Full Length Research Article

FORMULATION AND EVALUATION OF A MOISTURIZING DAY CREAM CONTAINING OLIVE LEAVES EXTRACT

*,1Al-Rimawi, F., 2Yateem, H. and 3Afaneh, I.

1Chemistry Department, Faculty of Science and Technology, Al-Quds University, P.O. Box 20002, Jerusalem, Palestine
2Raed Cosmetics Co., Aljanab St., Beit Sahour, P.O. Box 1395, Palestine
3Department of Food Technology, Faculty of Science and Technology, Al-Quds University, P.O. Box 20002, Jerusalem, Palestine

ARTICLE INFO	ABSTRACT
	The purpose of the current work is to investigate the incorporation of olive leaves extract standardized for oleuropein which have antioxidant, anti inflammatory, skin protectant and anti-aging properties into stable topical moisturizing day cream formulations. The physiochemical and rheological properties, and accelerated stability tests of three cream formulas containing different concentrations (0.1%, 0.4% and 1.0% w/w) of olive leaf extracts were assessed and compared with commercial cream product containing no olive leaf extract. A questionnaire was distributed to 100 volunteers to use the prepared creams containing olive leaf extracts and to compare with the commercial cream containing no olive leaves extract. All physical and rheological properties of the prepared formulations were found to be the same as the commercial cream product. Stability studies showed a stable homogenous appearance and effective cream during three months of storage at room temperature. Most of the volunteers are satisfied with the creams containing olive leaves extract, and have noticed significant differences between these creams and the cream containing no olive leaves extract.

INTRODUCTION

Human skin changes throughout the course of life due to physiological and external interactions. Either intrinsic or chronological ageing and extrinsic or photo ageing overlap during lifetime and both are more or less responsible for dysfunction of the skin's natural self-protection and repair. Areas of the body most exposed by the damaging effect of UV radiation, such as the face and the hands, are also the most visible in our social life. Increased lifespan and elevated life standards have increased the number of individuals with both need and desire for cosmetic amelioration (Vera *et al.*, 2012). Plant extracts are increasingly being used in cosmetic mostly because of the adverse effects of synthetic chemical products e.g. toxicity, allergy. etc. Plant extracts are widely used for many purposes in cosmetic applications such as moisturizing, whitening, tanning, color agent, sunscreen, radical-scavenging,

anti-oxidants, immune stimulants, washing, and preservatives. Polyphenols in olive leaves are of great interest to researchers as they have many activities e.g., antioxidant, antimicrobial, anti-inflammatory (Sawalha *et al.*, 2009, Somova *et al.*, 2003, Pooley *et al.*, 1997; Le Tutour *et al.*, 1992). Olive leaves are considered a cheap raw material and contains oleuropein in the range of 60-90 mg/g (dry weight) (Ansari *et al.*, 2011). Oleuropein is a natural product of secoiriodoid group; hetrosidic ester of elenolic deteracid and 3,4-dihydroxyphenel ethanol, containing a molecule of glucose, the hydrolysis of which yields elenolic acid glucoside and hydroxytyrosol. Many molecules isolated from Olea europea fruits or leaves are thought to have been originated from Oleuropein via aglycon, by opening of olenolic acid ring with a final rearrangement into the secoiridoid compound, such as hydroxytyrosol (Syed Haris 2010). The enormous interest in this natural polyphenolic compound (e.g.oleuropein) in recent years as additives or natural ingredient in food and cosmetics has gone beyond food preparation and conservation, into the new area, known as nutraceutics. This science studies the therapeutic effects of the components of foods and cosmetics.

*Corresponding author: Al-Rimawi, F.,
Chemistry Department, Faculty of Science and Technology, Al-Quds University, P.O. Box 20002, Jerusalem, Palestine

Oleuropein has high antioxidant activity in vitro, comparable to a hydrosoluble analog of tocopherols. Oleuropein scavenges superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and hydrochlorous acid-derived radicals (Visioli *et al.*, 2000). Recent studies showed that Oleuropein increases nitric oxide (NO) production in macrophages challenged with lipopolysaccharide through induction of the inducible form of the enzyme nitric oxide synthase, thus increasing the functional activity of these immune competent cells. It is well known that Oleuropein elicits anti-inflammatory effects by inhibiting lypoxygenase activity and the production of leukotriene B4 (Visioli *et al.*, 1998). Recent studies have shown that the phenol components of olive leave have a direct antioxidant action on skin, especially oleuropein, which acts as a free radical scavenger at the skin level (Ancora *et al.*, 2004).

Normal human fibroblasts undergo replicative senescence due to both genetic and environmental factors. The proteasome, a multicatalytic nonlysosomal protease, has impaired function during aging, while its increased expression delays senescence in human fibroblasts. Oleuropein enhances proteasome activities in vitro more effectively than other known chemical activators, possibly through conformational changes of the proteasome (Katsiki *et al.*, 2007). Importantly, Oleuropein-treated cultures exhibit a delay in the appearance of senescence morphology, and their life span is extended by approximately 15%. (Katsiki *et al.*, 2007). The extract of olive leaves are used in many cosmetics including antiaging creams and lotions. Some of the products that olive leaves extract are used in are shaving creams, muscle and joint balm, face masks along with anti-aging creams. Skin problems are common among people, and the development of new natural and safe moisturizing tropical cream preparation is the main objective of our study. The aim of the present study is therefore to formulate a natural, safe moisturizing day cream containing olive leaf extracts and to evaluate its physicochemical properties and stability.

MATERIALS AND METHODS

Chemicals

Glyceryl mono stearate was purchased from faci Italy. Stearyl alcohol, propylene glycol, mineral oil, isopropyle myristate, cetiol CC, propyl paraben, methyl paraben, imidazolidinyl urea and perfume were purchased from BASF, Germany. Oleuropein (40%) which used as a standard was obtained from Chengdu Biopurity Phytochemicals Ltd China. Chromatographic grade-double distilled water, HPLC grade acetonitrile (Merck), analytical grade acetic acid; and ethanol were obtained from Sigma – Aldrich company.

Olive leaf Samples collection

Olive leaves samples were obtained from trees localized in the sunshine area of Beit- Sahour /West Bank /Palestine. The collection was directly from the trees in the middle of November 2012.

Olive leaves extraction

Fresh olive leaves were dried at ambient temperature, and grinded to obtain olive powder which was stored at room temperature in dark until extraction. Ten grams of olive leaves powder were macerated in 100 mL of 80% ethanol at ambient temperature. The extracts were then filtered through a Whatman No.1 filter (Whatman, UK) to separate coarse particles from the solutions. The filtered extracts were then evaporated in rotary evaporator at room temperature under vacuum.

Preparation of the creams containing olive leaf extract

Base cream containing water and oil phases was prepared. The compositions and the amounts of the formulation ingredients are shown in table (1). For the preparation of the cream, all ingredients were added together according to the formula in table (1), then olive leaf extract was added in different concentrations (0.1%, 0.4%, 1.0% w/w).

Table 1. Amounts of the ingredients used to make 100 g of moisturizing day cream containing olive leaves extract

phase	Ingredients	Amounts in grams
Oil Phase	Glyceryl mono stearate	4.5
	Stearyl alcohol	1.50
	Steareth- 12	0.80
	Mineral Oil	5.00
	Isopropyl myristate	4.00
	Cetiol CC	1.00
	Silicon Oil	1.00
	Propyl paraben	0.10
Water Phase	Distilled water	Add to 100
	Carbomere	0.10
	Propylene glycol	3.00
	Methyl paraben	0.20
	Imidazolidinyl urea	0.20
	Triethanolamine	Qs
	Olive leaf extract	0.10 (Cream A), 0.40 (Cream B) and 1.0 (Cream C)
	perfume	Quantity sufficient

Evaluation of physical properties of the cream formulations

The prepared creams containing olive leaves extract and the commercial cream product were examined for their physical (pH, color, consistency and homogeneity) as well as rheological properties.

Determination of the pH: Determination of the pH of the creams was measured using pH meter (pH meter 211R hanna).

Homogeneity: Homogeneity of the creams was tested by visual observation and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good, and - = Poor.

Consistency: Consistency of the creams was evaluated as follows: The cone attached to holding rod was dropped from the fix distance to 10 cm such that it should be fall on the center of measuring cylinder travelled by cone was noted down after 10 sec.

Rheological properties: The cream preparations were evaluated for the following rheological characteristics:

Viscosity: A Brookfield viscometer was used to measure the viscosity (in mPa.s) of the creams. The spindle was rotated at 2.5 rpm. Samples of cream were allowed to settle over 30 min at the temperature of test ($25 \pm 1^{\circ}$C) before the measurement were taken.

Spreadability: Spreadability of the creams was performed by applying the cream on the skin and noticing whether spreading was good or not and was ranked as follows:

+++ = Excellent, ++ = Very Good, + = Good and - = Poor.

Accelerated Stability tests

Twelve sets of ten gram cream samples (0.1%, 0.4% and 1.0% w/w of olive leaves extract, and the commercial one without olive leaves extract) were stored at different temperatures (ambient temperature, 37 °C and 50 °C) for 3 months. After each month their stability was checked regarding oleuropein content, appearance, pH, color, homogeneity and viscosity.

Determination of Oleuropein in cream samples by HPLC

For determination of oleuropein in cream samples, reversed phase HPLC method was used with silca-based C_{18} bonded phase column (C_{18}, 250mm × 4.6 ID) with mobile phase consisting of a mixture of water and acetonitrile (80/20 volume ratio) containing 1% acetic acid at a flow rate of 1.0 mL/min. UV detector at 240 nm was used for oleuropein determination. The injection volume used is 20.0 µl for both standard and sample solutions. Identification of oleuropein in olive leaves extracts was based on retention times in comparison with standard of oleuropein. The quantitation was carried out using external standard method. The concentration of oleuropein in cream samples was calculated using peak area and the calibration curves obtained from oleuropein standard solution.

The questionnaire

The questionnaire consisted of two parts; personal data and product data. Personal data started from question number one to question number three. Product data started from question number four to question number six. Questions in personal data are about gender, age, and type of skin. While all product questions are about frequencies of the applications of the day cream, the sensorial evaluation of the cream (texture, color, odor, consistency, absorbance, after fed, fading and shinness), improvement in the skin upon use, allergic reaction to the cream, skin nourishment, and satisfaction with the cream.

RESULTS AND DISCUSSION

Creams are semisolid dosage forms intended mainly for external use and commonly consist of two immiscible phases; an oily internal phase and an aqueous external phase. Due to emulsified nature of skin surface, cream dosage form was chosen because cream interacts more effectively with skin and more readily penetrated through biological membranes. In this study, olive leaves extract were put in the cream dosage form to penetrate though membrane and keep it moist (moisturizing

day cream). A moisturizing day cream (o/w) emulsion was designed with different concentrations of crude olive leaf extracts. Each formula was given a letter which represented the concentration of the olive leaf extract as follows: Formula A contains 0.1 % crude olive leaf extract; formula B contains 0.4% crude olive leaf extract, and formula C contains 1.0% crude olive leaf extract.

Physical and rheological properties

As shown in table 2, physical properties (color, pH, homogeneity, and consistency) of the cream preparations containing olive leave extract were found to be equivalent to the commercial ones containing no olive leaves extracts. Consistency test showed no sticky and adhesion to skin and all the products are homogenous where no separation of the phases observed. Regarding the rheological properties (table 3), the spreadability of the prepared creams was excellent and the viscosities were within limit which indicated suitability of creams for applications on the skin.

Table 2. Physical properties of the three moisturizing day cream formulations containing olive leaves extract, and the commercial product

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)	Commercial product
Color	Creamy	Light brown	Brown	Creamy
pH	6.7	6.7	6.7	6.5
Homogeneity	+++	+++	+++	+++
Consistency (60sec)	5mm	5mm	5mm	5mm

Key: Homogeneity: +++ Excellent, ++ Very Good, + Good, - Un-satisfactory

Table 3. Rheological properties of the three moisturizing day creams containing olive leaves extract, and the commercial product

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)	Commercial product
Viscosity (mPa.s)	40.000	39.000	38.000	40.000
Spreadability	+++	+++	+++	+++

Key: Spreadability: +++ Excellent, ++ Very Good, + Good, - Un-satisfactory.

Stability studies

Accelerated stability tests (Tables 4 and 5) showed that pH of the creams was stable and suitable for skin application (pH cream A 6.3, pH cream B 6.1 and pH cream C 6.2). Viscosity was stable over the study period (40,000 mPa.s for all cream preparations). Color was stable where no significant change was observed of the color of creams at different concentrations. Stability studies (Table 4) showed a stable homogenous appearance during three months storage period and no separation phases occurred. The oleuropein contents (Table 5) decreased with increasing temperature and storage

Table 4. Effect of storage time on the physical properties of the three moisturizing day creams containing olive leaves extract after 3 months storage at room temperature

property	Commercial product	cream A (Freshly prepared)	cream B (Freshly prepared)	cream C (Freshly prepared)	cream A (3 months storage)	cream B (3 months storage)	cream C (3 months storage)
pH	5.9	6.7	6.7	6.7	6.3	6.1	6.2
color	Creamy	Creamy	Light Brown	Brown	Creamy	Light Brown	Brown
Homogeneity	+++	+++	+++	+++	+++	+++	+++
Viscosity (mPa.s)	40.000	40.000	40.000	40.000	40.000	40.000	40.000

Table 5. Oleuropein content determined by HPLC before and after accelerated stability tests

Parameter	Cream A (0.1%)	Cream B (0.4%)	Cream C (1.0%)
T1 at ambient temperature	95.2%	94.3%	95.7%
T1 at 37 °C	82.6%	84.7%	86.0%
T1 at 50 °C	72.5%	74.4%	76.0%
T3 at ambient temperature	94.5%	93.7%	94.2%
T3 at 37 °C	63.4%	66.5%	69.2%
T3 at 50 °C	54.1%	56.5%	59.5%

T1: first month, T3: third month.

time due to heat sensitivity of oleuropein. The results in table 5 show the degradation profile of oleuropein incorporated into cream and provide important data for the establishment of the shelf life of this product. One month stored at temperature 50°C equal to 8 months stored at room temperature and three months stored at temperature 37°C equal one year at room temperature. So the expiry date of the creams can be set to 1 year, and the consumer will still have a sufficiently active concentration for product efficacy even the concentration of the olive leaves extract is low (0.1 %).

Analysis of the Moisturizing day creams questionnaire

The study is concentrated on Raed cosmetics consumers from Bethlehem region in Palestine. Creams containing olive leaves extract (A, B, C), and the commercial cream product (without olive leaves extract) samples were given to each volunteer with different period of time; about 3 weeks between one sample and the other. This is to give the volunteers enough time to apply the cream sample. Principally the data were analyzed by SPSS version 16.0. In order to analyze all answers obtained from the questionnaires, all answers are summarized as descriptive statistic as follows:

Gender: the samples consisted of 100 volunteers, 100% were females.

Age: the samples consisted of 100 volunteers where 57% of them are between 31-45 years old, while 29% are between 18-30 years old, and only 14% are above 46 years old.

Type of skin: 39% of the volunteers have dry skin, while 33% have oily skin, and 28% have normal skin.

Sensorial evaluation of the creams: the volunteers have to give a mark from 1 to 10 to each evaluation where 1 is the lowest and 10 is the highest. Results showed that most of the consumers were satisfied with cream A, where about 46% gave a texture mark (8), 38% gave the color mark (9), 52% gave the consistency mark (8), 50% gave the absorbance mark (8), 44% gave Graceness mark (8), 48% gave the feeding mark (8) and 38% gave shinness mark (8). For the odor, 50% of the volunteers gave mark (8) and (9), and 50% gave mark (6) and (7) which implies that the consumers were satisfied with the odor of the cream. The same was applied for cream B, C, and D. For cream B, it was found that most of the consumers were satisfied with this cream, where 48% gave a texture mark (8), 50% gave the color mark (8), 52% gave the consistency also mark (8), 50% gave the absorbance mark (8), 47% gave Graceness mark (8) and 54% gave the feeding mark (8). For the odor, 52% of them gave mark (7), and 48% gave mark (8). Regarding the shininess of the cream, about 77% gave marks

between (6-9), which implies that most of the consumers have not felt their skin shinning which is considered as an advantage of the cream. Concerning cream C, 48% gave a texture mark (8), 27% gave the color mark (6), 26% gave the odor mark (7), 50% gave the consistency mark (8), 50% gave the absorbance mark (8), 47% gave Graceness mark (8) and 52% gave the feeding mark (8). Results showed also that about 87% of the volunteers have seen an improvement of their skin upon use of cream A,B, and C, and the majority (98-99%) of the volunteers have not experienced an allergic reaction to the these creams. Regarding skin nourishment upon use of the creams, 91% noticed skin nourishment upon use of cream A (from these 59% have noticed moisturized skin and 33% smooth skin). For cream B and C 90% noticed their skin nourishment (from these 53% have noticed moisturized skin and 37% smooth skin). However, for cream D which does not contain olive leaves extract, only 10% noticed their skin nourishment. In conclusion, about 91% are satisfied with cream A, B, and C. Additionally, most of volunteers noticed significant difference between the creams containing olive leaves extracts (A, B, C) and the one without olive leaves extract (D).

Relationship between type of skin and improvement of the skin

To see if there is a relationship between type of the skin and improvement of the skin, Chi-Square test was done for the data. It is found that 87% of the volunteers who have normal skin observed an improvement upon use, and 78% of the volunteers who have oily skin observed improvement upon use, and 90% of the volunteers who have dry skin have seen improvement upon use. Chi square results showed that there is no relationship between type of skin and improvement of the skin (Asy. sig. value is 0.397 which is larger than 0.05% at 95% confidence level).

Relationship between application of the cream and improvement of the skin

To check if there is a relationship between frequency of the cream application and improvement of the skin, chi square test was applied for the data. It is found that 92% of the volunteers who apply the cream once a day have seen improvement upon use, while 86% of the volunteers who apply the cream twice a day have seen improvement upon use, and 82% of the volunteers who apply the cream occasionally have seen improvement upon use. Results showed that there is a relationship between the application of cream and the improvement of the skin.

Conclusion

Olive leaf extract can be used as a moisturizing agent, antioxidant and anti aging for cosmetics products, such as anti aging cream or moisturizing day cream o/w emulsified non-ionic system, which is able to permeate the skin in small concentrations. Such cream preparations provide a satisfactory effect applied to the skin. Most of the volunteers have noticed significant difference between the creams containing olive leaves extract compared to the one without olive leaf extract. It was found a relationship between the application of cream and improvement of the skin.

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