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RESEARCH ON THE POTENTIAL COSMETIC APPLICATION OF A POLY-HERBAL PREPARATION

Alice GRIGORE[1], Svetlana COLCERU-MIHUL[2], Corina BUBUEANU[1], Lucia PIRVU[1], Roxana MATEI[1], Ionica BAJENARU[1], Ileana Paraschiv[1], Cornelia NICHITA[2]

[1]National Institute of Chemical-Pharmaceutical Research and Development, 112 Vitan St., Bucharest, Romania
[2]SC AGNES ITARA SRL, 13 Veronica Micle, Suceava, Romania

Corresponding author email: alicearmatu@yahoo.com

Abstract

Plants are widely used for the development of new products for cosmetics and pharmaceutical applications. In recent years, it is increasingly recognized that the active properties of plant extracts, which are usually complex mixtures of many components, are not due to the activity of a single component, but is the result of the synergistic effects of the elements of the mixture. The aim of this study was to demonstrate the feasibility of a poly-herbal formulation based on Daucus carota L. and Prunus armeniaca L. to be included in cosmetics as an active ingredient. Three types of extracts were taken into consideration: hydroalcoholic, hydroglycerin and macerate in grapeseed oil. HPLC analysis revealed that the hydroalcoholic extract contains polyphenolcarboxylic acids rather than flavonoids. As regards fatty acids quantified by GC, slight differences between sample and control were recorded. The potential effect of the three extracts on murine fibroblasts was evaluated and the proliferation rate was assesed by MTS test. It was showed that none of the extracts are not exhibiting cytotoxic effects. Except for the maximum concentration tested of the hydroglycerin extract, at which the cell viability was around 50%, most of the samples exceeded the 80% threshold. In the case of the hydroglicerin extract, it was observed a strong stimulating effect on cell proliferation compared to control group. It can be concluded that this poly-herbal combination could be used successfully as cosmetic ingredient for its wound healing and regenerative properties.

Key words*: Daucus carota L., flavonoids, polyphenolcarboxylic acids, Prunus armeniaca L.*

INTRODUCTION

Global market for skin-care products is characterized by constant growing in the period 2015 to 2021 with the highest share for the anti-aging segment (Research and Markets, 2015). Herbal preparations are a modern trend in the field of beauty but also in the pharmaceutical one. These agents are gaining popularity because these products provide the body with nutrients and enhance health, while also providing satisfaction, as they do not contain synthetic chemicals and have fewer side effects, compared to synthetic cosmetics. In recent years, it is increasingly recognized that the active properties of plant extracts, which are usually complex mixtures of many components, are not due to the activity of a single component, but is the result of the synergistic effects of the elements of the mixture. Currently, an important factor that determines the growth of the cosmetics market is innovation along the chain, which results in new products with diverse and innovative formulations and added functionality, produced in a sustainable way and in accordance with consumer expectations (Boeriu, 2015).

The plant metabolites with potential cosmetic applications include phenolics, polyphenols, flavonoids, terpenoids, steroids, steroidal saponins, sterols, sugars, polysaccharides, lignans, carotenoids, organic acids, anthocyanins and coumarins. A number of plant sources have been explored by the cosmetics industry to create innovative combination of ingredients with specific pharmacological actions such as antiallergy, antiinflammatory, moisturizing, procollagen, anticarcinogenic, antiageing, antihyperpigmentation and UV protective (Dorni et al., 2017).

The genus *Daucus*, belonging to the family Apiaceae (Umbelliferae), comprises about 60 annual and biennial species mostly distributed in north temperate regions of the world (Fu et al., 2010) and widely used în cosmetics for its astringent, vulnerary, antiinflammatory, maturative, analgesic, depurative, antiaging, tissue regenerative, beautifying properties

(Gilca et al., 2018). Wound-healing property of ethanolic extract of *Daucus carota* L. (carrot) root may be attributed to the various phytoconstituents like flavonoids and phenolic derivatives present in the root and the quicker process of wound healing could be a function of either its antioxidant or antimicrobial potential (Patil et al., 2012). High quality pectin also contributes to its soothing properties (Toma et al., 2019). In a recent study, among the species identified to have the highest dermatologic importance, *Daucus carota* L. had an index of 72.28 on a scale of 100, after *Brassica oleracea* L. (100), *Matricaria chamomilla* L. (79.17), *Arctium lappa* L. (74.82) (Gilca et al., 2018).

Apricot kernel oil (*Prunus armeniaca* Blanco), has been included in cosmetic preparations as moisturiser or emollient agent (Lube and Verpoorte, 2011). In traditional oriental medicine, apricot (*Prunus armeniaca* L.) seed has been used to treat skin diseases such as furuncle, acne vulgaris and dandruff due to its antimicrobial, antioxidant and antiinflammatory properties (Lee et al., 2014).

Fatty acid composition of the oils from the kernel samples of 42 wild apricot (*P. armeniaca*) genotypes collected from Kullu, Keylong, and Udaipur region of Himachal Pradesh and Nubra, Leh and Kargil region of Jammu and Kashmir (India) was determined and the principal fatty acid emerged as oleic acid (52.41-80.76%) and linoleic acid (12.19-39.79%) (Mandal et al., 2007).

The present study aimed to demonstrate the feasibility of a poly-herbal formulation based on *Daucus carota* L. and *Prunus armeniaca* L. to be included in cosmetics as an active ingredient. In this respect, three types of extracts were taken into consideration: hydroalcoholic, hydroglycerin and macerate in grapeseed oil and their rejuvenating potential was evaluated.

MATERIALS AND METHODS

Plant material

Sliced dried carrots and apricot kernel powder were purchased from a local market.

Chemicals

All reference compounds were purchased from Sigma Aldrich-Fluka. All other chemicals were analytical grade reagents.

Preparation of extracts

Hydroalcoholic extract was prepared by mixing 1 part vegetal material (equal parts of both herbs) with 10 parts of 50% hydroalcoholic solution (vegetal material/solvent ratio = 1/10 m/v) and further ultrasonicated for 30 minutes at 70°C. The resulted solution was filtrated and concentrated under reduced pressure to dryness. The residue was dissolved in ethylic alcohol 50% (v/v) to 0.25 g/ ml concentration.

Hydroglycerin extract was prepared by soaking 1 part vegetal material (equal parts of both herbs) with 10 parts of 70% glycerin (vegetal material/solvent ratio = 1/10 m/v) for 10 days at room temperature, in darkness, with occasional stirring, followed by filtration.

For the third type of extract, 1 part vegetal material (equal parts of both herbs) was soaked in 10 parts grapeseed oil and macerated for 10 days at room temperature, in darkness, with occasional stirring, followed by filtration.

Determination pf total phenolics

Total phenolics content of all three extracts was determined according to the Folin - Ciocalteu method adapted for microtitration format (Tatzber et al., 2020). The total phenolic content was expressed as gallic acid by reference to the gallic acid standard calibration curve in mM. Samples were determined in duplicate.

GC analysis

GC analysis was performed for identification of fatty acids in the oily extract was carried out by using an Agilent 6890N gas chromatograph equipped with a FID detector, 7683B autosampler and a capillary column HP INNOWax (60 m x 0.32 mm; film thickness 0.25 μm). The injector and detector temperatures were kept at 250°C. Nitrogen was used as carrier gas, a flow rate of 1.5 ml/min. Total analysis time was 31 min. Identification of the main components was carried out by the comparison of the GC retention times against those of the reference standards. The fatty acids contents were expressed as weight percentages, % *w*/*w* (g fatty acids/100 g of sample).

HPLC analysis

A method for quantification of polyphenols was developed for the hydroalcoholic extract. A Merck - Lachrom system with DAD L2455

detector was used. Phytochemicals were separated on a Intersil ODS3 analytical column (4.6 mm x 250 mm). The chromatographic separation was carried out using a mobile phase with phosphoric acid: water 0.05% as solvent A (pH = 2.8) and methanol as solvent B at a flow rate of 1-1.5 ml/ min. The injection volume for all samples and standard solutions was 10 µl.
A gradient flow of mobile phase and a gradient of mobile phase composition were applied: A:B (70:30) for 50 min, changing to A:B (30:70) for 10 min, then A:B (70:30) for another 10 min. Before use, all mobile phases were filtered through a 0.20 µm membrane (CHROMAFIL® O-20/25) and dis-aerated in an ultrasonic bath.

Cell viability assay

Cell viability was examined using an MTS assay kit (CellTiter 96 AQueous One Solution Cell Proliferation Assay, Promega, WI, USA). Murine fibroblasts (L929, ATCC) were cultivated in complete media (EMEM, 10% horse serum, 1% antibiotic, ATCC) and seeded into 96-well plates (1×10^4 cells/well). After 24 hours, different doses of each type of extract were applied in triplicate. Cell viability was measured 24 h after incubation. The results of MTS assay were obtained by measuring absorbance using a microplate reader (LKB Chamaeleon) at 492 nm. All experiments were repeated three times.

Scratch assay with fibroblast cell line

The fibroblasts L929 (1×10^4 cells) were seeded in 24-well cell culture plate. Linear scratch was made in confluent cell monolayer using 200 ml pipette tip. Cell debris were washed out with plain medium. The sample tested was hydroglycerin extract 5% and it was compared to a non-treated group. All experiments were made in duplicate. Images of cellular gap were captured periodically (0, 8 and 24 h) on Nikon inverted microscope.

RESULTS AND DISCUSSIONS

Three types of extracts suitable for inclusion in cosmetic formulations - oily, hydroglycerin, hydroalcoholic extracts - were analyzed.

Determination of total phenolics

In the three samples tested, the polyphenol content ranged between 0 in the oily extract and 2.2 mM in the hydroalcoholic extract. Hydroglycerin extract showed 1.34 mM polyphenols content.

GC analysis

The results are showed in Table 1 represent the percentage composition in fatty acids of the oily extract - determined by the ratio of the separate components on the chromatogram. No changes were recorded for myristic and linolenic acids. The variation in the fatty acid profile of the oily extract was insignificant comparing to control (grapeseed oil). Compared to the extraction solvent (grape seed oil), we noticed a slight decrease in the linoleic and palmitic acids contents while stearic and oleic acids contents increase. Several studies showed that apricot seeds oil have high content of monounsaturated oleic acid which is favourable in human nutrition and also have significant beneficial effect on the appearance and function of the skin (Stryjecka et al., 2019).

Table 1. Fatty acids composition of the oily extract

Fatty acids	Composition (%)	
	Grape-seed oil	Oily extract of *D.carota* and *P. armeniaca*
C14:0 (myristic acid)	0.06	0.06
C16:0 (palmitic acid)	8.16	8.13
C18:0 (stearic acid)	3.74	3.78
C18:1c (oleic acid)	26.52	27.03
C18:2c (linoleic acid)	60.10	59.65
C18:3α (α-linolenic acid)	0.68	0.68

HPLC analysis

As reference substances we selected ubiquitous phenolic compounds (polyphenolcarboxylic acids - caffeic, chlorogenic, rosmarinic, ferulic and flavonoids - rutin, quercetin, kaempferol, luteolin). The analysis was conducted only for the hydroalcoholic extract due to high viscosity of the other two extracts.
The results (Table 2) showed the absence of flavonoids (both aglycones and glycosides) and a high concentration of chlorogenic acid in the hydroalcoholic extract. Previously, Sharma reported that chlorogenic acid was the main hydroxycinnamic acid identified in different carrot tissues, accounting for 42.2% to 61.8% of total phenolics (Sharma et al., 2012).

Table 2. Chemical composition of alcoholic extract 50% (flavonoids and polyphenolcarboxylic acids) determined by high performance liquid chromatography

(HPLC-DAD)

Compound	Concentration mg/ 100 ml
Chlorogenic acid	3.970
Caffeic acid	0.210
Rutin	-
Quercetin	-
Kaempferol	-
Rosmarinic acid	0.072
Ferulic acid	0.163
Luteolin	-

MTS assay

Fibroblasts are critical in supporting normal wound healing, involved in key processes such as breaking down the fibrin clot, creating new extra cellular matrix (ECM) and collagen structures to support the other cells associated with effective wound healing, as well as contracting the wound (Bainbridge, 2013).

All extracts (hydroglycerin, hydroalcoholic, oily) based on *Daucus carota* - *Prunus armeniaca* combination had a strong stimulating effect on cell proliferation, comparing to controls. Except for the maximum concentration of the hydroglycerin extract, at which the cell viability was around 50%, most of the extracts exceeded the 80% threshold (Figures 1-3).

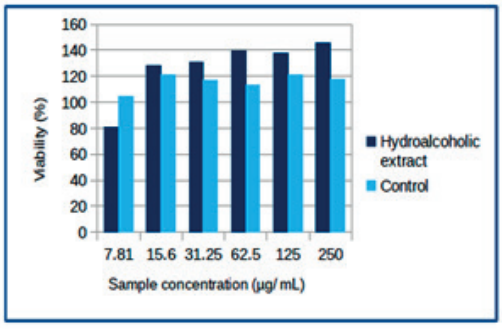

Figure 1. Viability of fibroblast cells after exposure to several concentrations of hydroalcoholic extract

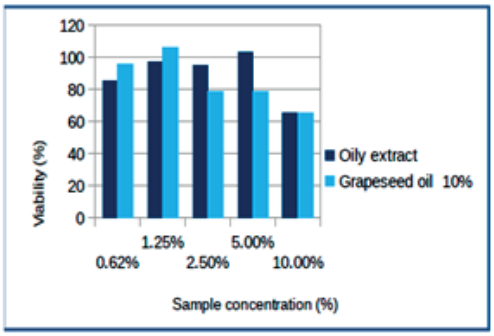

Figure 2. Viability of fibroblast cells after exposure to several concentrations of oily extract

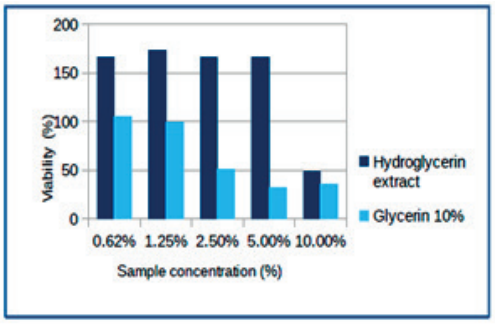

Figure 3. Viability of fibroblast cells after exposure to several concentrations of hydroglycerin extract

Scratch assay

In the present study, L929 (fibroblasts) cells were used in scratch assay. Using image analyzer, the time required to close the gap in the confluent cell monolayer in presence of 5% hydroglycerin extract was studied. The time taken to close the gap was compared with untreated cell culture.

The experimental results showed that formulation at 5% concentration closed the gap in the scratch of fibroblasts more efficiently as compared to control group.

Comparative cell migration at 0, 8 and 24 h in non-treated and formulation-treated is showed in Figure 4.

Figure 4. Scratch assay on L929 cell line

The hydroglycerin extract tested in this assay contains a high amount of polyhenols and it is widely known that plant phenolics play important roles in tissue repair mechanisms. As it was showed by Patil et al., wound-healing property of *Daucus carota* L. root extract may be attributed to the phenolic derivatives present in the root which accelerate the process of wound healing by antioxidant or antimicrobial effects (Patil et al., 2012).

CONCLUSIONS

The study proposed a poly-herbal formulation for cosmetic use based on *Daucus carota* L. and *Prunus armeniaca* L., two plants with long tradition of use in skin ailments.

All of the three extracts tested (hydroglycerin, oily and hydroalcoholic) had a strong stimulating effect on fibroblast cells proliferation, comparing to controls. Spectrofotometric and HPLC analysis showed high content of phenolic compounds in hydroglycerin and hydroalcoholic extracts that can contribute to pharmacological effects.

It can be concluded that preparations based on *Daucus carota* and *Prunus armeniaca* ingredients are suitable for inclusion in cosmetic preparations due to their antioxidant, anti-aging and wound healing properties.

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