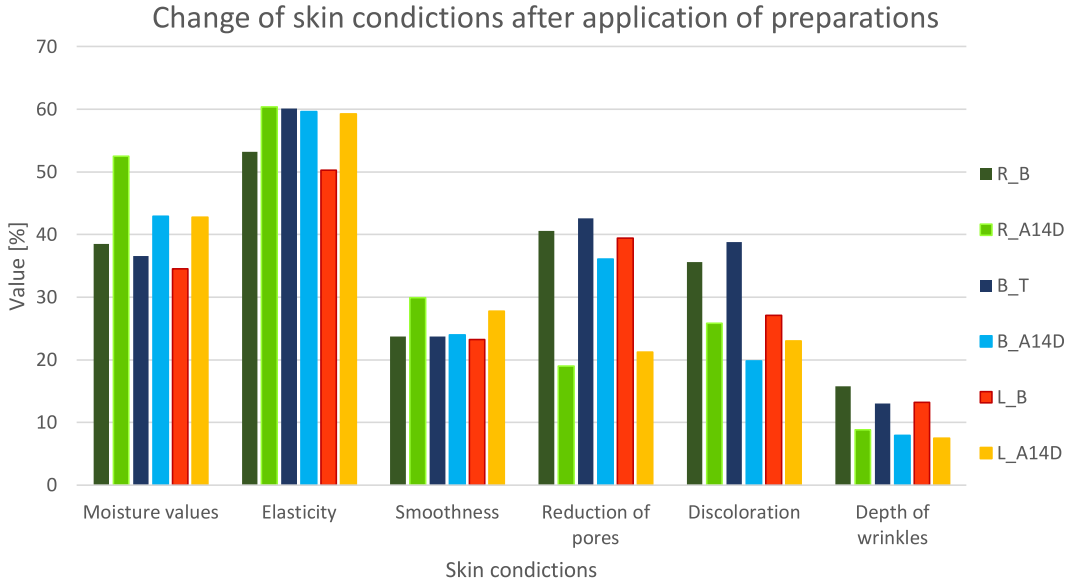

Figure 4. Results of apparatus tests (n = 10) conducted for developed cosmetics. R_B – Cosmetics with roots extracts before test; R_A14D – Cosmetics with roots extracts after 14 days; B_T – Base cosmetics before test; B_A14D – Base cosmetics after 14 days; L_B – Cosmetics with leaf extracts before test; L_A14D – Cosmetics with leaf extracts after 14 days

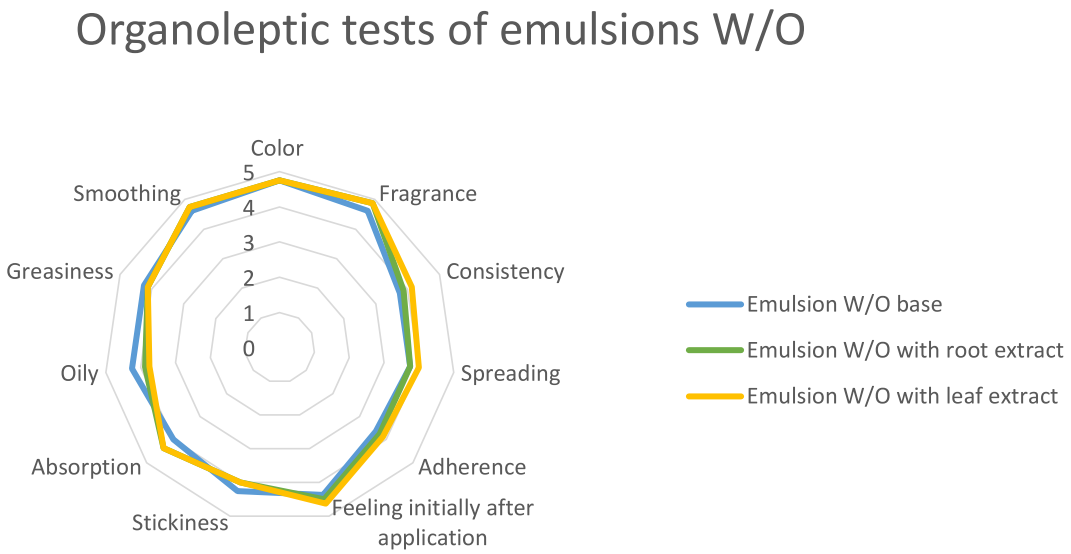

Figure 5. Organoleptic properties of the emulsions W/O

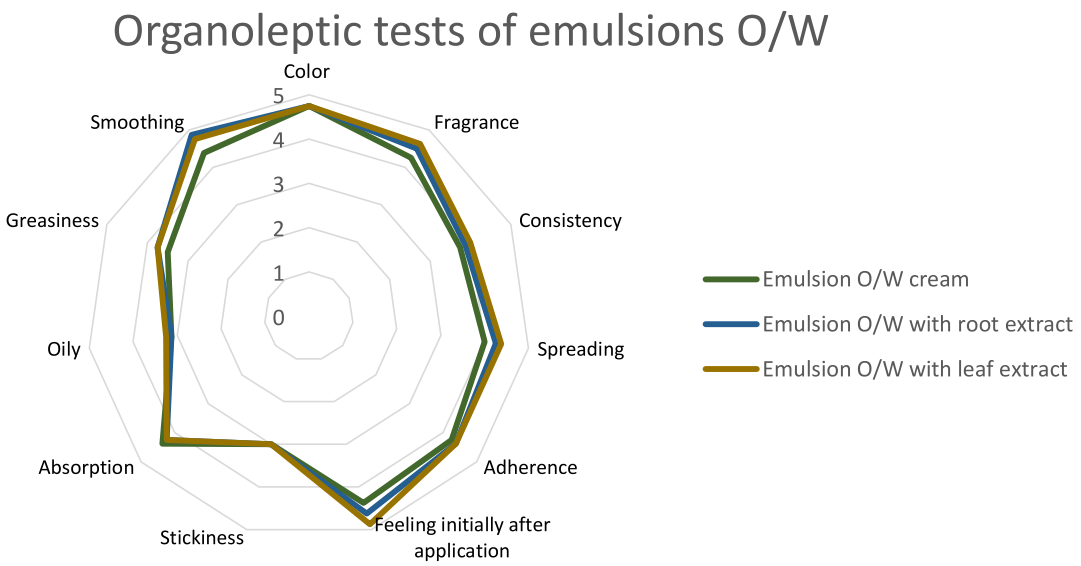

Figure 6. Organoleptic properties of the emulsions W/O

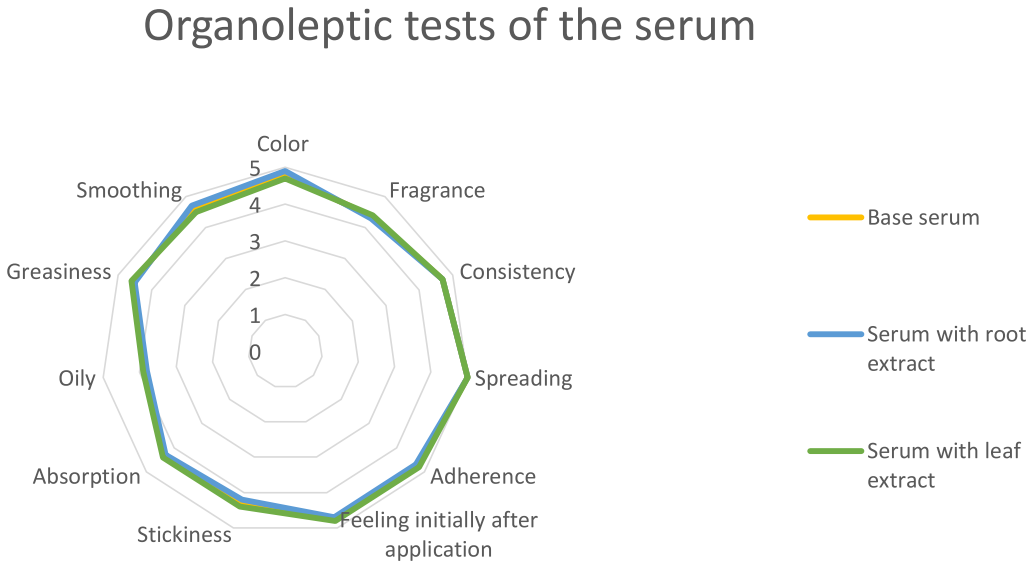

Figure 7. Organoleptic properties of the serum

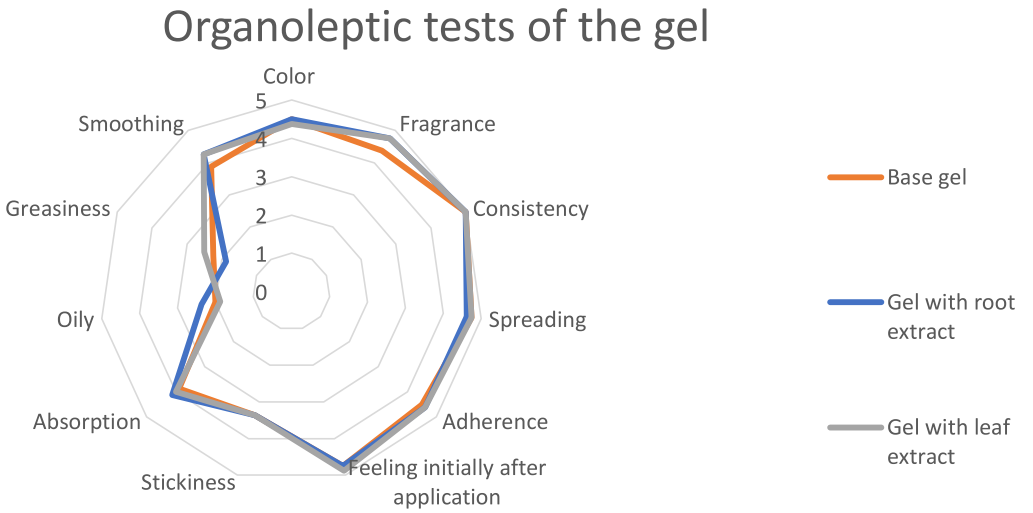

Figure 8. Organoleptic properties of the gels

CONCLUSION

Stable emulsions (O/W and W/O), gels, and serums, cotaining *Ajuga reptans* leaf or root extracts as active ingredients have been obtained. Based on the tests, it has been shown that the tested product physicochemical, microbiological, and dermatological point of view, all the developed formulations met the requirements for cosmetic products[22]. Products have no irritant or sensitizing effects on the skin of the subjects and meet the requirements of compatibility tests with the skin (Skin Compatibility Test). Moreover, the results of the consumer tests confirmed the positive effect of the *Ajuga reptans* extracts-loaded cosmetics on the condition

of the panelists skin. An improvement in the moisture content, elasticity, and smoothness of the skin, as well as a reduction in the visibility of pores, pigmentation, and depth of wrinkles, were observed. The obtained results confirmed that the extracts from *Ajuga reptans* (both leaves and roots extracts) could be applied as high-quality active ingredients in cosmetic products. It should be underlined that *Ajuga* root extract has not been used jet in the cosmetic formulations.

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