Extraction of algal lipid as a natural cosmetic component

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Purpose
The main aim of the present research is to use different methods for algal oil extraction with different organic solvents to be used as natural components in cosmetic formulations.
Materials and methods
Several extraction techniques using a cosolvent of *n*-hexane with isopropanol in a ratio of 3 : 2 (v/v) including conventional extraction, microwave-assisted extraction, ultrasound-assisted bath, and ultrasound-assisted probe extraction on three algae species (*Scenedesmus* spp., *Spirulina platensis*, and bloom) were studied against the percentage of algal lipid. The polarity effect of different solvents on the algal lipids, quantitative and qualitative, was studied with the ultrasound-assisted probe extraction method using, hexane, diethyl ether, acetone, ethanol, and chloroform–methanol. The content of essential fatty acids (FAs) in a series of omega-9 (oleic acid), omega-6 (linoleic acid), and omega-3 (linolenic acid and docosapentaenoic acid) was determined using gas chromatographic analysis.
Results and conclusion
The ultrasound-assisted probe extraction method recorded a higher lipid percentage for the species in the current study. The gravimetric analysis of lipid extractions proved that ethanol was the most producer solvent. The analysis of all FAs extracted using gas chromatography showed that there was no variance in both the classification and the mass of FAs for each algae species under study through either different techniques or with the use of different solvents. In our species of study, the FAs that were present in higher quantities were stearic (18:0), palmitic (16:0), oleic (18:1n9), linoleic (18:2n6), and α-linolenic (18:3n3). Very long rank FAs, lignoceric (24:0), and docosapentaenoic (22:5n3) acids were found. The high PUFA content of the investigated algae makes them a good source as natural materials for cosmetic industries.

Keywords:
cosmetics, microwave-assisted extraction, polar solvents, polyunsaturated fatty acids, ultrasound-assisted extraction

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Introduction

Technological development is an important path toward an environmental-friendly and sustainable way of life. Algae are phytoplankton that include a wide variety of forms ranging from microscopic unicellular organisms to multicellular organisms known as seaweeds or macroalgae. Algae have the ability of photosynthesis like plants. There are ~6.25×10^{25} algal cells in the oceans at any time that divide once per day [1]; these contribute about 40–50% of oxygen production in the atmosphere [2].

Microalgae have several advantages over terrestrial plants, for their ability to grow on nonagricultural land and as a source of a wide range of products, ranging from fine chemicals and pharmaceuticals, biofuel to foods, and feeds [3].

The success of algae cultivation is dependent on many factors; thus, variations in bioprocessing factors (i.e. temperature, pH, light, carbon source, salinity, nutrients, etc.) have been used to improve both the biomass and the productivity of bioproducts. The bioactive molecules from biological resources are a potential source of pharmaceuticals and nutraceuticals [4,5].

The main components of algal cells are proteins, carbohydrates, and lipids [6]. Microalgae naturally produce lipids as part of the structure of the cell, similar to fat stores in animals and humans [7]. Lipids produced by microalgae generally include neutral lipids, polar lipids, wax esters, sterols, and hydrocarbons, as well as phenyl derivatives such as tocopherols, carotenoids, terpenes, and quinines, and

pyrrole derivatives such as chlorophylls [8]. The most common lipids are composed of a glycerol molecule bound to three fatty acids (FAs), known as triacylglycerol, or to two FAs, with the third position taken up by a phosphate (phospholipids) or a carbohydrate (glycolipids) group. FAs consist of a long unbranched carbon chain. They are classified according to the number of carbon atoms in the chain and the number of double bonds, for example saturated fatty acid (SFA) (no double bonds), monounsaturated fatty acid (MUSFA) (one double bond), or polyunsaturated fatty acid (PUSFA) (>1 double bond). Microalgae usually contain FAs ranging from C12 to C24, often with C16 and C18 unsaturated [9,6]. Certain species contain significant amounts of PUSFAs. Storage lipids, generally in the form of triacylglycerol, accumulate in lipid vesicles called oil bodies in the cytoplasm.

Naturally, FAs are present in the skin. Palmitic acid is one of the most prevalent SFAs in body lipids. The level of palmitic acid in aging skin decreases as much as 56%; thus, it has many functions in cosmetics, from a detergent and a cleansing agent to an emollient. It helps reinforce skin's healthy barrier function for a smoother surface [10].

Oleic acid (C18:1, omega-9) is a MUSFA and distributed widely in nature. Cosmetic companies add it to lotions and soaps to boost their ability to nourish the skin. Oleic acid is often able to penetrate beyond the outer skin layer, which leads to a much longer-lasting and more intense moisture [10].

Linoleic acid (C18:2, omega-6) and linolenic acid (C18:3, omega-3) are PUSFAs that are essential in nutrition and are used as an emollient and thickening agent in cosmetics.

Some research has shown it to be effective in cell regulation and skin-barrier repair, as well as an antioxidant and an anti-inflammatory agent [10].

Generally, FAs are very important in cosmetology because of their beneficial influence, especially on the skin. They are increasingly being in many cosmetic formulations for daily care of the face and body. The use of FAs as a cosmetic base prevents water loss through the skin by creating a protective layer on the epidermis. In addition, they soften the stratum corneum and reduce inflammation of the skin, thereby decreasing the sensation of pain [11].

Several methods have been used for the extraction of lipids from microalgae, but the most common methods are liquid–liquid extraction (solvent extraction), supercritical fluid extraction, microwave, and ultrasound [12–14]. Solvent extraction proved to be successful for extraction of lipids from microalgae. In this approach, an organic solvent, such as *n*-hexane, acetone, chloroform, methanol, or ethanol, was added to algal cells (as a paste or dry). The solvent destroys the algal cell wall, and extracts lipid from the aqueous medium because of its high solubility in organic solvents. The solvent extract can then be subjected to a distillation process to separate oil from the solvent [15]. Ideally, the solvent used for the extraction process should be inexpensive and nontoxic.

Nowadays, the Soxhlet apparatus is used most frequently for extraction because of its ease of operation. The advantage of this method is its safety and the ability to scale up [16]. Another promising method used in the extraction of microalgae lipid is the application of ultrasound [17]. This method exposes algae to a high-intensity ultrasonic wave, which creates tiny cavitation bubbles around cells; the collapse of bubbles emits shockwaves, shattering the cell wall and releasing the desired compounds into the solution. Although extraction of lipid from microalgae using ultrasound is already in extensive use at the laboratory scale, sufficient information is not available on the feasibility or the cost for a commercial-scale operation. This approach seems to have high potential, but more research is needed [12,18].

Another potential extraction method worth noting is the use of pulsed electric field technology, in which cells are processed by exposing them to brief pulses of a strong electric field. Electric pulses penetrate cell walls, enhancing mass transfers across cell membranes [19], which makes it a promising pretreatment before solvent or mechanical extraction methods, or both, to recover lipids. Pulsed electric field technology is a relatively benign nonthermal process and has been used in applications such as oil extraction from rapeseed, and oil recovery from other plant products, such as maize, olives, soy beans, and juice from alfalfa [20].

The microwave technology is the simplest, and the most rapid, safe, and economical method for extracting lipid and does not require dewatering of algal biomass [21,22]. A dielectric or a polar material introduced in a rapidly oscillating electric field, such as that produced by microwaves, will generate heat because of the frictional forces arising from intermolecular and

intramolecular movements [23]. Intracellular heating results in the formation of water vapor, which disrupts the cells from within. The main aim of the present research is to use different methods for algal oil extraction with different organic solvents as natural components in cosmetic formulations. Furthermore, the study aimed to evaluate which method and solvent are safer and more economical.

Materials and methods

Three algae species were used in our investigation:

(1) *Scenedesmus* spp., which was obtained from the Algal Biotechnology Unit, National Research Council, Giza, Egypt.
(2) *S. platensis*, which was obtained from the Microbiology Department, Soils, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt.
(3) Dry biomass of microalgae community (bloom), which were collected from the high-rate algal pond constructed to treat municipal wastewater at Zinin Wastewater Treatment Plant, Giza, through the 'Biodiesel production from microalgae in stabilization pond for municipal wastewater treatment' project by Water Pollution Research Department, National Research Council, Giza, Egypt.

All chemicals and solvents used through the experimental work were analytical grade. The microalgae species mentioned above were subjected to the same extraction methods.

The conventional extraction method

A mixture (100 ml) of *n*-hexane and isopropanol (3 : 2, v/v) was added to 10 g of algal powder. The Model Wise Tis HG-150, Germany was used for algal cell disruption as a pretreatment method for 5 min at 800 rpm, and then subjected to magnetic stirring at room temperature (30–35°C) for 2 h under a reflux condenser to avoid solvent evaporation. Cells residues were removed by filtration. The filtrate was transferred to a separating funnel and sufficient hexane and water (~20 ml each) were added to induce biphasic layering. After settling, the solvent mixture was divided into two distinct phases: a top dark-green hexane layer containing most of the extracted neutral lipids and a bottom light-green aqueous-isopropanol layer containing most of the coextracted nonlipid contaminants and polar lipid. The hexane was evaporated using a rotary evaporator to enable gravimetric quantification of the lipid extract. The crude lipid was redissolved in hexane (~5 ml) for further analysis.

Microwave-assisted extraction

Dry algae (10 g) were mixed with 20 ml from 100 ml cosolvent mixture [*n*-hexane and isopropanol (3 : 2, v/v)] and subjected to pretreatment using a homogenizer; the extraction process was performed in a microwave oven (Arclike Intellewave MD-599, Japan) at 900 W power and a 5 s interval time for a total duration time of 10 min. The cosolvent was decanted and another quantity of cosolvent mixture was added. The extraction process was repeated until the solvent mixture became colorless. The neutral lipid was separated to calculate the lipid percentage of the studied species.

Ultrasound-assisted bath extraction

Ten gram of algal powder was mixed with 60 ml mixture of *n*-hexane and isopropanol (3 : 2, v/v) using a homogenizer (described above). The extraction process was performed in an ultrasonic bath (Model WUC-D10H, Germany, 60 Hz, 230 V, 665 W, 3 Amp) at room temperature (30–35°C) under a reflux condenser for 30 min. The crude lipid was obtained and the percentage of lipid based on dry weight for algal species was calculated.

Ultrasound-assisted probe extraction

The experiment of ultrasound-assisted bath was conducted using an ultrasonic probe Model Sollics Vibra Cell V500, Germany at room temperature for 15 min excluding the pretreatment step (homogenize). The extraction process by the ultrasonic probe was repeated using different organic solvents such as acetone, diethyl ether, hexane, ethanol, and a cosolvent of methanol–chloroform (1 : 1, v/v). The crude lipid percentage extracted with each solvent was determined for all algal species.

The lipid samples, after all the production processes, were transferred to sealed glass vials and stored at −20°C for further analysis.

Gas chromatography analysis of extracted oil

For all extraction processes, the FAs profiles for algal species in the present study were determined using gas chromatography (GC) provided with a split automatic injector and a silica capillary column DB-5 (length: 60 m; internal diameter: 0.32 mm). Helium was used as a carrier gas at a flow rate of 1 ml/min. The column was held at 150°C for 1 min and ramped to 240°C, at a rate of 30°C/min, and held at 240°C for 30 min. Standards

were used to obtain well-individualized peaks to enable the identification of the FAs composition.

Results

Lipid content

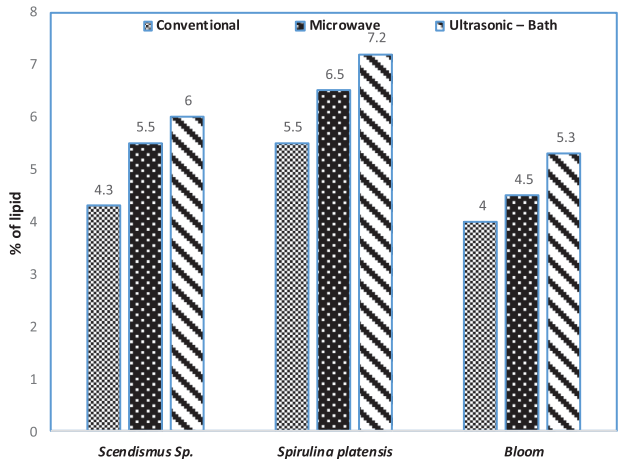
The gravimetric analysis of lipid from three microalgae species, *Scenedesmus* spp., *S. platensis*, and bloom with different techniques of lipid extraction proved that the microwave and ultrasonic bath-assisted extraction techniques led to a higher lipid yield percentage on the basis of dry microalgae than the conventional method as shown in Fig. 1.

It is clear that the lipid percentage of dry weight biomass obtained using an ultrasonic bath is higher than that obtained by the microwave-assisted technique, which recorded an oil percentage higher than the conventional method for the three tested species of microalgae. In a previous study, the microwave was the most effective in oil extraction from *Botryococcus* spp., *Chlorella vulgaris*, *Scenedesmus* spp., and *Crypthecodinium cohnii* [22,24].

Microwave technology is based on the release of very little heat to the environment and directly affects polar solvents and/or materials; thus, even when they are used on dried material, they affect trace amounts of moisture in cells. Moisture is evaporated, generating a significant amount of pressure that exerts pressure on the microorganism's cell wall, rupturing it and releasing the lipid contents [25].

The advantages of the microwave-assisted extraction method over conventional ones for algal lipid extraction are that it requires less solvent and less extraction time, but it is still difficult to scale up [24].

Figure 1



Algal lipid percentages with different techniques.

The ultrasonic-assisted bath technique improved lipid extraction through cavitation; this is in agreement with the extraction results of *C. cohnii* microalgae species [24] by the same method.

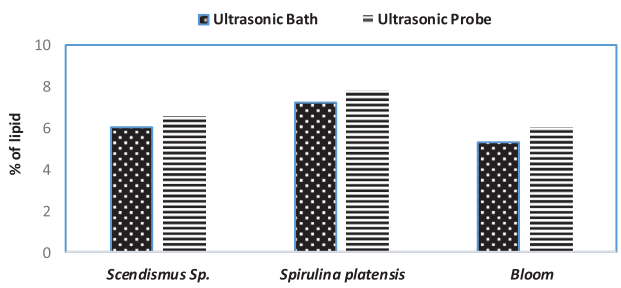
Cavitation occurs when the pressure of the liquid is lower than the pressure of the vapor bubbles in the liquid. These bubbles grow at low pressure and compress under high pressure, which leads to collapse of the bubbles. If the bubbles collapse near the cell walls, damage can occur and the cell contents are released, enhancing mass transfer and facilitating solvent access to the cell contents. This effect is much stronger at low frequencies (18–40 kHz) [26,27]. Ultrasonic-Assistant Extraction (UAE) has been proven to be a versatile technique that can be scaled up for industrial production.

Both ultrasound-assisted and microwave-assisted methods improve extractions of microalgae significantly with higher efficiency, reduced extraction times, and increased yields, as well as low to moderate costs and negligible added toxicity.

The ultrasonic bath and probe systems are based on an electromagnetic transducer (i.e. a device capable of converting mechanical or electrical energy into high-frequency sound) as a source of ultrasound power, commonly operating at a frequency of 20 kHz.

The pretreatment step is excluded using an ultrasound-assisted probe extraction technique and the probe-type sonicators can deliver up to 100-fold greater power to the extraction medium than the ultrasonic bath, so that better performance can be achieved at a low cost. The results in Figure 2 confirm this, where the lipid percentage, extracted from the dry biomass of *Scenedesmus* spp., *S. platensis*, and bloom, performed by an ultrasonic probe system using a cosolvent of hexane–isopropanol, is higher than that obtained using the ultrasonic bath system because the

Figure 2



Lipid % extracted from algae species by an ultrasonic bath and a probe.

ultrasonic probe energy is introduced directly into the extraction vessel and not transferred through the liquid medium to the system [28,29].

Effect of different solvents on lipid percentage extracted by an ultrasound probe

The yield percentages of lipid extracted from the dry biomass of algae species under investigation by an ultrasonic-probe system using different organic solvents and binary mixtures are reported in Table 1.

The results showed that ethanol has a higher extraction efficiency among the other solvents used for the tested algae biomass. This can be attributed to the fact that the efficient extraction of lipids from microalgae is highly dependent on the polarity of the solvent used [30].

In the present study, ethanol had polarity higher than the other solvents used in the following order:

Ethanol>chloroform–methanol>acetone>hexane–isopropanol>diethyl ether>hexane.

The polar solvents can open the cell walls and thus allow more solvent extraction of the cell contents [31,32]; thus, it is the most promising biorenewable solvent for lipid extraction. Ethanol and isopropanol have been proposed as polar solvents of short-chain alcohols in several studies as alternative extraction solvents because of their greater safety [33,34]. Alcohols tend to extract more unsaponifiable compounds than hexane and the lipids contain more phosphatides because of their greater polarity [35]. However, increasing the polarity of the extraction solvent accelerates the destruction of lipids' associations with cell membranes or with lipoproteins [32]. Polar solvents decrease the difference between the surface tensions in the phase boundary and improve phase separation [32]. However, the solvent polarity should be optimized because further increase could limit the solubility of lipids and lead to hydrolysis (solvolysis) of some lipids [32,34].

Table 1 Lipid percentage with different solvents by an ultrasonic probe

	Scenedesmus spp.	*Spirulina platensis*	Bloom
Hexane–isopropanol	6.5	7.8	6
Hexane	5	6	4.5
Chloroform–methanol	6.8	8	6.2
Ethanol	7.6	9	7.3
Diethyl ether	6	6.5	5
Acetone	6.3	6.8	5.5

Acetone produces a lower lipid yield because it is a polar aprotic solvent that lacks O-H or N-H bonds; thus, it does not participate in the reaction to form a hydrogen bond, serves only as a medium, and easily extracts more polar constituents such as phospholipids. It is a biorenewable solvent has a boiling point that is lower than that of hexane, and is not considered a hazardous air pollutant under the Federal Clean Air Act [34].

Sometimes, a cosolvent is used to increase the polarity of the liquid phase and to enable extraction of more components with different polarities in the same process, such as chloroform–methanol and hexane–isopropanol [32].

Diethyl ether is considered a good organic extracting solvent because it has a low polarity according to the University of Alberta's Organic Web Chem. Organic substances are generally soluble in ether and ether has a low boiling point, making its removal after the extraction very easy. This can explain the higher value of oil extracted using diethyl ether than that by hexane [32].

Fatty acid profile

GC analysis of extracted lipid showed slight or negligible differences in the FA composition of the lipid extracted either by different techniques or with various organic solvents for the same algae strain. This result is in agreement with previous studies [28,29,36].

According to our results, ethanol yields a higher percentage of oil. Furthermore, it has some advantages compared with other solvents in terms of environmental and financial benefits [33]. Table 2 shows the FAs composition of algal oils in the three investigated samples that were extracted by an ultrasonic probe using an ethanol solvent.

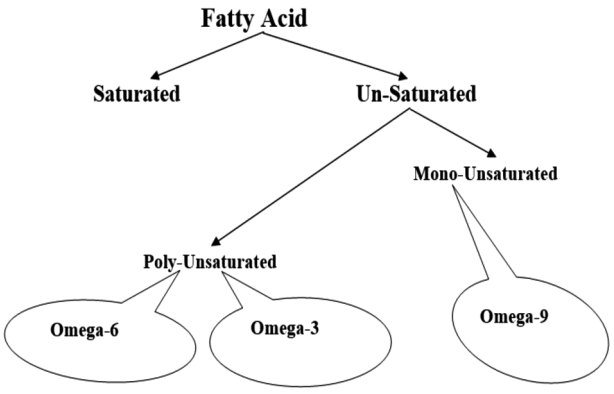
The relative amounts of FAs varied among the alga species. According to the presence and the number of bonds, FAs can be classified into saturated, monounsaturated, and polyunsaturated (including the omega-3, omega-6, and omega-9 family) (Fig. 3). This typology dictates the usefulness of cosmetic triglycerides [37,38].

A total of six SFAs were detected in the lipid of the three samples with a carbon chain from 12 to 24. Palmitic (16:0) and stearic (18:0) acids were the most abundant SFAs, but it was present in high amounts in *Scenedesmus* sp. and *S. platensis* than bloom as shown in Table 2. Small amounts of a very

Table 2 Fatty acids' composition of oil extracted by an ultrasonic probe using ethanol

Fatty acids	Mass fraction % of *Scenedesmus* spp.	Mass fraction % of *Spirulina platensis*	Mass fraction % of Bloom
Lauric acid C12	2.2	1.5	2.3
Myristic acid C14	3.2	1.4	2
Palmitic acid C16	42	40	38
Margaric acid C17	1.6	1.5	1.9
Stearic acid C18	5.9	9.7	4.2
Lignoceric acid C24	0.7	0.8	0.6
Palmitoleic acid C16:1 (omega-7)	4.8	6.3	5.2
Oleic acid C18:1 (omega-9)	13.2	10.7	12.5
Linoleic acid C18:2 (omega-6)	13.5	12.3	13.2
Linolenic acid C18:3 (omega-3)	9.2	10.8	16.4
Docosapentaenoic acid C22-5 (omega-3)	1.3	1	2.4
Saturated fatty acid	55.6	55.9	49
Unsaturated fatty acid	42	41.1	49.7
Monounsaturated fatty acid	18	17	17.7
Polyunsaturated fatty acid	24	24.1	32
Total fatty acid	97.6	97	98.7

Figure 3



Classification of fatty acids.

long-chain SFA lignoceric (24:0) acid were present in all the samples with similar values. Similar amounts of lauric (12:0), myristic (14:0), and margaric (17:0) acids were detected in algal samples and higher than lignoceric acid. These results are in agreement with these found by Muller *et al.* [39] and Terry *et al.* [40]. FAs with a chain of more than 10 aliphatic carbon atoms are nonvolatile and insoluble in water [41].

Palmitoleic and oleic acids are the MUSFAs present in the algal lipid with 16 and 18 carbons, respectively. Oleic acid is essential FA omega-9 and palmitoleic acid is omega-7 FA.

The PUSFAs are found as essential FAs omega-6 such as linoleic acid (18:2n6) and omega-3 such as α-linolenic acid (18:3n3) and docosapentaenoic acid (22:5n3).

Discussion

The main SFAs of the lipid from all algal species in the current study were found to be similar to those of shea butter, which is mainly used in cosmetics [42].The percentages of unsaturated fatty acids (USFA) as shown in Table 2, about 40% of the total FAs, are the most important factor in cosmetology and medicine [37,41] because of the fact that the free FAs on the skin can be generated as a result of decomposition of triglycerides by bacteria. On destruction of the protective layer of the transepidermal and loss of water, the epidermis produces more lipids in the lamellar granules of the stratum granulosum. Usually, with age, the reproduction of lamellar granules slows down. It has been shown that the use of lipids containing FAs, in particular, those belonging to the omega-6 series, accelerates the reproduction of lipids in lamellar granules [43].

Linoleic acid is a natural component of sebum; a decrease in the linoleic acid content leads to blocked pores and formation of comedos and eczemas. The use of linoleic acid for oily skin and problematic skin care leads to improved function of sebaceous glands, unblocking of pores, and a decrease in the number of comedos [11].

Recently, it has been proven that the omega series acids omega-9 (oleic), omega-6 (linoleic), and omega-3 (α-linolenic) can alleviate the effects of sunburn, stimulate healing processes, and soothe irritations [44].

USFAs present in algal lipids such as that in vegetable oil have found wide applications in many branches of industry, in particular, in the cosmetic industry and cosmetology, pharmacy, and medicine because of their beneficial and diverse effects on the skin.

Conclusion

The microalgae often have unusual levels of lipids with a unique structure and valuable properties.

The pretreatment of biomass is an important step to increase the lipid yield; ultrasound and microwave-assisted methods improve the extractions of microalgae lipid significantly, with higher efficiency, reduced extraction times, and increased yields. Probe-type sonicators can deliver a higher percentage of lipids than that obtained using the ultrasonic bath system at a low cost; because of the exclusion of the pretreatment step, the solvent polarity affects the lipid yield, and polar solvents such as ethanol, chloroform–methanol, and acetone had higher lipid yields than nonpolar solvent such as *n*-hexane. GC analysis showed that for algal species in the current study, there were no significant differences in FAs compositions of each species, either by different techniques or with different solvents; we have established that a variety of FAs can be found in the studied species. SFA and USFAs, palmitic (C16:0), and stearic (C18:0) acids are the most abundant saturated acids; the essential FAs are present in a series of omega-9 (oleic acid), omega-6 (linoleic acid), and omega-3 (linolenic acid and docosapentaenoic acid), with a high percentage. The USFAs play an important role in immune function by regulating inflammation and enabling the body to fight infections; the USFA present in triglycerides are used not only as base and active ingredients in cosmeceutics but also as carrier oils and penetration enhancers for the absorption of other bioactives. They make the skin look smooth and properly moistened.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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