

Impact of Sesame Oil Source: A Quality Assessment for Cosmeceutical and Pharmaceutical Use

Abdel Naser ZAID* , Nidal JARADAT* , Numan MALKIEH** ,
Salah AL-RIMAWI ** , Fatema HUSSEIN* , Linda ISA* , Iyad ALI*** ,
Rana MANASRA* , Ghada SILWADI* , Othman SADDER* ,
Mohammed HAWASH*

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Susam Yağı Kaynağının Etkisi: Kozmesötik ve Farmasötik Kullanım için Kalite Değerlendirmesi

SUMMARY

Sesame oil has many cosmeceutical and pharmaceutical benefits. These can be exploited to produce cosmeceuticals such as sunscreens and wound healing creams according to their sun protection factor (SPF) value and β -sitosterol content. The aim of this study was to assess the quality of sesame oil available on the Palestinian market for cosmeceutical and pharmaceutical use. A phytochemical qualitative analysis was executed using standard tests like Molisch's test, Fehling's test and Benedict's test. Moreover, 2,2-diphenyl-1-picrylhydrazyl (DPPH) inhibition percentage and elastase inhibition percentage calculations were applied using the standard references Trolox and oleanolic acid. Furthermore, the β -sitosterol (w/w) component was measured for each oil type. The DPPH inhibition, elastase inhibition and SPF values of Indian, Turkish and Palestinian sesame oil were ($6.7 \pm 0.64 \mu\text{g/mL}$, $9.3 \pm 0.37 \mu\text{g/mL}$, $9.77 \pm 0.44 \mu\text{g/mL}$), ($50.11 \pm 0.70 \mu\text{g/mL}$, $56.23 \pm 0.37 \mu\text{g/mL}$, $79.43 \pm 0.48 \mu\text{g/mL}$) and (3.2, 3.0, 2.2), respectively. In addition, the β -sitosterol concentrations were Turkish 0.194% w/w, Palestinian 0.196% w/w and Indian 0.153% w/w. The results show that Indian sesame oil was the strongest antioxidant and had the highest elastase inhibition activity and SPF value for use in sunscreens and anti-ageing products. Turkish and Palestinian sesame oils are best used in wound healing creams.

ÖZ

Kozmetik ve farmasötik açısından Susam yağının birçok faydası bulunmaktadır. Bunlar, güneşten korunma faktörleri (SPF) değerlerine ve β -sitosterol içeriğine göre güneşten koruyucular ve yara iyileştirici kremler gibi kozmesötiklerin üretiminde kullanılabilir. Bu çalışmanın amacı, Filistin piyasasında kozmesötik ve farmasötik kullanım için mevcut olan susam yağı kalitesini değerlendirmektir. Bir fitokimyasal kalitatif analiz, Molisch testi, Fehling testi ve Benedict testi gibi standart testler kullanılarak yapılmıştır. Ayrıca, standart referanslar Trolox ve oleanolik asit kullanılarak 2,2-difenil-1-pikrilhidrazil (DPPH) inhibisyon yüzdesi ve elastaz inhibisyon yüzdesi hesaplamaları uygulanmıştır. Ayrıca, her bir yağ tipi için β -sitosterol (a/a) bileşeni ölçülmüştür. Hint, Türk ve Filistinli susam yağının sırasıyla DPPH inhibisyonu, elastaz inhibisyonu ve SPF değerleri ($6.7 \pm 0.64 \mu\text{g/mL}$, $9.3 \pm 0.37 \mu\text{g/mL}$, $9.77 \pm 0.44 \mu\text{g/mL}$), ($50.11 \pm 0.70 \mu\text{g/mL}$, $56.23 \pm 0.37 \mu\text{g/mL}$, $79.43 \pm 0.48 \mu\text{g/mL}$) ve (3.2, 3.0, 2.2) olarak bulunmuştur. Ayrıca β -sitosterol konsantrasyonları sırasıyla Türk, Filistin ve Hint susam yağında % 0.194, % 0.196 ve % 0.153 oranındadır. Sonuçlara gelince Hint susam yağının en güçlü antioksidan olduğunu ve güneşten koruyucularda ve yaşlanma karşıtı ürünlerde kullanım için en yüksek elastaz inhibisyon aktivitesine ve SPF değerine sahip olduğunu göstermektedir. Türk ve Filistinli susam yağları yara iyileşmesinde kullanılan en iyi kremlerdir.

Key Words: Sesame oil, SPF, Elastase inhibition, Antioxidant, Wound healing, β -sitosterol.

Anahtar Kelimeler: Susam yağı, SPF, Elastaz inhibisyonu, Antioksidan, Yara iyileşmesi, β -sitosterol

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* ORCID: 0000-0002-3176-6581, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

** ORCID: 0000-0003-2291-6821, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

*** ORCID: 0000-0003-3662-5996, Jerusalem Pharmaceuticals Co, Al Bireh-Ramallah, Palestine.

**** ORCID: 0000-0002-4481-0369, Jerusalem Pharmaceuticals Co, Al Bireh-Ramallah, Palestine.

***** ORCID: 0000-0002-6712-4302, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0002-1103-2505, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0002-3921-6859, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0001-9375-8512, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0001-8512-5766, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0001-6250-4376, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

***** ORCID: 0000-0001-5640-9700, Department of Pharmacy, Faculty of Medicine and Health Sciences, An-Najah National University, Nablus, State of Palestine. P.O. Box 7.

° Corresponding Author; Abdel Naser Zaid

Tel: +970 9 2345113 Ext (2159)

Email:anzaid@najah.edu

INTRODUCTION

Over the last 60 years, plants have been considered as source of oils in the food industry. Fixed oils contain unsaturated acids, which usually tend to be in a liquid state (Evans, 2009). Several natural oils have captured growing attention over the years due to their antioxidant capacity. Sesame (*Sesamum indicum* L.) is a crop cultivated mainly in India, China and other tropical countries; oil can be obtained from the seeds (sesame oil, teel oil, gingelly oil) (Evans, 2009). Sesame oil (SO) has been used in the food industry as a cooking oil, in cosmetics for making soap and in pharmacy as a carrier for pharmaceutical active ingredients (Evans, 2009). In fact, it has been found that SO contains about 1% lignin phenols, which play a role in cholesterol reduction and vitamin E activity. Some of these lignans include sesamin, sesamol and sesamol. In addition, SO contains variable amounts of linoleic acid and oleic acid (Bopitiya and Madhujith, 2015; Kanu et al., 2010). Considering the different components of SO, several nutraceutical uses have been established. In addition, SO contains a considerable amount of linoleate and myristic acid (Osier and Lindroth, 2001). SO, has also found to be rich in β -sitosterol, which has wound healing capacities (Reshma et al., 2010).

β -sitosterol is known for his benefits on the skin due to its anti-oxidant, anti-bacterial, and anti-inflammatory effects, which explain the use of SO as an effective therapeutic agent to treat skin dermatitis and as burns and wounds healer (Han et al., 2014).

In traditional medicine, SO used as demulcent, emollient, antifungal antiviral, anti-inflammatory and mildly laxative. In addition, for thousands of years SO has been utilized to expedite the healing of wounds due to its antibacterial property specially against skin pathogens such as *Streptococcus aureus*.

Moreover, in the Ayurvedic medicine, the oil has been utilized in the treatment of various chronic diseases including migraine, diabetes and hepatitis (Anilakumar et al., 2010). In pharmaceutical industries, SO included in several available pharmaceutical forms such as Auromyose[®] which used as anti-inflammatory agent, Dronabinol[®] and Marinol which indicated for the treatment of anorexia and vomiting (Calhoun et al., 1998; Ten Wolde et al., 1997)

As with many natural products, the SO phytochemical content is variable based on the source or place of the seed's cultivation. These differences are due to environmental aspects such as nutrient availability in the soil and rainfall. Moreover, interactions between the environment and genotype are associat-

ed with variations in plants and herbs (Szakiel et al., 2011). These differences in the phytochemical composition can be exploited in cosmetic, nutraceutical and pharmaceutical formulations. Three sources of sesame seeds, i.e. Turkish, Indian and Palestinian, are commercially available on the Palestinian market.

The aim of this study was to assess the phytochemical compositions of these oils. Conclusively, these differences will be exploited in detecting the most suitable SO to be included in formulations for use as sunscreens, moisturisers, anti-stretch mark creams and wound healing ointments.

METHODS AND MATERIALS

The Palestinian market was investigated for the availability and cost of the different types of sesame seeds. Then, the seeds were subjected to cold press extraction at room temperature. Precisely, 1 kg of each dry sesame seeds were ground into paste, the paste was then further mixed appropriately. After that, pressure is applied to the paste to remove the oil and the obtained oil was weighed.

The obtained oils were assessed for their phytochemical component, antioxidant activity, SPF value, anti-lipase inhibition activity and β -sitosterol content.

Instrumentation

A UV-visible spectrophotometer (Jenway 7135, England), filter papers (Whatman no.1, USA), a shaker device (Memmert shaking incubator, Germany), rotary evaporator (Heidolph OB2000 Heidolph VV200, Germany), grinder (Moulinex model, Uno, China), balance (Rad wag, AS 220/c/2, Poland), cold press machine (China) were used.

Collection of plant material

Various types of *Sesamum indicum* seeds were purchased from the markets of Nablus in Palestine. The seeds were botanically classified by Dr. Nidal Jaradat and deposited in the Pharmacognosy Laboratory, Department of Pharmacy at An-Najah National University.

Chemicals

Methanol, NaOH, n-hexane and acetone were purchased from Loba Chemie (India). Millon's reagent, Benedict's reagent, ninhydrin solution, iodine solution, H₂SO₄ and Molisch's reagent were obtained from Alfa-Agar (UK). Folin-Ciocalteu's reagent, HCl, AlCl₃, potassium acetate, chloroform and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (Germany). Magnesium ribbon, acetic acid, FeCl₃ and dimethyl sulfoxide (DMSO) were purchased from Riedeldehan (Germany). Trolox ((s)-(-)-

6 hydroxy-2,5,7, 8-tetramethylchroman-2-carboxylic acid) and quercetin were brought from Sigma-Aldrich (Denmark). N-succ-(Ala)3-p-nitroanilide (SANA) and porcine pancreatic elastase (PPE) were purchased from Sigma (USA).

Qualitative analysis of the phytochemical content

Crude Indian Sesame oil (ISO), Turkish Sesame oil (TSO) and Palestinian Sesame oil (PSO) were subjected to analysis. This analysis was performed to check for the presence of phenols, tannins, flavonoids, saponins, glycosides, steroids and terpenoids. Tests were performed using the following standard phytochemical tests (Harborne, 1998).

Phenols and tannins assessment

For this test, 2 mL of 2% FeCl₃ was added to the three test tubes, then mixed together with 2 mL of each oil. If phenols and tannins were present, a black or dark green colour

Shinoda's test: Magnesium appeared.

Flavonoid assessment

Ribbon and HCl were mixed along with the three different obtained oils using 2 mg of each oil separately. Mixtures were left for a few minutes. The appearance of a pink colour indicated the presence of flavonoids.

Alkaline reagent test: 2 mL of 2% NaOH was added to the three obtained oils using 2 mg of each oil separately. An intense yellow colour formed, then became colourless after the addition of two drops of diluted acid. This indicated the presence of flavonoids.

Saponin assessment

Foam formation is evidence of the presence of saponins. This was checked by the addition of 5 mL of distilled water to the three test tubes containing 2 mL of each oils.

Glycoside assessment

Liebermann's test: 2 mg of each oil was mixed with 2 mL of acetic acid and 2 mL of chloroform. The mixture then was cooled and concentrated H₂SO₄ was added to each test tube. The appearance of a green colour indicated the presence of steroidal aglycones, a type of glycoside.

Salkowski's test: About 2 mL of concentrated H₂SO₄ was added to each test tube containing 2mg of each oil. The production of a reddish-brown colour indicated the presence of steroidal aglycone glycosides.

Keller-Kilani test: 2 mL of glacial acetic acid and two drops each of 2% FeCl₃ and concentrated H₂SO₄

were added to form a mixture with 0.5mg of each oil. The formation of a brown ring between the layers indicated the presence of cardiac steroidal glycosides.

Steroid assessment

Concentrated H₂SO₄ and 2 mL of chloroform were mixed along with 1 mL of each oil. The formation of a red colour in the lower chloroform layer indicated the presence of steroids.

Terpenoid assessment

A mixture was prepared of 2 mL of chloroform and each oil. The mixtures were evaporated on a water bath, followed by boiling after the addition of 2 mL of concentrated H₂SO₄ to each test tube containing 0.5mL of each oil. A grey colour indicated the presence of terpenoids.

Assessment of antioxidant activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities for each oil were assessed by the following method. Altogether, a stock solution of each oil was prepared at a concentration of 100 µg/L of methanol (by dissolving 10mg of each oil up to 100ml methanol in volumetric flask). After that, different concentrations were prepared through a serial dilution of the stock, followed by mixing with 1 mL of methanol plus 1 mL of 0.002% DPPH. Samples were incubated in the dark for 30 mins at 25°C. Then, absorption was recorded at λ_{max}= 715 nm. Another serial dilution series of Trolox stock solution from 10 mg/100 mL methanol was prepared. After repeating the previous tips, 1 mL of each concentration was mixed with 1 mL of methanol and 1 mL of 0.002% DPPH. All samples were incubated in the dark for 30 mins at room temperature, then absorption was recorded at λ_{max}= 715 nm. A blank solution was prepared by mixing DPPH solution with methanol at a ratio of 1:1. The absorbance was used to calculate the percentage inhibition. The following equation was used to obtain these percentages (Davies et al., 1988):

$$\% \text{ of inhibition} = (B-A)/B * 100\%$$

where A is the absorbance of the blank and B is the absorbance of the tested sample.

Assessment of elastase inhibition

Porcine pancreatic elastase (PPE) was assayed using a spectrophotometric method. This assessment was conducted using SANA as the substrate. The release of p-nitroaniline for 15 minutes at 25°C was monitored by measuring the absorbance at 410 nm. The following reagents were prepared to carry out the reaction: 2 mM Tris-HCl buffer (pH 8.0), 10 µg/mL elastase enzyme dissolved in 10% DMSO, 5 mM

SANA and 1000 µg/mL stock solution from each oil type (by dissolving 100mg from each oil up to 100ml of 10% DMSO in volumetric flask); different dilutions (10, 20, 30, 50, 80, 100, 200 µg/mL) were prepared from this stock solution. For each reaction tube, 5 mL of Tris-HCl buffer was mixed with 0.5 mL of elastase and 1 mL of each oil dilution, then pre-incubated for 10 min at 25°C before adding 0.5 mL of the SANA substrate, followed by another incubation for 15 min at 25°C. Blanks contained all the components except the seed oils. The percentage of inhibition was calculated using the following equation (Jaradat et al., 2017):

$$\% \text{ of Inhibition} = (1-B/A) \times 100$$

where A is the enzyme activity without the inhibitor and B is the activity in the presence of the inhibitor.

Assessment of the SPF value

Three samples of the three different oils were prepared to assess the sun protection factor (SPF). This test was conducted by adding 1 g of each oil to a 100

mL volumetric flask. The oils were then diluted to 100 mL with 99.9% ethanol, then subjected to ultrasonication for 5 minutes. Then, 5 mL from each oil was transferred to a 25 mL volumetric flask and the volume was adjusted using absolute ethanol (99.9%v/v). The SPF value is measured using a previously verified spectrophotometric method (Zaid et al., 2018). For this test, hydroalcoholic dilutions of ISO, TSO and PSO were prepared. The in vitro photoprotective activity was assessed using a UV spectrophotometric method in the range of 290-320 nm according to the following equation (Dutra et al., 2004; Moon et al., 2010):

$$SPF_{\text{spectrophotometric}} = CF \times \sum_{320}^{290} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

CF is the correction factor, which is equal to 10

EE(λ) is the erythemal effect spectrum

I(λ) is the solar intensity spectrum

The stated values were obtained from Table 1.

Table 1. Normalised product function used in the calculation of SPF.

Wavelength (λ nm)	EE x I (Normalised)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180

Assessment of the β-sitosterol content

The content of β-sitosterol was determined using N HPLC method. The following chromatographic conditions were adopted: mobile phase acetonitrile: ethanol (90:10), C18 column 300 mm x 4.6 mm, wavelength 210 nm and flow rate 1.8 mL/min. The products were analysed against USP β-sitosterol standard reference and the samples were diluted with hexane before injection.

RESULTS AND DISCUSSION

Skin aging is considered as one of the most common dermatologic concerns that patients are facing worldwide. In fact, in the modern culture which is usually called “youth-obsessed,” patients more and more look for cosmetic formulations and procedures that could improve the look of their skin (Helfrich et al., 2008). Actually, there are extrinsic and intrinsic factors that may cause skin aging to follow. Accordingly, the options for treatment include laser rejuvena-

tion, cosmeceuticals, chemical peels, and microdermabrasion. The use of cosmeceutical involves creams or other topical formulations that might prevent these factors including sunscreens, antioxidants, elastase inhibitors as well as wound and burn healers (Korać and Khambholja, 2011). Sesame oil is one of the most popular natural products that have been prescribed for its benefits in the cosmeceutical industries. In fact, according to the popular alternative medicine, sesame oil is used for many cosmetic purposes, especially on the skin. In fact, its antibacterial and anti-inflammatory characteristics make it effective enough to reduce pimples and prevent skin rashes. In addition, it is also prescribed as wounds healing agent. In fact, sesame oil is filled with phytochemical components that are able to provide a velvety and feel to the skin, due to the presence of fatty acids along with linoleic acid which gives it an effective moisturizing properties skin. In addition, it is also rich in vitamin E, D and B complex which makes it effective in reducing scars and other

rashes on the skin. In this contest, the inclusion of sesame oil in cosmeceutical preparation might result in a multipurpose formula that compact oxidative stress, prevent the elastase activity, and attenuate the effect of UV light. In addition, this formulation may act as burn healer especially those caused by sunlight exposer. However, this should implement the best quality of sesame oil available on the market, in order to perform the desired outcome. Accordingly, in this study we tried to evaluate the quality of sesame oils available on the market in terms of antioxidant activity, elastase inhibition, sunprotection activity as well as their content in B- sitosterols.

Market assessment

Three different sources of sesame seeds were found on the Palestinian market, from Indian, Turkish and local sources. The local (Palestinian) seeds were the most expensive at 8.5 USD per kg, while the Indian and Turkish seeds were approximately 4.3 and 3.7 USD per kg, respectively. In addition, the extraction yield for ISO, TSO, and PSO was 32%w/w, 29% w/w, and 28%w/w respectively.

Phytochemical qualitative analysis

The current study on ISO, TSO and PSO revealed the presence of medically phytochemical active components, as reported in Table 2.

Table 2. Summary of the phytochemical and nutritional content of sesame oil from different sources

Phytochemical group	ISO	TSO	PSO
Phenols and tannins	-	-	-
Flavonoids	-	-	-
Saponins	-	-	-
Glycosides (steroidal)	+	+	+
Steroids	+	+	+
Terpenoids	+	+	+

(+ Presence, - Absence)

The results demonstrated the presence of steroidal glycosides, steroids and terpenoids in all the investigated sesame oils. Steroidal glycosides have been shown to have cytotoxic effects and anticancer activity (Mansur et al., 1986). In addition, terpenoids have been found to be useful in the prevention of various diseases like cancer, and are effective as antiviral, antibacterial, anti-hyperglycaemic and anti-inflammatory agents (Nakamura et al., 1996). Accordingly, this may be of great importance when using these oils for the treatment of burns and for wound healing. However, these results showed no significant differences

between the three oils regarding the phytochemical components. Accordingly, this factor should not have any impact on the selection of oil for any cosmetic formulations.

Anti-oxidant activity

The assessment of DPPH inhibition activity showed the ability of these oils to act as free radical scavengers (Chen et al., 2005). In fact, there is a significant relationship between oxidative stress and skin aging (Rinnerthaler et al., 2015). Accordingly, any cosmeceutical formulation that include SO with the highest antioxidant activity would result in an improvement of the obtained formulation. The results of the antioxidant activity of ISO, TSO, and PSO are reported in Figure 1 and Table 3. It seems that ISO had the highest antioxidant activity, while PSO and TSO had almost identical activity. This is an important finding due to the old belief that local sesame oil may have the best activity and its nutraceutical and cosmeceutical use may justify its high price when compared with the other two sources. However, it is not recommended to pay double the cost for this oil with lower antioxidant activity.

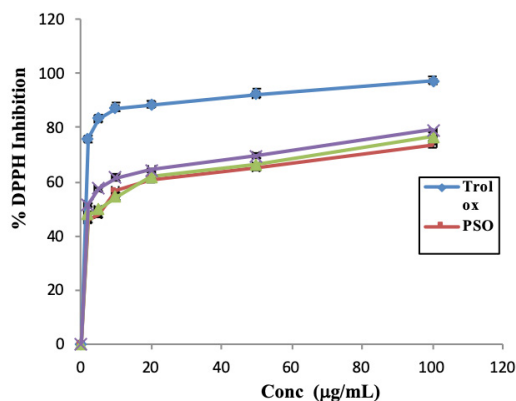


Figure 1. Antioxidant activity of the three sesame oils from different sources

Table 3. DPPH inhibition IC₅₀ value (µg/mL) of Trolox and three sesame oils from different sources

Compound	DPPH inhibition IC ₅₀ (µg/mL)
ISO	6.7±0.64 (moderate)
TSO	9.3±0.37 (weak)
PSO	9.77±0.44 (weak)
Trolox (reference standard)	1.9±1.74

Elastase inhibition

Elastase is a proteinase enzyme responsible for elastin degradation; elastic fiber accumulation in the skin dermis increases its elasticity. Inhibiting this enzyme has an anti-aging effect on the skin (Ayoola et al., 2008). In fact, skin aging and exposure to UV radiation promotes the generation and activation of human elastase. This enzyme is known to break down the extracellular matrix which promotes formation of skin wrinkles. Accordingly, the inhibition of this enzyme by sesame oil would provide the formulation with additional value in terms of skin aging retardation and protection.

In this study, elastase inhibition was studied using several concentrations of a stock solution of oleanolic acid and the three types of sesame oil. As reported in Figure 2 and Table 4, ISO showed the highest activity while PSO had the lowest activity. Accordingly, ISO is the ideal candidate for elastase inhibition in anti-stretch mark cream.

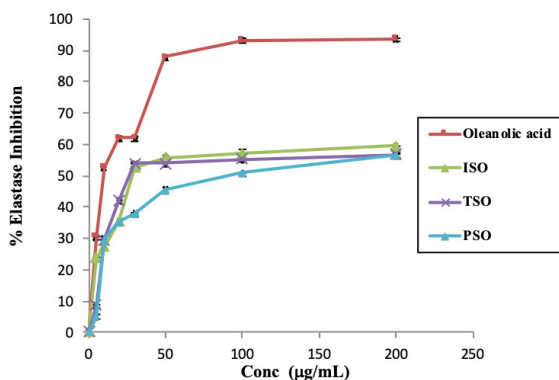


Figure 2. Elastase inhibition profile of seame oils from different sources

Table 4. Elastase inhibition by sesame oils from different sources

Compound	Elastase inhibition IC ₅₀ (µg/mL)
ISO	50.11±0.70 (moderate)
TSO	56.23±0.37 (moderate)
PSO	79.43±0.48 (weak)
Oleanolic acid	11.75±0.3 (standard reference)

Assessment of the SPF

Sunscreens are very important in cosmeceuticals. They are widely used to achieve skin protection from the hazard of UV exposure. There has been considerable effort in the cosmetic and pharmaceutical industries to produce natural sunscreens. Accordingly, any edible natural product such as fixed oils would be of

great importance in this context. In fact, this would lead to better exploitation of the oil vehicle, since it can be used as the oily phase in a cream and provide SPF value. Accordingly, sesame oil was assessed for this property with the hope of employing this vehicle to make natural sunscreens.

Table 5 shows that ISO had the highest SPF value (close to 3.2), followed by TSO and PSO. Based on these findings, we can suggest the use of ISO as it has the best properties for anti-stretch mark creams or ointments with moderate to low SPF properties. Sunscreen efficiency is assessed by the SPF value (Bernerd et al., 2003)UVB or UV-solar simulated radiation Eur. J. Dermatol. 242-249, 13-3, 2003, 1167-1122

Table 5. SPF values of sesame oils from different sources

Oil	SPF Value
ISO	3.2
TSO	3.0
PSO	2.2

Therefore, the production of a cosmeceutical agent that include seame oil would be able to have synergistic acitvities toward prevention of skin aging and keeping the skin in good conditions, since this oil would contribute not only to the nourishment of the skin but also due to its antioxidant.

Assessment of the β-sitosterol content

β-Sitosterols are phytosterols that have chemical structures analogous to cholesterol and are included in topical ointments for burn and wound healing. In fact, some commercial anti-burn products such as MEBO and AVOMEB are based on sesame oil. These oil-based natural ointments are currently used in many countries, including Palestine. These products should be rich in β-sitosterols since they promote skin epithelialisation (Jewo et al., 2009). Sesame oil is a source of β-sitosterols, which soothe, moisturise wounds and relieve pain (Ang et al., 2000; Kim et al., 2009). Accordingly, it was worthy to investigate the β-sitosterol content of sesame oils available on the Pal-estinian market, since the one with the highest sterol level should be used in making ointments for wound healing. Interestingly, ISO had the lowest β-sitosterol content (0.153% w/w), while the local source had the highest content (0.196% w/w), followed by TSO (0.194% w/w). Accordingly, TSO is the best candidate for wound healing ointments due to its β-sitosterol content and lower price when compared to PSO. In addition, including these oils in sunscreens would be beneficial not only as protection from sunburn but also as healer in case of mild sunburns.

CONCLUSION

The use of SO in the cosmeceutical and pharmaceutical industries is based on its content in several phytochemical agents that improve the quality and health of the skin. However, this depends on the level of these agents in the oil. Accordingly, three different sources of sesame oil, available on the Palestinian market, were assed for their price and their various phytochemical activities. ISO was the least expensive and showed the highest antioxidant activity, elastase inhibition and SPF value. Accordingly, it can be used in the production of sunscreens and anti-ageing creams. TSO and PSO had comparable β -sitosterol content. Accordingly, TSO can be used instead of PSO as a therapeutic agent for wound healing creams (MEBO, CALMEX and AVOMEB creams) since it is less expensive than PSO.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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