RESEARCH ARTICLE

The Beneficial Effect of Cannabis Sativa Seed Oil on the Epidermis

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Abstract: Background: Cannabis Sativa seed oil has become more and more popular in cosmetic industry mainly due to the high content of antioxidants and unsaturated fatty acids that are desirable in formulations because they prevent moisture loss and reduce the occurrence of dry skin.

Objective: The aim of this study was to determine the effect of Cannabis Sativa seed oil on skin parameters such as hydration and transepidermal water loss.

Methods: The in vivo tests on volunteers with combination skin were performed by using corneometer and tewameter.

Results: The obtained results proved that Cannabis Sativa seed oil improved skin condition. The transepidermal water loss decreased because the lipophilic components of the oil formulation tend to form an occlusive layer on the epidermis surface. The highest increase in skin hydration was observed after one week of treatment.

Conclusion: These results confirmed that Cannabis Sativa seed oil has strong moisturizing properties and can be recommended as a natural-based skin conditioning agent.

Keywords: Hemp seed oil, true hemp, skin parameters, skin hydration, TEWL, polyunsaturated fatty acids.

1. INTRODUCTION

Recently, it has been observed a growing awareness of healthy lifestyle that led to an increase in the importance of natural products. Living in harmony with nature is a philosophy that applies not only to nutrition but also to cosmetic formulations. Costumers are more and more willing to buy ecological products based on natural ingredients. Therefore, both small producers and also large companies extended their offer with organic cosmetics. Very often, various types of oils derived from plants are used in skin care. They have a broad spectrum of action and improve skin condition providing sufficient moisture. The effect of plant oils on skin has been widely described in literature. Agero and Verallo-Rowell determined that coconut oil improve skin hydration and no significant changes in TEWL values were observed after its topical application [1]. Similar results have been obtained when peanut oil has been tested [2]. Sunflower seed oil has also shown moisturizing effect on the skin of adult volunteers [3]. Almond oil and jojoba oil meaningly increased hydration when it was tested on patients with xerosis [4].

Another study has demonstrated that daily usage of argan oil also improved skin hydration by preserving the water holding capacity and repairing its barrier function [5]. On the other hand, it has been shown that topically applied olive oil exhibited unfavorable effects on both skin barrier function and stratum corneum integrity [6]. The TEWL values increased when it was tested on patients with and without atopic dermatitis and also when the experiments were performed on mice [7]. These results proved that plant oils depending on their compositions, may exhibit various effects on skin. In recent years Cannabis Sativa seed oil has become more and more popular in the cosmetic industry. Cannabis Sativa belongs to the mulberry family and is wind-pollinated annual plant. It is a precious source of food, fiber, medicine and oil [8]. Its roots are 2 meters long and can dig into the ground to a depth of 115 cm. The plants reach a height of 4 to 7 meters. Cannabis Sativa has large and palm edged leaves. On their surface it secretes resin that contains cannabinoids. Selected cannabinoids that belong to the group of tetrahydrocannabinol (THC) are psychoactive and have an intoxicating effect [9, 10]. Cannabinoids are psychoactive components of Cannabis Indica L. such as marijuana or cannabis resin (hashish). After their chronic use, tolerance and psychological dependence develop, which neuronal mechanisms is still not fully defined [11]. In ancient times Cannabis Sativa was considered as “the elixir of immortality”, giving long and healthy life. The non-drug types of Cannabis Sativa L. also known as hemp has been used as a source of nutrition and food in China for many years [12, 13]. It was also applied in traditional Chinese medicine in cardiovascular, gastrointestinal, and dermato-
logical diseases [14, 15]. Cannabis Sativa seed oils were used to overcome vomiting, menstrual pains and reduce ulcers, burns and hair loss. They have an incredibly high content of nutrients and antioxidants, which is especially important nowadays in cosmetic products. It was proved that hemp seed extracts exhibit anti-ageing and antioxidant effects [16]. The greatest concentration of beneficial chemicals such as unsaturated fatty acids and antioxidants (such as lignanamides) can be found in the seeds [17-19]. The presence of antioxidants in seeds is very important because they prevent oxidation of sensitive fats [20]. Moreover, the shell which surrounded the seed provides an additional protection from oxidation and light. The growing interests of cosmetic industry in Cannabis Sativa is mainly due to the high content of unsaturated fatty acids that are desirable in formulations because they prevent moisture loss and reduce the occurrence of dry skin [21]. Table 1 presents the profile of fatty acids in Cannabis Sativa seed oil. Cannabis Sativa seed oil is rich in essential fatty acids such as linoleic acid (C18:2), α- and γ-linolenic acid, which constitute over 80% of the total fatty acids [22, 23].

Table 1. Profile of fatty acids in Cannabis Sativa seed oil [24, 25].

<table>
<thead>
<tr>
<th>Fatty Acids</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linoleic C18:2</td>
<td>50-70</td>
</tr>
<tr>
<td>α-linolenic C18:3</td>
<td>15-25</td>
</tr>
<tr>
<td>Oleic C18:1</td>
<td>10-16</td>
</tr>
<tr>
<td>Palmitic C16:0</td>
<td>6-9</td>
</tr>
<tr>
<td>Stearic C18:0</td>
<td>2-3</td>
</tr>
<tr>
<td>γ-linolenic C18:3</td>
<td>1-6</td>
</tr>
<tr>
<td>Arachidic C20:0</td>
<td>0.79-0.81</td>
</tr>
</tbody>
</table>

First double bond in α-linolenic acid is positioned at the third carbon atom from CH2 end of the chain, therefore it is known as polyunsaturated n-3 or omega-3 fatty acid. While in linoleic acid and γ-linolenic acid the first double bond is located at the sixth position from the n end and therefore they are called omega-6 fatty acids. These compounds have a positive effect on dry and rough skin. It should be highlighted that the amount of the omega-3 and omega-6 fatty acids in Cannabis Sativa seed oil is very high compared to the seed oil of other plant species. Deficiency of linoleic and α-linolenic acid is associated with symptoms of dry skin. Polyunsaturated fatty acids are very desirable in skincare because they are the structural components of phospholipids in the cell membrane [26]. Therefore, they have an impact on its functions such as: fluidity, electrolyte transport and hormone activity. The seed oil has a perfectly balanced ratio (3:1) between linoleic and linolenic acids because of this, it is an appropriate component for light body oils and lipid-enriched formulations [27]. Apart from the unsaturated fatty acids, it contains a number of other fatty acids, including palmitic acid, which creates a microscopic film on the surface of the skin, that prevents water loss. It comprises stearic acid, which moisturizes and softens the epidermis. Cannabis Sativa is usually grown without using plant protection products, as the cannabinoids provide protection against harmful pest insects. Therefore, Cannabis Sativa seed oil is a perfect ingredient for natural cosmetics [28, 29]. In addition to polyunsaturated fatty acids, it also contains other valuable compounds, including phospholipids, sitosterols, tocopherols, carotenoids, terpenes, and methyl salicylate. Their presence supports the beneficial effects of essential unsaturated fatty acids. Due to sitosterols, especially β-sitosterol, Cannabis Sativa exhibits antimicrobial and anti-inflammatory properties [30]. Apart from the high content of fatty acids and antioxidants, it contains also vitamins (vitamin E, B, A) and microelements (copper, zinc, manganese) that affect the condition of the skin. An important ingredient of Cannabis Sativa seed oil is cannabidiol. It does not cause narcotic intoxication and is widely used in medicine and cosmetology. It exhibits anti-inflammatory, antibacterial and antifungal properties. It slows down the growth of microorganisms and alleviates allergy symptoms. Additionally, it also has an antioxidant property, thanks to which it delays the aging process of cells and protects against free radical reactions. The aim of this study was to determine the effect of Cannabis Sativa seed oil on skin parameters such as hydration and transepidermal water loss (TEWL). The in vivo tests on volunteers were performed by using non-invasive methods.

2. MATERIALS AND METHODS

The study enrolled 20 females in the age range of 20-40 years. It was conducted with bioethics (Poznań University of Medical Sciences) and regulatory approvals, and in compliance with the principles of the Declaration of Helsinki and in accordance with Good Clinical Practice (GCP). The informed consent forms were obtained from all participants of this study. Volunteers were qualified on the basis of a detailed questionnaire and preliminary tests. All women had combination skin, with no signs of inflammation. They did not take any drugs and did not apply topical formulations for a period of 7 days before starting the assay. The reliability of the analyzes was increased by the proper selection of the tested group. Therefore, the volunteers with intolerance to the active substances present in the product and the coexistence of diseases that may affect the course of the investigation (data from the interview of the test subjects) were excluded. In addition, based on initial tests, women whose skin was in a bad or critical condition and exhibited the signs of inflammation were disqualified from participation in the study. The formulation was subjected to physicochemical, microbiological and dermatological tests as well as stability tests before measurements were taken. Pseudomonas aeruginosa, Staphylococcus aureus and Candida albicans were not detected in 0.1 g of Cannabis Sativa seed oil. The total number of mesophilic aerobic microorganisms was below 500 CFU/ml, therefore the product was qualified as class I cosmetics.

The experiments were carried out for 5 weeks during which the volunteers applied on the forearm the Cannabis Sativa seed oil (CAS 89958-21-4, Hemp Oil/The Kerfoot Group) obtained from Latech Bernard Latanowicz company (Poland).
The measurements were carried out using a Tewameter® TM 300 (to obtain TEWL values) and Corneometer® CM 825 (Courage & Khazaka, Cologne, Germany) that determined the level of skin hydration. In order to assess the safety of the formulation the systematic exposure dose was calculated based on the following equation:

$$SED = \left( A \text{ (g/day)} \times 1000 \text{ mg/g} \times C(\%)/100 \times \text{Dap (\%)/100} \right)/60 \text{ kg}$$

where

- $C$ - the concentration (%) of ingredient in finished product,
- $\text{Dap}$ – dermal absorption expressed as % of the test dose,
- $A$ – daily dose of a cosmetic product.

The systemic exposure dose of a cosmetic ingredient is the amount expected to enter the bloodstream per kg body weight and per day.

### 3. RESULTS AND DISCUSSION

The *Cannabis Sativa* seed oil was subjected to physicochemical studies. The results are presented in Table 2. The data obtained proved that cosmetic was stable under different storage conditions. The product was assessed as microbiologically safe. The *Cannabis Sativa* seed oil was kept in a dark glass in order to provide additional protection from solar light and oxidation of different components of the product, such as essential fatty acids. Additionally, systematic exposure dose for *Cannabis Sativa* seed oil was calculated, and the results are presented in Table 3. Based on the data obtained, the oil was considered safe. Before starting the treatment with *Cannabis Sativa* seed oil, each volunteer was subjected to preliminary tests to obtain information about the condition of their skin.

Additionally, a detailed survey was conducted including questions about skin condition, skin susceptibility to irritant, skincare habits, problems with skincare, lifestyle (physical activity, stimulants, sleep, stress), diet, digestive problems, allergies, diseases and drugs used.

Fig. (1) depicts the sensitivity of volunteers’ skin to external factors, including temperature, climate and wind. 45% of women participating in the tests had skin moderately sensitive to external factors. On the other hand, very high skin sensitivity was declared by 15% of volunteers. 25% of female claimed that they had high skin susceptibility to external conditions.

Volunteers did not experience any side effects or allergic reactions while using *Cannabis Sativa* seed oil. After analyzing the results of preliminary tests, the extreme cases were discarded. Next, the volunteers were divided into 2 groups taking into account the effects obtained after the application of *Cannabis Sativa* seed oil. Two non-invasive procedures were applied to determine the skin condition after the treatment with oil formulation. Both tewameter and corneometer are considered safe and nonharmful probes which maintain the integrity of skin [31].

#### 3.1. Transepidermal Water Loss (TEWL)

Tewameter is used to assess the transepidermal water loss resulting from the secretion of sweat from the sebaceous glands and the evaporation of water through epidermis [32]. The TEWL value is influenced by the number of corneocyte layers and their size. The larger they are, the longer the evaporation path and the lower the TEWL value. The smallest number and diameter of corneocytes are found in the facial skin, which is the reason of increased water evaporation. Different factors have an influence on the TEWL value such as gender, age of volunteers, sweat gland activity, as well as temperature and humidity. The higher the humidity, the higher TEWL value. Additionally, the time of day also affects the results of transepidermal water loss, as

### Table 2. Physicochemical properties of *Cannabis Sativa* seed oil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Visual</td>
<td>Liquid without mechanical impurities</td>
</tr>
<tr>
<td>Color</td>
<td>Visual</td>
<td>Green to dark green</td>
</tr>
<tr>
<td>Odor</td>
<td>Visual</td>
<td>Characteristic for the raw material</td>
</tr>
<tr>
<td>Stability (5 °C, 37 °C)</td>
<td>Climatic chamber</td>
<td>Stable</td>
</tr>
<tr>
<td>Total acid number</td>
<td>Titration</td>
<td>2 mg KOH/g</td>
</tr>
<tr>
<td>Iodine value</td>
<td>Titration</td>
<td>99 g I₂/100 g</td>
</tr>
<tr>
<td>Saponification number</td>
<td>Titration</td>
<td>190.7 mg KOH/g</td>
</tr>
<tr>
<td>Peroxide number</td>
<td>Titration</td>
<td>6.4 meq O₂/kg</td>
</tr>
</tbody>
</table>

### Table 3. Data used to calculate the systematic exposure dose.

<table>
<thead>
<tr>
<th>Application Site</th>
<th>C (%)</th>
<th>Dap (%)</th>
<th>A (g/day)</th>
<th>SED (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body</td>
<td>100</td>
<td>100</td>
<td>7.82</td>
<td>130.33</td>
</tr>
</tbody>
</table>
they are much lower in the morning than in the evening. High TEWL values signify the skin barrier abnormalities which can be associated with atopic dermatitis and ichthyosis vulgaris [33-36]. Elevated TEWL values are due to lower skin hydration, which may be a result of skin dysfunction [37]. Low transepidermal water loss values give information about intact skin function [38, 39]. The measurement principal is based on Nilsson’s Vapor Pressure Gradient theory. The teawmeter constitutes a hollow cylinder with temperature and hygroscopic sensors that determine the density gradient of water evaporation pressure in the selected areas on the skin surface. Fick law of diffusion is applied to estimate the variances between to measurements point [40]. As the stratum corneum exhibits affinity to water, the Fick’s law was adjusted by a partition coefficient Km (1) that takes into consideration ratio between water concentration in the lower horny layer and water concentration in the intercellular space of living epidermis. Table 4 presents the relationship between the TEWL value and skin condition. It shows that as the TEWL value increases, the skin condition deteriorates.

![Fig. (1). The sensitivity of volunteers’ skin to external factors (temperature, climate, wind). (A higher resolution/colour version of this figure is available in the electronic copy of the article).](image)

**Table 4. Interpretation of TEWL measurements [33].**

<table>
<thead>
<tr>
<th>TEWL Value (g/h/m²)</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>Very healthy skin</td>
</tr>
<tr>
<td>10-15</td>
<td>Healthy skin</td>
</tr>
<tr>
<td>15-25</td>
<td>Normal skin</td>
</tr>
<tr>
<td>25-30</td>
<td>Skin in poor condition</td>
</tr>
<tr>
<td>Above 30</td>
<td>Skin in critical condition</td>
</tr>
</tbody>
</table>

The results of preliminary test showed that the volunteers had healthy skin (TEWL values 12.9 ± 2.0 g/h/m²). Fig. (2) presents the changes in TEWL values after application of Cannabis Sativa seed oil. The transepidermal water loss decreased in groups A and B. In group A the greatest decrease in TEWL value (ca. 30%) was observed after the first week of product’s application. The lipophilic components of the oil formulation tend to form occlusive layer (which prevents from water evaporation) on the epidermis surface. Moreover, as a result of regular application of Cannabis Sativa seed oil on the skin, physicochemical effect on the intercellular spaces in the stratum corneum could be noticed.

![Fig. (2). Changes in transepidermal water loss after application of Cannabis Sativa seed oil. (A higher resolution/colour version of this figure is available in the electronic copy of the article).](image)

Due to the incorporation of lipids into the structure of the so-called intercellular “cement”, the changes in the properties of the epidermal barrier are possible. The amount of lipids in Cannabis Sativa seed oil (mainly long chain fatty acids) affects the tightness of the occlusive layer. In the following weeks both in group A and group B the decrease in TEWL values was lower than in the first week of the measurements, which may be due to a change in weather conditions. When the experiments were carried out, the temperature dropped down, that could lead to reduced sebum production. In this case, the epidermal protective barrier was affected and thus the transepidermal water loss was higher.

3.2. Skin Hydration

A proper skin hydration is essential for healthy skin, so moisturizers are important components of skin care formulations. A corneometer enables to determine the water content in the epidermis by measuring the electrical capacitance of the skin surface. The increase or decrease of the hydration level in epidermis causes proportional changes in electrical capacitance. The results obtained by corneometer give information about the current hydration of the upper layers of the skin and determine the condition of the epidermal barrier [41]. Various external conditions such as relative air hu-
midesty, temperature and direct air flow may have influence on the hydration values [42]. Additionally, other factors such as age, gender, sweat and temperature on the skin surface, may affect the barrier-related parameters [40, 43]. Table 5 presents the interpretation of skin hydration measurements.

<table>
<thead>
<tr>
<th>Skin Hydration Value</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 30</td>
<td>Very dry skin</td>
</tr>
<tr>
<td>30-45</td>
<td>Dry skin</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>Skin sufficiently moisturized</td>
</tr>
</tbody>
</table>

The results of preliminary test showed that the volunteers had dry skin (33.43 ± 3.04). Fig. (3) shows the changes in skin hydration after application of Cannabis Sativa seed oil. The volunteers were divided into two groups. The highest increase in skin hydration (ca. 25%) was observed in group A (that corresponds to 70% of all participants) after 1 week of treatment. During the following weeks of oil’s application, the increase in hydration was maintained at the same level of ca. 20%. These results confirm that Cannabis Sativa seed oil has strong moisturizing properties. The presence of long-chain unsaturated fatty acids including linoleic acid, linolenic acid and oleic acid may strengthen the intercellular cement and renew the lipid layer of the skin. The long-chain fatty acids incorporate into the intercellular lipids in stratum corneum and form an occlusive layer. Additionally, other components of Cannabis Sativa seed oil such as the derivatives of vitamin E (α-tocopherol linoleate) also have an influence on the increase in skin hydration [44]. By their incorporation into lipids in the epidermis, the long-lasting moisturizing effect is provided. The increase in skin hydration is also directly related to the reduction in water evaporation from the epidermis, which results in lower TEWL values. In group B, which constitutes 30% of all volunteers, a decrease in skin hydration in each week was observed. It may be explained by the high initial level of skin hydration of volunteers who applied other moisturizing formulations before taking part in the test. Thus, the results of the next measurements were disturbed because the actual hydration level of epidermis was not as high as it was determined. In the following weeks, the decrease in degree of skin hydration was smaller and smaller (did not exceed 8%).

3.3. Subjective Assessment of Cannabis Sativa Seed Oil

The impact of cosmetics on our senses is depends on different factors and is a complex matter [45]. Each person can perceive the skincare product in an individual way. Therefore, apart from apparatus measurements, the subjective volunteer assessment was also performed after the application tests. The participants evaluated the following parameters: viscosity, absorbability, spreadability, smoothing, moisturizing, skin feeling, tolerance and skincare effect. Fig. (4) collects the results of conducted surveys. Volunteers rated their level of satisfaction as average, good or very good. Based on the performed questionnaires it could be stated that the majority of respondents positively assessed all parameters of Cannabis Sativa seed oil. 80% of volunteers considered that the tested oil formulation had very good spreadability. 70% of the respondents assessed the skin tolerance at very good level. The hydration, viscosity and care effect were rated very good by 50% of volunteers.

CONCLUSION

The study performed proved that Cannabis Sativa seed oil improved skin hydration and reduced the transepidermal water loss. The positive moisturizing effect of this oil is
similar to the results obtained in the case of other oils derived from coconut, peanut, almond and sunflower. The Cannabis Sativa seed oil act as a protective barrier by forming an occlusive layer on the skin. Therefore, its application in cosmetics is fully justified.

The formulation exhibited beneficial effect on the skin mainly due to the high content of fatty acids, in particular polysaturated fatty acids such as linoleic acid or linolenic acid. It protects the skin barrier by preventing the damage of the lipid layer, and it strengthens the integrity of the dermis. As a powerful source of fatty acids, the Cannabis Sativa seed oil has a positive effect on dry and rough skin. The appropriate amount of fatty acids in Cannabis Sativa seed oil may prevent or reduce skin inflammations such as: psoriasis, atopic dermatitis, and acne. Moreover, fatty acids have impact on the skin conditions because they are components of phospholipids in the cell membrane. They are also responsible for the regeneration and proper skin tone, including the lack of discoloration.

ETHICS APPROVAL AND CONSENT TO PARTICI­PATE

Bioethics approval for analysis was obtained from the Bioethics Committee at Poznań University of Medical Sciences (No. 143/18). All volunteers gave consent to participate in the studies.

HUMAN AND ANIMAL RIGHTS

No animals were used in presented studies. All humans research procedures were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013 (http://ethics.iit.edu/edudes/nodes/3931).

CONSENT FOR PUBLICATION

This study obtained the consent of the participants and authors to publish the data.

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

FUNDING

None.

CONFICT OF INTEREST

The authors have no conflicts of interest to declare.

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