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Facial Acne Therapy by Using Pumpkin Seed Oil with Its Physicochemical Properties

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Abstract
The herbal remedy individually or in combination with standard medicines has been used in diverse medical treatises for the cure of different diseases. Pumpkin seed oil is one of the recognized edible oil and has substantial medicinal properties due to the presence of unique natural edible substances. Inflammation is an adaptive response that is triggered by noxious stimuli and conditions, which involves interactions amongst many cell types and mediators, and underlies many pathological processes. Unsaturated fatty acids (UFAs) can influence inflammation through a variety of mechanisms, and have been indicated as alternative anti-inflammatory agents to treat several inflammatory skin disorders. Pumpkin seed oil is rich in (UFAs), that its topical anti-inflammatory properties have been investigated. For that reason, the goal of this article was to evaluate the effects of pumpkin seed oil on acute and chronic cutaneous inflammation experimental models. The extracted pumpkin seed oil had an acceptable initial quality, when it was extracted using soxhlet extraction method and was characterized using standard methods .The physicochemical parameters of purified oil were determined. The boiling point of pumpkin seed oil was (158.90 ^{o}C) that equal to the values obtained in literature for some oil seeds, but lower than the boiling point of the oils studied, plus the melting point of pumpkin seed oil was (15.39 ^{o}C) that lead to a characteristic in cold cream manufacture. The iodine value was (104 ± 0.03 mg of KOH/g) of oil, indicated a high degree of unsaturation. The saponification value was (181± 3.2 mg KOH/g), this value indicated the pumpkin seed oil had fatty acids with higher number of carbon atoms. As a final point, the acid value was low (0.67 ± 0.09 mg KOH), while the peroxide value was low (10.03 ± 0.59 meq peroxide /kg).
Keywords: The therapy for acute and chronic facial acne from the extracted pumpkin seed oil, the physicochemical parameters of extracted pumpkin seed oil.

1. Introduction

Pumpkin is a storehouse of vitamins, mineral and other healthy nutrients. Whether, it is the pulp or the seed, pumpkin is splendid for your health and can offer some incredible advantages [1]. Pumpkin seed oil has been used traditionally as medicine in many countries such as China, Yugoslavia, Argentina, India, Mexico, Brazil, and America. It is applied in treatment of small disorders of the prostate gland and urinary bladder caused by hyperplasia (BHP)[2,3]. The extracted pumpkin seed oil, that has been reported to have antidiabetic, antitumor, antibacterial, anticancer, and antioxidant activities. Furthermore, the health benefits of pumpkin seeds are attributed to their macro- and micro-constituent compositions. They are a wealthy natural source of antioxidative phenolic compounds [4] .

Pumpkin owes its bright orange color to the high quantity of carotenoids present in it. Carotenoids assist in staving off the free radicals in the body, and help in banning premature aging, cardiovascular diseases and other infections [5,6]. Pumpkin seed oil has high amount of phytosterols or plant-based fatty acids which can help in reducing the blood cholesterol levels [7,8]. Pumpkin seed oil is a rich provenance of essential fatty acids, that has numerous health benefits, when it affords the protection against serious health diseases such as high blood pressure, arthritis and promoting healthy skin [9,10]. An analysis of the oil extracted from the seeds of each of twelve cultivars of *C. maxima* yielded the following ranges for the percentage of several fatty acids (Table 1) [11].

Table 1: The percentage of several fatty acids

n:unsat	Fatty acid name	Percentage range
(14:0)	Myristic acid	0.09-0.27
(16:0)	Palmitic acid	12.6-18.4
(16:1)	Palmitoleic acid	0.12-0.52
(18:0)	Stearic acid	5.1-8.5
(18:1)	Oleic acid	17.0-39.5
(18:2)	Linoleic acid	18.1-62.8
(18:3)	Linolenic acid	0.34-0.82
(20:0)	Arachidic acid	0.26-1.12
(20:1)	Gadoleic acid	0-0.17
(22:0)	Behenic acid	0.12-0.58

Pumpkin is a rich resource of Vitamin A. Regular consumption of pumpkin seed oil can reinforce the health of your eyes and boost your immune system remarkably. Vitamin C helps fight free radicals, improves immunity and reinforces the production of collagen. The high Vitamin C content in pumpkin seed oil also offers protection against various forms of cancer [12]. The seed oil of pumpkin is abundant in a mixture of minerals such as magnesium, potassium and Zinc which are important minerals required for various biological functions. Consequently, these minerals make pumpkin seed oil a superb choice for those who want a healthy and garish skin, also prevent semblance of wrinkles and to keep your skin hydrated and nourished (Table 2) [13,14].

Table 2: The weights and their percentages of minerals that exists in pumpkin's seed

Minerals	Weight in pumpkin's seed	Percentage %
Calcium	52 mg	5%
Iron	8.07 mg	62%
Magnesium	550 mg	155%
Manganese	4.49 mg	214%
Phosphorus	1174 mg	168%
Potassium	788 mg	17%
Sodium	256 mg	17%
Zinc	7.64	80%

Inflammatory skin disorders can be handled with some success by pharmaceutical agents, such as corticosteroids and non-steroidal anti-inflammatory drugs (NSAIDs). However, they can regularly reason a set of undesirable side effects [15]. Because of these risks, alternative bioactive molecules are being intensely investigated. In this scenario, fatty acids are highlighted as influential effectors and regulators molecules in the immune-inflammatory response [16]. As a result of, the Beta carotene present in pumpkin seed oil has anti-inflammatory features. Accordingly, pumpkin seed oil has been known to supply relief from inflammation quickly, without the hurtful side-effects of anti-inflammatory medicines. The present study is intended to investigating physicochemical properties of extracted oil from pumpkin seed, besides screening its effects on acute and chronic facial inflammation models.

2. Materials and Methods

2.1 Materials

The dried pumpkin seeds (*C. pepo* subsp. *pepo* var. Styriaca) were obtained from a local market in Suliemanyah – Iraq, and taxonomically identified and authenticated by a taxonomist at the Department of Agricultural, Faculty of Horticulture, Suliemanyah University, in Suliemanyah – Iraq. Approximately (1kg) of pumpkin seed was milled fine and then ethanol extracts were given pumpkin seed oil or natural product (100 ml). All chemicals and solvents, and fatty acid methyl ester (FAME) standards used in this study were of analytical reagent grade and were purchased from Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MO).

2.2 Extraction of oil

Hot extraction of the oil was done according to (AOAC 1980), Pumpkin seed (50 g) was milled and extracted by adding (200 ml) ethanol (96%) (boiling between 70–78 ◦C) with a soxhlet extractor for (3-4) h .Whatman No.1 filter paper was placed in the thimble of the Soxhlet extractor. The oil was extracted with Ethanol (1:4 w/v) and at the end of this period, the mixture was filtered and the liquid part was evaporated by using a rotary evaporator to remove excess solvent used in the oil, cooled and preserved for further analysis [17].

2.3 Fixing of boiling point

A capillary tube of about (3-4) cm long was sealed at one end and placed in a glass tube with its open end downwards. A little quantity of the oil samples was inserted into the tube with a dropper. The tube was then tied to a thermometer and immersed in a bath of liquid paraffin used for determination of boiling point. The bath was heated slowly with continuous stirring until a rapid and incessantly stream of bubbles evolved from the capillary tube and passed through the liquid. The flame was eliminated and the system was allowed to cool while continuously stirring until a point was reached which the bubbling ceased and the oil initiated to rise in the capillary tube. The temperature at which the oil just came into the capillary tube was marked as the boiling point of the oil. The measures were repeated three times and the mean temperatures were recorded.

2.4 Fixing of melting point

The estimation of melting point of the oil, the oil samples were left in the refrigerator to solidify and the solidified samples was placed in a capillary tube. The tube was inserted into the hole of the electro thermal melting point apparatus. The temperature of the instrument was set and the instrument was allowed to stand until the lipid samples melted as monitored through the lens of the instrument.

2.5 Fixing of iodine value

Preceding to the determination of the iodine value of the oil, Hanus reagent, potassium iodide (KI) and starch solution were prepared as follows; (13.2) g of Iodine crystals were dissolved in (100) mL of glacial acetic acid (AcOH gl.). The solution was put in a water bath until the iodine dissolved. The solution was cooled and (3) mL of bromine (Br_2) was added to double the halogen content. The solution was stored in a dark cupboard for using. (1) g of (KI) was weighed and dissolved with (20) mL of distilled water. The solution was made up to the (100) mL mark and stored in a reagent bottle. The starch solution was also prepared by dissolving (1) g of soluble starch in (10) mL of distilled water and made up to mark in a (100) cm^3 standard volumetric flask. In the determination of iodine value of the oil, (0.5) g of each of the oil sample was dissolved in (100) mL of chloroform contained in a (500) cm^3 conical flask. (25) mL of Hanus solution was added into each flask, stoppered and let to stand for 30 minutes in the dark. A blank test was carried out without the samples using exactly the same quantity of chloroform and Hanus solution, stoppered and also allowed to stand for 30 minutes. (15) cm^3 of 10% (KI) solution and (10) mL of distilled water were added to each flask mixed by gentle shaking. The content of the flask was titrated with (0.1) N $Na_2S_2O_3$ to pale yellow before the addition of (2) mL of starch indicator. The titration continued until the blue black color was completely discharged.

Calculation

1 cm^3 of 0.1 N $Na_2S_2O_3$, 0.01269 g of iodine 1.26 (a-b)/w, w = weight of the sample,
b = volume of 0.1 N $Na_2S_2O_3$ for the sample, a = volume of 0.1 N $Na_2S_2O_3$ for the blank,
1 cm^3 of 0.1 $Na_2S_2O_3$ 0.01269 g of 12\1000 cm^3 0.01269x1000, 12.69x0.1 N = 1.269.

2.6 Fixing of saponification value

(2 g) of each of the oil sample were respectively weighed into the different conical flasks and (25) mL of ethanedioic potash was added. A blank was prepared by adding the same quality of the ethanedioic potash (without the oil sample) to another flask. All the flasks were boiled in a water bath for 30 minutes with frequent shaking. Two drops of phenolphthalein "**phph**" indicator were added to each flask and titrated with (0.5) M HCl with vigorous shaking to the end point.

Calculation

1 cm^3 of 0.5M of HCl 0.02805 g KOH\1000 cm^3 0.02805x1000 = 28.05, SV
(Saponification value) = (B-S) 28.05/w; where B = Average blank titre, S = Average sample
titre and W = weight of the sample.

2.7 Fixing of acid value

(2) g each of the different oil samples were weighed and were added to (25) cm^3 of (CCl_4) in different conical flasks. Two drops of phenolphthalein "**phph**" indicator were then added to the mixture. A similar titration was performed without the sample to determine the blank and titration was carried out with (0.1) N alcoholic potash until the color change occurred in the different conical flasks.

Calculation

Av = (sample titre – blank) 0.1x56.1/w. where W = weight of sample. Estimation of ester value, The ester value of the oil was calculated from the equation; EV = SV – AV; where EV is the ester value, SV is the saponification value and AV is the acid value.

2.8 Fixing of peroxide value

(2) g each of the oil samples were respectively weighed into various conical flasks and(15) mL of the mixture of [CH_3COOH – $CHCl_3$] in the ratio of (3:2) respectively was added to the oil sample. (0.5) mL of saturated (KI) was added to each conical flask and allowed to stand for 5 minutes, thereafter; (15) mL of distilled water was added and titrated with (0.1) N ($Na_2S_2O_3$) until the yellowish color almost disappeared, then (0.5) mL of starch was added and the titration continued to a colorless end-point.

Calculation

Peroxide value =1000 (V_2 - V_1) T / M

Where M = mass of oil taken (2 g), V2 = volume of 0.1 N $Na_2S_2O_3$, V1 = volume of 0.1 N blank and T = normality of $Na_2S_2O_3$ (0.1 N).

3. Results and Discussion

3.1 Pumpkin might be used to solve facial problems:

Though pumpkin is a recognized ripe plant, most parts of this plant are also used in traditional systems of medicine around the world. Additionally, pumpkin seed oil has been deemed to provide a momentous source of vitamin E in Japanese diets [18]. Thus, diseases caused by bacteria, viruses, fungi and other parasites are major causes of facial problems for millions of individuals. In spite of the existence of safe and effective interventions, many individuals lack access to needed preventive and treatment care. Increasing drug resistance in infectious micro-organisms has warranted the development of new drugs against pathogenic micro-organisms. In this heed, natural source has been considered as the best option to isolate new anti-microbial component. Anti-microbial component has been isolated from pumpkin seed oil. Pumpkin seed oil restrains *Acinetobacter baumanii, Aeromonas veronii* biogroup *sobria, Candida albicans, Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Propionibacterium, Pseudomonas aeruginosa, Salmonella enterica* subsp. *enterica* serotype *typhimurium, Serratia marcescens* and *Staphylococcus aureus* at the concentration of 2% (v/v) [19]. A noteworthy inhibitory effect of a purified protein (MW 28 kDa) against the fungal growth of *Fusarium oxysporum* was exerted in an agar disc plate at a concentration greater than 2mM. This protein owned a synergistic effect with nikkomycin, a chitin synthase inhibitor, for the growth inhibition of *Candida albicans* [20]. Three pumpkin seed oil basic proteins, MAP2 (MW 2·2 kDa), MAP4 (MW 4·6 kDa) and MAP11 (MW 11·7 kDa), have been shown to inhibit the growth of yeast cells, with MAP11 being the most effective inhibitor. Nevertheless, MAP2 and MAP4 did not inhibit the growth of the Gram-negative bacterium *E.coli* [21]. Moreover, it has been reported that phloem exudates from pumpkin seed oil has anti-fungal activity via inhibition of pathogenic fungal proteases [22]. Pumpkin seed oil has been utilized for various cosmetic applications such as skin scrubber, massage oil, lotion and dry facial masque [23].

3.2 General Considerations

A. Description of Acne

Acne, also famed as acne vulgaris, is a long-term skin disease that occurs when hair follicle are clogged with dead skin cells and oil from skin [24] .It is distinguished by black heads or white heads, greasy skin, pimples, and possible scarring [25-27] . It essentially influences on the skin areas with a relatively high number of oil glands, including the face, upper part of the chest, and back [28]. The resulting appearance can lead to anxiety, reduced self-esteem and, in extreme cases, depression [29,30]. Genetics is thought to be the primary cause of acne in 80% of cases [26]. The function of diet is unclear, and neither cleanliness nor uncover to sunlight emerges to play a part [31,32]. During puberty, in both sexes acne is often brought on by an increase in hormones such as testosterone [33]. Excessive growth of the bacterium *Propionibacterium* acnes, which is ordinarily existent on the skin, is often involved [34].

B. Study design

This cross-sectional study was conducted at the Department of Medical Analysis of the Suleimani Polytechnic University, Kurdistan region/ Iraq. All patients were recruited as they presented or were referred for facial acne care. The study was carried out from March 2017 to June 2017. Male and female subjects (18 to 25 years old) . Results which have received acne topical treatment in three months were analyzed. Evaluation of patients' subjective response to curative was performed by a questionnaire ranking the degree of satisfaction as highly satisfied, satisfied, neutral, or dissatisfied. Lesion counts and the standard deviation at baseline and at each subsequent treatment session were compared using the paired Student's **t**-test. As such, the paired t-test was appropriately picked for analysis to account for initial variation in lesion counts [35].

C. Method

This study was performed on (20) patients with acne vulgaris. Inclusion criteria include having acne vulgaris above the 18 years. Exclusion criteria include having skin diseases. The target of the survey was demonstrated the significance of orientating to utilize natural products to solve facial disease. Moreover, the responders benefit to rid of their acne vulgaris plus participate in this study.

D. Results

All twenty patients demonstrated reductions in their acne lesion counts. On the whole, the mean acne lesion count diminished from a baseline of 36.2±29.6 to 22.7±23.1 after the first month's treatment ($p< 0.01$). After the second and third months treatments, the mean acne lesion count diminished to 15.3±15.4 ($p< 0.01$) and 6.2±6.9 ($p< 0.01$), respectively . This harmonized with a 38% reduction in mean acne lesion count after one month's treatment, a 59% diminished after two months treatment, and an 84% diminished after three months treatments figures (1,2). There was no difference in enhancement between male and female patients ($p=0.44$), as both illustrated statistically significant enhancements ($p< 0.01$). Figures (3–6) revealed representative pretreatment and photographs of patients. In the group with mild inflammatory acne (n=6), the mean acne lesion count minimized from a baseline of 10.1±4.2 to 5.1±7.2, after the first month's treatment ($p=0.12$). After the second and third months treatments, the mean acne lesion count minimized to 9.3±12.5 ($p=0.84$) and 3.3±5.8 ($p=0.20$), respectively. In the group with moderate inflammatory acne (n=7), the mean acne lesion count minimized from a baseline of 28.1±15.2 to 19.3±12.1 after the first month's treatment ($p< 0.01$). After the second and third months treatments, the mean acne lesion count minimized to11.9±8.9 ($p< 0.05$) and 6.1±4.8 ($p=0.06$), respectively. In the group with severe inflammatory acne, the mean acne lesion count reduced from a baseline of 70.3±20.7 to 37.8±29.6 after the first month's treatment ($p< 0.05$). After the second and third months treatments, the mean acne lesion count reduced to 25.9±20.3 ($p< 0.05$) and 10.6±13.5 ($p< 0.05$), respectively .

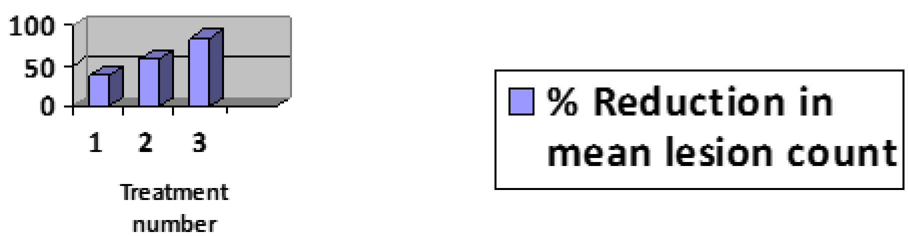

Figure 1. Percentage reduction in mean inflammatory acne lesion count after one, two, and three months treatments with the pumpkin seed oil

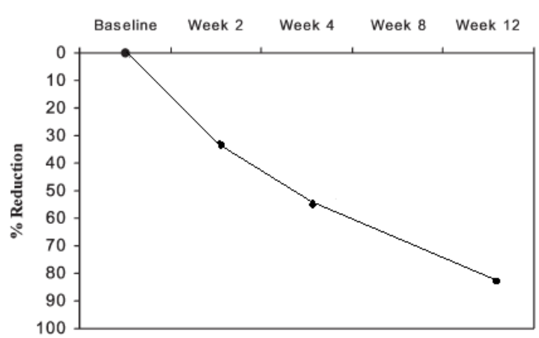

Figure 2. Mean percentage reduction in total lesion count

When questioned concerning treatment, 50% of patients reported being 'highly satisfied' and 50% reported being 'satisfied' with their outcome. Significantly, there were no patients who reported dissatisfaction with their treatment. In addition, 90% of patients stated that they would recommend the treatment to others. No harmful effects such as pigmentary alteration, scarring or infection were remarked.

Before (A) After (B)

Figure 3. (A) acne traces. (B) a 82% clearance of acne traces after three months treatments with the pumpkin seed oil

Before (A) After (B)

Figure 4. (A) acne traces. (B) a 84% clearance of acne traces after three months treatments with the pumpkin seed oil

Before (A) After (B)

Figure 5. (A) Multiple inflammatory papules and few scattered pustules pretreatment. (B) After two months treatments with the pumpkin seed oil, a 57% decrease in active acne lesions was observed compared with baseline.

Before (A) After (B)

Figure 6. (A) Multiple inflammatory papules and pustules. (B) After three months treatments with the pumpkin seed oil, an 83% decrease in lesion counts from baseline was observed.

E. Discussion

In our research, there was a statistically significant improvement in inflammatory facial acne lesion counts overall, patients were uniformly satisfied with their treatment. This may, in part, be due to the fact that our patients included previously refractory cases who responded significantly to pumpkin seed oil treatment. We deem that the effectiveness, convenience, and adaptability of this treatment contribute to its high patient satisfaction rate. In our experience, this oil has been equally safe and effective when used to treat inflammatory acne. Although this study is not conducive to a cost-effectiveness analysis and was not carried out over a long period of time, this therapy should be deemed as an alternative for the treatment of acne in patients who are noncompliant with or resistant to standard acne therapies.

3.3 Physicochemical properties of the pumpkin seed oil

The boiling point of pumpkin seed oil was (59.50 ºC). The boiling point is equal to the values obtained in literature for some oil seeds [36] but lower than the boiling point of the oils studied [37]. The melting point of pumpkin seed oil was (15.39 ºC) comparable with the melting point that reported for some seed oils [38,39]. The melting point of the seed oils is an advantage in cold cream manufacture. The lower melting point of the seed oil, would exhibit the capability for making oil cream [40]. The iodine value (104 ± 0.03 mg of KOH/g) of oil, indicating a high degree of unsaturation . This value was close to (103.2, 107.0, and 105.1) reported by, respectively [41], but higher than 80.0 [42], plus lower than 123.0 [43], and (116.0-133.4) [44] for *Cucurbita* species. It also lied within the range reported for cottonseed, canola, rapeseed, and corn oils [45]. The iodine value of the oil reduce the risk of oxidative rancidity, also pumpkin seed oil rich in unsaturation fatty acids have been related as alternative anti-inflammatory agents on skin disorders [46-48] . The saponification value was (181± 3.2 mg KOH/g), this value indicated that the pumpkin seed oil had fatty acids with higher number of carbon atoms in comparison with coconut (248–265) and palm kernel (230–254) oils [45]. This result was in good agreement with the (185.5-195.3) range [44], however, it was lower than (200-218) range [49] and was higher than (132.3) [44] for *Cucurbita* species. Furthermore, it fell in the range reported for olive, canola, corn, and sunflower oils [45]. The acid value was low (0.67 ± 0.09 mg KOH), while the peroxide value was low (10.03 ± 0.59 meq peroxide /kg). The extracted pumpkin seed oil had an acceptable initial quality (Table 3). The Codex Alimentarius Commission expressed the permitted maximum acid values of (10) and (4) mg KOH/g oil for virgin palm and coconut oils, respectively [50]. It has been shown that oils the peroxide value ranges from (20.0 to 40.0 meq peroxide/ kg oil) [51]. Otherwise, in relation to the Codex Alimentarius Commission [50], the peroxide value for unrefined olive oil may be maximum (20) meq/kg oil [44]. This illustrates the commercial potential of the oil, which is enhanced by the low peroxide and acid values.

Table 3: Physicochemical characteristics of pumpkin seed kernel oil at 25 ºC

Properties	Mean value

Boiling point ºC	59.50 ±0.26
Melting point ºC	15.39 ±0.15
Iodine value, gI_2/100 g	104 ± 0.03
Saponification value, mgKOH/g	181± 3.2
Acid value, mgKOH/g	0.67 ± 0.09
Peroxide value, meq peroxide/ kg oil	10.03 ± 0.59

Conclusion

Pumpkin seed oil performs as a topical anti-inflammatory agent, and it is effective against acute and chronic skin inflammatory processes. In this study, documenting the safety and efficacy of pumpkin seed oil treatment for inflammatory facial acne . Besides, medical enhancement was seen in all patients. This scientific study further supports and suggests the use of this plant oil as an adjuvant along with commonly used anti-inflammatory agent.

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