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ORIGINAL ARTICLE

CHARACTERIZATION OF SEABUCKTHORN OIL EMULSION

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Summary

External application of seabuckthorn oil (*Hippophae rhamnoides* L.) is difficult due to its liquid state in spite of its benefits for damaged skin. In order to overcome this inadequacy the semisolid emulsion with seabuckthorn oil was prepared. Previous research showed that this emulsion possessing an enhanced structure with liquid crystals showed a higher wound healing potential than seabuckthorn oil. The aim of this investigation was to characterize suitability of this emulsion for topical use. The emulsion was prepared by combining emulsifiers that form liquid crystals. Two different quantities of seabuckthorn oil were incorporated. Samples were prepared with 10% and 40% of seabuckthorn oil. Organoleptic characteristics were estimated visually and by smearing samples on a thin glass plate. Type of emulsion was determined by a conductometric method, while a pH value of the emulsion was measured by a pH meter. Samples of seabuckthorn emulsion were orange, semisolid, shiny, easily spreadable on skin, and the smear on the glass plate was homogeneous. There was an absence of smell and the emulsion could be rinsed by water after the application on skin, which is a desired characteristic of oil/water emulsions. Results of an electrical conductivity confirmed that an outer phase is water. Samples possessed an acceptable pH value for an external topical use. This research confirmed that constituents and a method used were suitable for preparing semisolid emulsion with seabuckthorn oil. Organoleptic properties, a pH value and a type of obtained emuslion appear to be adequate for topical use.

Key words: Seabuckthorn oil; Characteristization; Emulsion; Topical use

INTRODUCTION

Information about *Hippophae rhamnoides* L. is scarce in the oldest botanical literature, because it was not described well until 1753, when Linne chose its name connecting it to the meaning of genus *Rhamnus* (thorny) [1]. It is a bushy tree (1.5 - 4 m), with big thorns on branches. It grows in Europe and Asia, up to 2000 m altitude in Europe, 4500 m in Tibet, while in Himalayas it reaches 5000 m. The biggest habitats are in the mountain chains in central Asia where it reached optimal ecological adaptation. Habitats in Asia are connected with Europe by two branches - there are northern habitats in Germany near the sea and the southern habitats near the Black sea. It can be found in Caucasus, in the Alps and in the Carpathians as well as in the delta of Danube. It gets cultivated, especially in Germany and Russia [2]. In Serbia it was found

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near the Danube close to Belgrade [3], which is confirmed by presence of several traditional names [4].

Fruits of seabuckthorn are researched for medical use, although many research papers indicate possible use of leaves [5 - 7]. Fruits are small, round and fleshy, tightly connected to the branch. A dominant colour is orange, but they come also yellow and rarely reddish [4]. A smell of the fruits is mild and aromatic. Fruits' pulp is watery and oily at the same time. There is one seed in the pulp ellipsoid, brown, smooth and shiny. The fruits are ripe in autumn (September-October), at that time containing the biggest quantity of vitamin C and fatty oil. Though it was used traditionally for a long time, this plant became popular in medicine only after papers on its medicinal properties started appearing at the end of the twentieth century [8 - 9]. Harvesting of the fruits is very difficult due to a presence of big thorns. It was not part of human diet for a long time, due to a sour and an astringent taste. A content of the fruit pulp and oil was characterized by Li and Schroeder 1996 [10], and it varies depending on a subtype of seabuckthorn and time of harvesting [11]. Fatty oil from the fruits was thoroughly researched for therapeutic uses due to a unique combination of constituents and a high content of vitamin E, carotenoids, phytosterols and unsaturated fatty acids [12].

Seabuckthorn fatty oil could be used externaly and internally [12]. Orally applied, it showed a positive effect in atopic dermatitis (double blind randomized placebo study) [13]. Exeternally applied, it enhances an epitalization of damaged skin - wounds, burns and radiation dermatitis [12, 14 - 16]. It is assumed that a mechanism of this action is regenerative and anti-inflammatory by a competitive inhibition of series of leukotriens from an arachidonic acid. The most recent research shows that a topical use of seabuckthorn in burns provoked a stimulation of a proliferation of fibroblasts, synthesis of collagen and an expression of a specific matrix metalloproteinase, and that it shows an angiogenic activity [15]. It was suggested that this effect was in a correlation to a specific high content of unsaturated omega-3 and omega-6 fatty acids, carotenoids and tocopherols in oil [14].

Seabuckthorn fatty oil is deeply coloured, orange, lipophilic liquid. Its topical application is unsuitable and connected to dripping, leaking, difficult dosing and absorption. These inadequacies could be improved by formulation of a topical dosage form - emulsion. A semisolid emulsion containing seabuckthorn oil was prepared. On an animal model this emulsion showed a wound healing potential which was better than the one of seabuckthorn oil [17]. The aim of this study was to characterize two samples of this emulsion containing 10% and 40% of seabuckthorn oil, so that suitability for a human use could be estimated.

MATERIALS AND METHODS

In order to prepare semisolid emulsion with seabuckthorn oil we used substances listed in Table 1:

Table 1. Substances used for preparing semisolid emulsion with seabuckthorn oil.

Substance/ INCI	*Brand name/Producer*
Hexadecyl (cetyl) alcohol	Lanette 16®, Cognis, Nemačka
Octadecyl (stearyl) alcohol	Lanette 18®, Cognis, Nemačka
Polyoxyethylene (21)stearyl ether (steareth-21)	Brij® 721P, Croda, SAD
Polyoxyethylene(2)stearyl ether, (steareth-2)	Brij® 72, Croda, SAD
Polyoxypropylene(15)stearyl ether	Arlamol E®, Croda, SAD
Methyl hydroxybenzoate	Nipagin®, Nipa, SAD
Aqua purificata	Zorkapharma, Serbia
Propylenglycol	Zorkapharma, Serbia
Sea buckthorn oil	*ex tempore*

All ingredients were separated based on a lipophilic and a hydrophilic affinity in an inner/oily and an outer/watery phase. The outer phase consisted of purified water, propylenglycol and a preservative (methyl hydroxybenzoate), while the inner oily phase was represented by a mixture of seabuckthorn oil, cetyl and stearyl alcohol, emulsificans polyoxyethylene(21)stearyl ether, polyoxy-ethylene(2)stearyl ether and polyoksipro-pylenglykol(15)stearyl ether. Two different samples were prepared containing 10% and 40% of seabuckthorn oil. The emulsifying method was in accordance with general rules for emulsification [17, 18]. Standard laboratory equipment was used – a digital balance (Chyo, MP-3000, Japan), a water bath (Sutjeska, Belgrade, Republic of Serbia) and a laboratory mixer (Velp, EU).

Organoleptic properties were evaluated visually - colour, smell, brightness, consistency and homogeneity. Homogeneity was also tested by smearing it on a glass plate which allowed observing trapped air or other inhomogeneous parts. Tactile characteristics of the emulsion (absorption, stickiness, softness, occlusive film on skin) were evaluated after an application on skin. These characteristics were again followed 24h, 30, 90 and 365 days after the preparation [19, 20].

Two-phase emulsion comes as oil in water and water in oil type. A conductometric method is the most often used method for distinguishing which type of emulsion was formed. Oil in water emulsion has a specific conductivity of less than 1 μS/cm because the outer phase is oil and this acts as isolation. Emulsion having a specific conductivity of more than 50 μS/cm has water as the outer phase which conducts electricity. In order to determine a type of emulsion we used a conductometric method of a direct immersing of conductometric electrodes in samples [18]. Measurements were performed in room temperature (20 ± 2°C), 48h and 30 days after the preparation again in room temperature, by a conductometer Dist, Hanna Hi 98311, SAD. The results are average values of three measurements.

pH values of samples were measured 48h and 90 days after the preparation in room temperature (20 ± 2°C) and in different storage temperature (5°C and 45°C) after 90 days. We used a pH meter, Hanna Instruments H8417, SAD [18]. The pH meter was calibrated before measuring by standard buffers (pH = 7.0 and pH = 4.0). The results obtained are average values of three measurements.

The study was approved by the Ethics Committee of Medical Faculty, University of Kragujevac. Statistical descriptive and analytical methods were used.

RESULTS

Both samples of emulsion containing 10% and 40% of seabuckthorn oil were semisolid, easily spreadable, with shiny surface and of orange colour. The sample containing 40% of seabuckthorn had more intensive orange colour. Homogeneity of samples was noted in both types and there was no pronounced smell present. Both samples showed to be easily spreadable on skin, not sticky and forming a mild occlusive film on skin after the application. The sample with 40% of oil formed a more occlusive film. Both samples could be washed out from the skin. Organoleptic characteristics remained unchanged 30 and 90 days after the preparation. The only feature that had changed during 365 days was colour. It has diminished after 365 days of storage in room temperature while other organoleptic properties remained the same. The change of colour intensity was more evident in the sample with 40% of seabuckthorn oil.

Electrical conductivity of both samples was the following (Table 2):

Table 2. Results of electrical conductivity of emulsion with seabuckthorn oil.

	Electrical conductivity μS/cm (t = 20±2°C)	
Emulsion sample	10% of seabuckthorn oil	40% of seabuckthorn oil
After 48 h	310.5	332.3
After 30 days	300.5	284.2

pH values of both samples of emulsion with seabuckthorn oil measured after 48h, 90 days and after 12 months in room temperature as well as after 90 days in different storage temperature (5°C and 45°C) are given in Table 3.

Table 3. pH values as a function of time and temperature of storage.

	pH value				
Emulsion sample	after 48 h (20±2°C)	after 12 months (20±2°C)	after 90 days (20±2°C)	after 90 days (5°C)	after 90 days (45°C)
10% of oil	4.44	5.19	5.11	5.13	5.33
40% of oil	4.65	4.95	5.2	5.2	5.25

DISCUSSION

Lately there has been an intensified search for non-toxic topical formulations containing natural substances which stimulate regeneration of damaged skin [21]. A careful selection of substances is needed in order to formulate topical emulsion [22]. Previous research on seabuckthorn oil showed anti-inflammatory and epitelization stimulating properties [14, 15]. Its oily nature makes it ideal for incorporation in a semisolid topical formulation. Pharmacological research of this formulation showed its wound healing potential in an animal model, implying that seabuckthorn oil in this emulsion preserved its epithelization stimulating properties, while the enhanced structure of emulsion synergistically contributes to healing properties [17]. Studying organoleptic characteristics and pH properties of this emulsion helps in determining its stability and suitability for a human topical use.

A consistency of emulsion varies from liquid (lotion) to a semisolid cream [22]. An external application of semisolid emulsion requires good organoleptic features (colour, smell, shiny surface, homogeneity), which should be stable during an application and storage time. It is desired that medical emulsion possesses a good tactile characteristic besides its medical effect on skin [23]. Our study showed that the proposed formulation in both its varieties (samples containing 10% and 40% of seabuckthorn oil) possesses suitable organoleptic and tactile properties for an external application. The samples were naturally colored which eliminates the need for using synthetic colors, often unwanted for damaged skin. The consistency was in accordance with a desired type of oil in water emulsion. An absence of smell makes this formulation suitable for medical use. The homogeneity test showed that the emulsion is homogenous throughout its consistency and that there is an absence of trapped air. The diminished colour of both samples after 12 months, but more prominent at the one containing 40% of seabuckthorn oil, indicates the need of quantification of carotens throughout time and a possible use of some antioxidants.

The conductometric measurements higher than 50 μS/cm confirmed that it was formed as oil in water emulsion. An oil in water type of emulsion has advantages in easier application, is more comfortable for a patient and well washable from skin due to a hydration of *stratum corneum* without forming a sticky occlusive film [24]. The choice of emulsifiers that could form oil in water emulsion and at the same time could form a phase of liquid crystals [17] was led by the fact that such emulsions are more stable and possess enhanced hydrating properties on skin [25, 26].

An external application of topical formulations could change a natural pH value of skin, therefore weakening a major skin function as a barrier. Literature suggests that a pH value of a formulation that stays longer on skin should range between 3.5 - 8.0 [28]. Since pH values of both samples depending on conditions of storage were 4.440 - 5.33, we could conclude the components and methods were appropriate and the emulsion could be applied on skin.

The proposed formulation overcomes disadvantages of a liquid application of seabuckthorn oil. Based on these findings, we characterize semisolid emulsion with seabuckthorn oil as suitable for a topical use.

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