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PREPARATION AND CHARACTERIZATION OF ANTIBACTERIAL CREAMS CONTAINING PLANT EXTRACTS FOR TOPICAL APPLICATION

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Abstract

The use of herbal cosmetics is increasing in the world market, due to the good activity with no side effects of the active ingredients from plant extracts as compared to synthetic compounds. The aim of the present study was to formulate and characterize herbal creams containing several plant extracts (Arctium lappa, Magnolia virginiana, Tiliae flos) in different combinations. The creams were prepared with different ratio of plant extracts and essential oils, while the composition of the cream base was kept the same. The characterization of the formulated creams was carried out by standard methods, for evaluating the organoleptic characteristics, physicochemical properties, microbiological contamination level and in vitro antibacterial activity against Staphylococcus aureus. Also, the plant extracts were evaluated in terms of cytotoxicity using MTS/MTT assay on L-929 fibroblasts. The creams were homogeneous, non-irritant and easily removable. The pH of creams was in the range of 5.0-5.5 which is safe for human skin. The samples were found to be populated with aerobic bacteria, yeast and fungi up to 10 CFU/g and showed moderate antibacterial activity.

Key words*: plant extracts, antibacterial creams, Arctium lappa, Magnolia virginiana, Tiliae flos.*

INTRODUCTION

Arthritis is a term used to describe more than 100 different pathological conditions of the skin and joints, being a chronic inflammatory systemic disease characterized by persistent swelling and stiffness of the joints and destruction of the synovial joints, leading to severe disability with early mortality (Sayah & English, 2005; McInnes, & Schett, 2011). Among the most common causes are trauma, metabolic disorders, genetic factors (HLA class II -DR1 and DR4), infections, immune factors (Harth & Nielson, 2019). It is also one of the most common chronic diseases and one of the most common causes of disability in the world's population. The incidence of the disease is estimated at 95-150 new cases per 100,000 inhabitants annually (Harth & Nielson, 2019). The prevalence of the disease is about 1% in Caucasians, and ranges from 0.1% in rural Africans to 5% in Indians (Smolen et al., 2016; Coates & Helliwell, 2017). It is well known that topically applied medication products are preferred over their systemic counterparts for treating topical illnesses due to the numerous benefits they provide (Benson et al., 2019; Tang, 2019). Because there is no systemic absorption, topical therapies have no systemic negative effects. They are also simple to make, administer, and have a higher level of patient compliance (Chang et al., 2012; Benson et al., 2019). Currently, on the dermatocosmetics market, there is a wide range of topical products with an adjuvant role in the treatment of inflammatory diseases of the skin and joints (e.g. arthritis), such as creams, lotions, gels, and ointments, mainly based on plant extracts (Panda & Ghosh, 2010; Oltean et al., 2014; Ahuja et al., 2021). Most of these products contain highly aggressive synthetic chemicals for the skin (e.g. salicylic acid, urea), synthetic

surfactants (tween, borax, cetyl alcohol), preservatives and/or stabilizers (Singh Malik et al., 2016; Rodriguez-Merchan, 2018). Also, plant extracts used in most skincare products are not standardized. The aim of this study was to formulate and characterize herbal creams containing several standardized plant extracts (*Arctium lappa, Magnolia virginiana,* and *Tiliae flos*) in different combinations.

MATERIALS AND METHODS

Materials. The materials used include: white wax purchased from MAYAM Pure Cosmetics, Romania, cocoa butter (*Theobroma cacao*), shea butter (*Buthyrospermum parkii*) procured from Herbavit, Romania, vitamin E (α-tocopherol) acquired from Sigma-Aldrich, Germany, grapeseed oil (*Vitis vinifera*), sweet almond oil (*Prunus amygdalus dulcis*) and cinnamon essential oil (*Cinnamomum zeylanicum*) purchased from Sabio Cosmetics, Romania.
The lanolin used for cream base was purified by Pharmaceutical Technologies's team of the National Institute of Chemical-Pharmaceutical R & D (ICCF Bucharest), Romania. The plant extracts used for cream formulations were obtained and characterized by the botanist's team of the National Institute of Chemical-Pharmaceutical R & D (ICCF Bucharest), Romania. Each plant extract of burdock (*Arctium lappa)* leaf and linden inflorescence (*Tiliae flos*) had a concentration of 5 mg gallic acid equivalents [GAE] *per* 1 mL, and magnolia (*Magnolia virginiana*) petals extract had a concentration of 2.5 mg gallic [GAE] acid equivalents *per* 1 mL. All plant extracts were standardized in 50% glycerin. The composition of herbal cream formulations is shown in Table 1.

Formulation. The required quantities of the cream base constituents, namely, lanolin (12 g), white wax (6 g), cocoa butter (2.2 g), shea butter (3.8 g) were accurately weighed, heated in a water bath up to 60-70^{0}C and stirred continuously. Grapeseed oil (8.1 g), sweet almond oil (8.1 g) were also weighed accurately and were added to the base cream constantly until homogenous product was attained. The mixture of plant extracts (*Arctium lappa* leaves: *Tiliae flos* 1:1 w/w for F1 and *Arctium lappa* leaves: *Magnolia virginiana* petals 1:1 w/w for F2) was then incorporated into the cream base under continuous stirring. A homogeneous mixture was obtained, to which essential oil (1.5 g of cinnamon oil) and 1.5 g of vitamin E were added. The composition of the two different herbal cream formulations is given in Table 1.

Table 1. Composition of herbal cream formulations (F1 and F2)

No.	Constituent	F1 % w/w	F2 % w/w
1	Lanolin	28	28
2	White wax	14	14
3	Cocoa butter	5	5
4	Shea butter	9	9
5	Mixture of burdock leaf extract : linden inflorescence extract (1:1 w/w)	3	-
6	Mixture of burdock leaf extract : magnolia petals extract (1:1 w/w)	-	3
7	Grapeseed oil	19	19
8	Sweet almond oil	19	19
9	Cinnamon essential oil	1.5	1.5
10	Vitamin E	1.5	1.5

Organoleptic characterization. The formulations were examined for colour, odour, texture and homogeneity. A small amount of each formulation was split into two glass slides and examined visually.
Results for texture and homogeneity were presented as follows: (+++) = excellent, (++) = very good, (+) = good and (-) = poor.

pH determination. pH has been measured using a digital pH meter (MP 220, Mettler Toledo). 1 g of each formulation was mixed in 100 ml distilled water (1% w/v), then warmed, vigorously stirred and stored for two hours. The electrode was inserted three times into the sample for pH recording.

Stability study. The formulations were packed in foldable aluminium tubes and placed in an accelerated stability chamber at 40°C and 75% relative humidity for three months according to the international conference on harmonization (ICH) guidelines (ICH Harmonized Tripartite Guidelines, 2003). Samples were removed after 1, 2, and 3 months storage to evaluate the following parameters: texture, colour, odour, and pH.

Microbiological evaluation. Microbial counts and detection of microorganisms were performed according to the European Pharmacopoeia

(Ph.Eur. 9th edition). The test sample was prepared by suspending 1 g of the formulation in 9 mL of buffered sodium chloride peptone solution and shaking it for 15 minutes in a vortex blender.

Total Aerobic Bacteria Count

1 mL of test liquid was aseptically removed from the supernatant layer and spread over solid plates of soybeancasein digest agar medium (Sigma-Aldrich, Germany) containing amphotericin B (2.5 µg/mL) (2 ml/L of medium) to prevent the fungal growth, and incubated at 37 °C for 24 hours. The colony-forming unit (CFU) of each plate was counted using the colony meter and the CFU/g of the formulation was recorded.

Total number of yeasts and fungi

1 mL of test liquid was spread on solid Sabouraud dextrose agar medium (Sigma-Aldrich, Germany) containing a chloramphenicol (50 mg/L of medium) to prevent bacterial growth, and incubated at 25°C for 72 hours. The colony-forming unit (CFU) of each plate was counted using the colony meter and the CFU/g of the formulation was noted.

In vitro evaluation of antibacterial activity

In vitro antibacterial activity was evaluated against *Staphylococcus aureus* ATCC 6538 using the agar well diffusion technique in accordance with the requirements of Ph. Eur. 9th edition.

Determination of cell viability and cytotoxicity of cream formulations components (plant extracts). The experiments were performed on the L-929 cell line (ATCC CRL-6364), the murine fibroblast line. Cell cultures were performed in Eagle's Minimum Essential Medium (EMEM) adjusted with 10% equine fetal serum, 1% fetal bovine serum, penicillin-streptomycin-neomycin mixture in 0.9% NaCl solution (10.000 µg/mL/10.000 U/mL). At 75% confluence, cell cultures were harvested by trypsin-EDTA treatment to remove the cell monolayer, after which trypsin was neutralized with fetal bovine serum, and the cells were homogenized. The cell suspension was then harvested in 15 mL centrifuge tubes and the cells were centrifuged at 1200 rpm for 10 minutes. The cells were then resuspended in culture medium and adjusted to 10^6 cells/mL. 96-well plates were inoculated at a density of 8 x 10^3 cells/well. After 24 hours, the culture medium was replaced with fresh medium (180 µL/well). The cells were then incubated in the presence of the above samples for 24 hours at 37°C in an atmosphere with 5% CO_2, at concentrations of 100 µg/mL, 50 µg/mL, 25 µg/mL, 10 µg/mL and 5 µg/mL medium, after which cell viability was determined by a colorimetric method using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay Kit (Promega, USA). After 24 hours of exposure to the above concentrations, the medium was replaced with 100 µL of MTS reagent, diluted 1:10 with fresh medium. The cells were incubated for 3 hours in an incubator with 5% CO_2, then the optical densities were measured at 490 nm using a Microplate Reader (Chameleon V Plate Reader, LKB Instruments).

Optical densities were recorded and related to the values of the control samples, considered to be the maximum values of cell viability.

All tests were performed in triplicate.

RESULTS AND DISCUSSIONS

This study was geared to obtain cream formulations based on natural ingredients:

(1) *Lanolin* is a waxy, anhydrous substance obtained by extraction after processing sheep's wool, used in a wide range of cosmetics, especially for skincare, and pharmaceuticals. It was used in these formulations because it is easily absorbed into the skin, reduces transepidermal water loss and has an emollient, protective and repairing role on the skin (Chang et al., 2012; Rodriguez-Merchan, 2018; Singh Malik et al., 2016).

(2) *White wax* is a natural ingredient widely used in the preparation of cosmetics and dermatocosmetics because it gives consistency and stability to the compositions, has an emollient, soothing role and helps maintain an optimal level of skin hydration (Chang et al., 2012; Singh Malik et al., 2016).

(3) *Cocoa butter* (*Theobroma cacao*) has a moisturizing, calming effect, stimulates the production of collagen, which leads to the fading of wrinkles, as well as their prevention and helps to treat and fade scars (Rodriguez-Merchan, 2018; Singh Malik et al., 2016).

(4) *Shea butter* (*Buthyrospermum parkii*) is anti-inflammatory, antibacterial, skin regenerating,

epithelializing, facilitates healing and soothes irritations, protects the skin from external factors, has a nourishing role and maintains the optimal level of skin hydration (Chang et al., 2012).

(5) *Vitamin E* (α-tocopherol) has an antioxidant effect, leading to a high stability of topical products. It is included in many cosmetics products, because it increases the hydration and elasticity of the skin (Chang et al., 2012; Singh Malik et al., 2016).

(6) *Burdock leaf extract* (*Arctium lappa*, family Asteraceae) has a high content of scaffeol-quinic acids, derivatives of quercetin and luteolin, and bitter sesquiterpene principles with germacranic structure (arctiopicrin); the natural complex provides antioxidant, antibacterial and anti-inflammatory properties. It is also strongly emollient, regenerating and moisturizing (Chan et al., 2011; Pirvu et al., 2017).

(7) *Magnolia petals extract* (*Magnolia virginiana*, family Magnoliaceae) contains a series of chemical compounds with a complex structure, including lignans, neolignans, terpenoids, alkaloids, polyphenolic acids (syringic acid), the most studied being neolignans, such as magnolol, honokiol and obovatol, with anti-inflammatory and antinociceptive effect (Ding et al., 2018).

(8) *Linden inflorescence extract* (*Tiliae flos*, family Malvaceae) presents polyphenols and flavonoids derived from cvercetol and kaempferol, gallic and catechin tannins, small amounts of fraxoside and esculoside, a non-hemolytic saponin, called tiliadin. Due to the hydroxyl groups presence in the structures of the compounds that neutralize reactive oxygen species, involved in inflammatory oxidative processes, linden inflorescence extract has antioxidant properties (Barreiro Arcos et al., 2006).

(9) *Grapeseed oil* (*Vitis vinifera*, family Vitaceae), *sweet almond oil (Prunus amygdalus dulcis*, family Rosaceae) and *cinnamon oil* (*Cinnamomum zeylanicum*, family Lauraceae) have antioxidant, invigorating, tonic, anti-inflammatory and moisturizing effect on the skin, maintaining the elasticity of epithelial cells in optimal parameters (Ahuja et al., 2021; Panda & Ghosh, 2010).

Also, the obtained herbal cream formulations were evaluated for organoleptic parameters, pH, microbial contamination level, antimicrobial activity, and for plant extracts was assessed the cytotoxicity effect.

Organoleptic characterization. The herbal creams (F1 and F2) showed similar organoleptic characteristics (Table 2). Generally, the results obtained for all characteristics were acceptable as all have light yellow consistent color, agreeable odour, highly homogenous and an excellent semi-solid texture. The odour was aromatic plant for both formulations.

Tabel 2. Organoleptic and physical characteristics of herbal cream formulations F1 and F2

No.	Characteristics	Formulation code	
		F1	F2
1	Texture	+++	+++
2	Homogeneity	+++	+++
3	Colour	Light yellow	Light yellow
4	Odour	Aromatic plant, pleasant	Aromatic plant, pleasant
5	*p*H	5.2 ± 0.2	5.4 ± 0.1
6	Stability	Stable	Stable

pH evaluation. F1 and F2 formulations had pH values ranged between 5.0 and 5.5 as displayed in Table 2. Such values of pH would guarantee physiological compatibility with human skin since pH of the human skin is ranged between 4.0 and 7.0 and underneath or over this range will unfavourably influence the human skin.

Stability study. Both herbal cream formulations (F1 and F2) showed stability at a temperature of 40^0C and 75% relative humidity for three months. It was not observed any differences in texture, colour, odour, or pH.

Microbiological evaluation. All herbal cream formulations exhibited Total Aerobic Bacteria Count < 10 CFU/g, Total Number of Yeasts and Fungi < 10 CFU/g (Table 3), and showed moderate *in vitro* antibacterial activity against *Staphylococcus aureus* (mean diameter of 18.44 ± 1.43 mm for F1 and 18.05 ± 1.09 mm for F2; Figure 1). The obtained results are in accordance to the Ph.Eur. requirements and indicated the good hygienic preparation, good microbial limit of raw materials and efficient preservation.

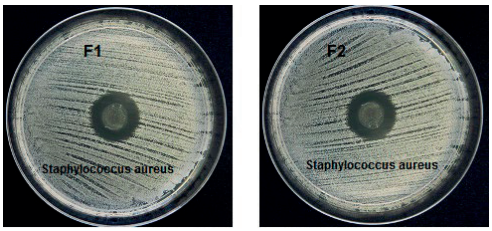

Figure 1. *In vitro* antibacterial activity against *Staphylococcus aureus*

Tabel 3. Microbiological contamination level and *in vitro* antibacterial activity of herbal cream formulations F1 and F2

Formulation Code	Characteristics	Results (CFU/g)
F1	Total Aerobic Bacteria Count	< 10
	Total number of yeasts and fungi	< 10
F2	Total Aerobic Bacteria Count	< 10
	Total number of yeasts and fungi	< 10

Evaluation of cytotoxicity effect of plant extracts. Following the experiments performed on the L-929 murine fibroblast line, given the dose levels used, the plant extracts (*Tiliae flos* and *Arctium lappa*) are practically free of cytotoxicity. Cytotoxic effects were observed only in the case of the plant extract of *Magnolia virginiana*, at concentrations higher than 25 µg ± 0.20 GAE/mL.

The IC_{50} value for *Magnolia virginiana* plant extract of 61.26 ± 0.30 µg GAE/mL, a difficult dose to achieve *in vivo*, indicates that therapeutic doses can be used safely.

The results lead to the conclusion that all the extracts tested are either non-toxic or have a very low cytotoxicity and can be used safely as ingredients for dermatocosmetics (Figures 2-4). The results are in accordance to the literature (Barreiro Arcos et al., 2006; Chan et al., 2011; Ifeoma & Oluwakanyinsola, 2013; Ding et al., 2018).

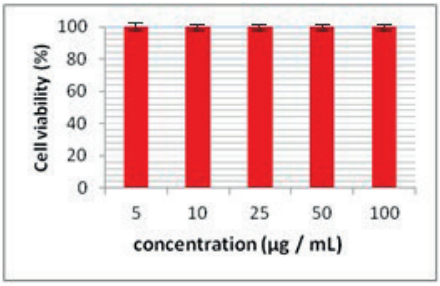

Figure 2. Effect of *Tiliae flos* extract on L-929 fibroblasts

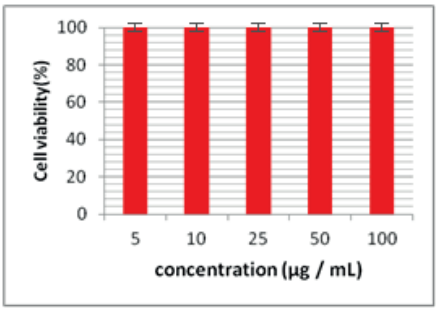

Figure 3. Effect of *Arctium lappa* extract on L-929 fibroblasts

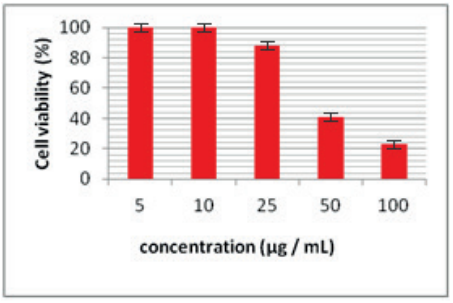

Figure 4. Effect of *Magnolia virginiana* extract on L-929 fibroblasts

CONCLUSIONS

Out of the numerous topical drug delivery systems, herbal cream formulations have shown favourable properties over other semisolid dosage forms, such as easily spreadable, greaseless, easily removable, non-staining, bio-friendly and have long shelf life. In this study two formulations were obtained containing several plant extracts (*Arctium lappa, Magnolia virginiana, Tiliae flos*) in different combinations, while the composition of the cream base was kept the same. The herbal cream formulations were homogeneous, non-irritant and easily removable. The pH of creams was in the range of 5.0–5.5 which is safe for human skin. The samples were found to be populated with aerobic bacteria, yeast and fungi up to 10 CFU/g and showed moderate antibacterial activity.

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