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Review Article

Nutraceutical and Therapeutic Applications of Squalene

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Abstract

Squalene, an isoprenoid molecule present in large quantities in deep sea shark liver oil and in smaller amounts (0.1-0.7%) in palm, wheat-germ, olive and rice bran oils, is well known for its antilipidemic, antioxidant and membrane stabilizing properties. Squalene is a key precursor molecule in the biosynthesis of vitamins, cholesterol and steroid hormones in animals. In human, presence of this highly unsaturated hydrocarbon keeps the skin healthy and protects it from ultraviolet radiations mediated carcinogenesis. Recently, squalene has drawn major attention as high valuable compound due its potential applications in the field of pharmacological, cosmetic, and nutraceutical industries. The present review includes a brief description about the natural sources, properties and applications of squalene.

Keywords: Squalene, isoprenoid, antioxidant, nutracetical, applications

Introduction

Squalene is a colourless and odourless compound with low viscosity widely distributed in nature and also produced in our body. Liver oil of deep-sea sharks are the major source of squalene and smaller quantities are found in oils extracted from olive, amaranthus, palm, rice-bran, wheat-germ etc. Squalene has been reported to possess antilipidemic, antioxidant and membrane-stabilizing properties (Farvin et al., 2006). Natural products have gained considerable attention with regard to health benefits and different functional as well as active components, which are being extensively studied for exploring the effect of these compounds in protecting many disorders. Squalene is a greatly valued natural compound because of its biological and therapeutic importance. Squalene is a poly unsaturated hydrocarbon compound (C30H50) with six ð bonds and acts as physiological substance for the biosynthesis of photosterol/cholesterol in plants or animals and humans (Huang et al., 2009).

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History of squalene

Mitsumaru Tsujimoto, a Japanese Industrial Engineer successfully extracted the nonsaponifiable matter from the liver oil of sharks (“kuroko-zame’’) and identified the presence of squalene in the year 1906 (Tsujimoto, 1906). Approximately after ten years Tsujimoto perfected vacuum distillation of the unsaponifiable oil fraction from the liver of two deep sea shark species (Tsujimoto, 1916). Squalene was mostly sourced from the shark species belonging to the family Squalidae hence the name squalene was derived. After this successful innovation Dr. Keijiro Kogami reported the most beneficial effects of squalene on human health in 1930 (Fatma, , 2013). In addition a Nobel Prize winner, Paul Karrer, revealed that squalene was found in human body (Heller et al., 1963). An article in the scientific journal Nature demonstrated that squalene stimulates the activity of immune cells (macrophages) that founds in the inner and outer layer of human bodies (Heller et al., 1963). Detoxifying action of squalene was demonstrated in several experimental studies in 1982 (Richter et al., 1982b) and its radio-protective effects were discovered in 1993 (Storm et al., 1993). These discoveries pointed that the use of squalene in therapeutic preparations. Squalene can inhibit the oxidation of lipids in skin surface induced by UV radiation (Kohno et al., 1995) a significant discovery in 1995 that finally placed this compound in the scientific limelight. In the upcoming years, further studies were conducted with the aim of exploring

the beneficial properties of squalene for its potential pharmaceutical applications.

Chemical structure of squalene

Structural formula of squalene was explained by Paul Karrer, a Swiss Chemist working at Zurich University, Switzerland in the year 1936 (Fatma, 2013). Structurally squalene (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20tetracosahexane) is a 30 carbon nonpolar compound with an extremely unsaturated linear chain that cannot be produced at cellular level, but made available through squalene rich diets. It is essential for the steroids and an isoprenoid with six isoprene units, so it acts as a naturally occurring isoprenoid compound (Fig. 1). The long carbon chain structure of squalene is molded into three interrelated closed rings with six carbon atoms in each ring and attached to a five-carbon ring with a prenyl side-chain in the presence of enzymes. Squalene is structurally similar to several other compounds which include coenzyme Q10, beta carotene, vitamins A, vitamins E, and vitamins K1and the hydrocarbon composition make this compound very hydrophobic in nature and is extremely competent for suppressing the progression of tumor cells (Tomita, 1983).

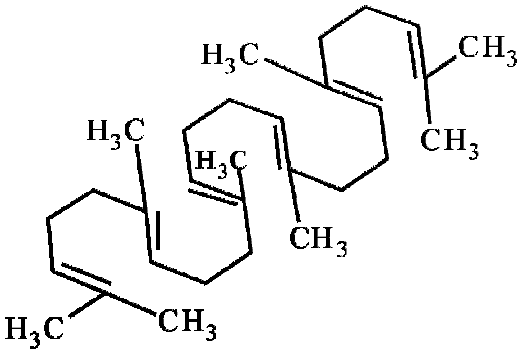

Fig. 1. Squalene– Chemical Structure

Physico-chemical properties

Squalene is glassy-white in colour, nonpolar in nature and demonstrate low viscosity (Table 1.) The oil has a pleasant bland taste with almost no odor. Squalene is not stable as it is highly unsaturated and easily get oxidised on exposure to atmosphere oxygen. Oxidized squalene has very little biological properties and nutritional value. The most common factors that promote the oxidation process of squalene include temperature, metals, water, oxygen and light.

Table 1. Chemical and Physical Properties of squalene

1	Molecular weight	410.7 g mol^{-1}
2	Solubility	Basically insoluble in water, lightly soluble in alcohol and glacial acetic acid, easily soluble in fat solvents, petroleum ether, carbon tetrachloride, acetone etc.
3	Melting point	-75°C
4	Viscosity at 25^0C	12cP(centipoises)
5	Specific gravity	0.8 - 0.86
6	Boiling point	285°C
7	Iodine number	$381g^{-1}$
8	Saponification value	30
9	Refractive index	1.492
10	Calorific value	19,400 BTU/pound
11	Flash point	110°C
12	Density	0.858 mol^{-1}
13	Infrared peak	2728, 1668, 1446, 1380, 1150, 1180, 964, 835 cm-1
14	Surface tension	32 m Nm^{-1}

Different Sources of squalene

Squalene from animal sources

The highest concentration of squalene in the living world is found in the liver of shark, with a squalene content of 40% of its body weight, especially the deep sea sharks living in the marine environment at a depth under 400 m. Nonsaponifiable fraction in deep sea sharks accounts for 50 – 80% of the liver, major portion thus represents squalene followed by some fat soluble vitamins. In deep sea sharks, the liver is the main organ for lipids storage which serves as main energy source, provides hydrostatic lift and aids in maintaining the buoyancy. Sharks inhabiting in deep sea waters (depths between 600 and 1000 m) have high-level of squalene (Deprez 1990), reported to a maximum of 80% (Wetherbee & Nichols, 2000). For example, *Centrophorous artomarginatus* a deep sea shark lives in sea at a depth in the range of 600 to 1000m and its liver accounts for 25-30%. The shark,*Centrophorous squamosus* accounts for 18.1% liver by weight and its squalene percentage is 79.6%. Similar results have been obtained for *Centroscymnus crepidater*. The deep sea shark, *Cetorhinus maximus* (basking shark) contain about 7-45% of liver oil which is composed

of squalene (Lovern, 1962). The shark, *Centrophorus scalpratus* found in Indian Ocean particularly off Andaman & Nicobar Islands contains about 80-90% unsaponifiable matter, of which 80% is squalene and the species represented the richest source of squalene. Thankappan & Gopakumar, 1991 described that the other species of sharks which is rich in squalene are Gulper shark (*Centrophorus granulosus*), Taiwan gulper shark (*Centrophorus niaukang*), Mandarian dogfish (*cirrhigaleus barbifer*), Kite fin shark (*Dalatias licha*), Bird peak dogfish (*Deania calcea*), Small-toothed sand tiger (*Carcharias ferox*) and Crocodile shark (*Pseudocarcharias kamoharai*). Squalene is secreted in the skin suface tissue (300-500 $\mu g\ g^{-1}$) and protect the skin from the UV radiation (Kohno et al., 1995). However liver oils extracted from sharks is the abundant natural source of squalene, the presence of organic pollutants limits its use as natural sources which can still be found in the purified squalene.

Squalene from vegetable sources

In addition to shark liver oil, squalene is also identified in many plant oils, especially among the oils extracted from olives. Olive oil contains a saponifiable fraction of 98-99% of its total weight as well as the nonsaponifiable matter accounts for 0.5-2%. Squalene is also found in oils extracted from wheat germ, rice bran and also found in other different sources such as, carrots, amaranthus, Phycomyces blakesleeanus mold, alfalfa, elderberry, and lettuce (Deiana et al., 2001). The highest quantity of squalene in vegetable kingdom is seen in the oil collected from *Amaranthus* sp. Extracted and purified form of squalene from Amaranth seeds accounts about 5.1-7.7% (Bhattacharjee et al., 2001). Other important vegetables sources of squalene are shown in Table 2.

Recent studies demonstrated that deodorisation distillate derived from palm oil, olive oil and soybean oil becomes the valuable natural source for squalene. Squalene content in above mentioned oils represents higher than that in other oil distillates taken out by purifying different vegetable oils. Czaplicki et al., 2012 studied the three different extraction methods like chloroform/methanol extraction, cold pressing extraction and super critical fluid extraction; for obtaining the best squalene concentration from amaranthus oil and showed that super critical fluid extraction gives best results in terms of yield and purity.

Table 2. The level of squalene concentration in various oils

Oil	mg 100 g^{-1}
Olive oil	564
Rice bran oil	332
Seal oil	35
Cod liver oil	31
Peanut oil	28
Hazelnuts	27.9
Corn oil	27.4
Grape seed oil	14.1
Soybeen oil	9.9
Butter	7
Coconut oil	2

Squalene from microbial sources

In recent years constant effort has been made to isolate squalene from microorganisms. Experimental reports demonstrated that squalene has been successfully isolated from microorganisms like yeast, bacteria and microalgae which therefore serve as an alternate source for squalene. Chang et al., 2008 revealed that a new yeast strain (*Pseudozyma* sp) from sea environmental produces a higher concentration of squalene. *Pseudozyma* sp successfully produced squalene at optimal conditions and is considered a good candidate for the commercial production of squalene. Micro algae of fresh water and marine origin are also another important source of squalene isolation (Jinang et al., 2004). Microalgae (*Schizochytrium mangrovei*) is considered as a viable alternative source of squalene and the strain *Schizochytrium* sp. CCTCC M209059 produces squalene which is similar to the squalene extracted from fish oil.

Pharmacokinetics of Squalene

Liver produces squalene and is distributed to various cells via chylomicrons in human body. Availability of squalene content in serum is directly from endogenous cholesterol synthesis and also from squalene-rich diets. Post-absorptive metabolism of squalene has not been well studied, however the existing proof indicates that squalene is absorbed 60-85%in humans (Miettinen & Vanhanen, 1994).Compared to cholesterol, orally administrated squalene is quickly absorbed into systemic circulation and is distributed to various tissues. Extremely

high concentrations of squalene content are presented inhuman adipose tissue as well as muscle tissues. Majority (80%) of the squalene in the fat cells of adipose tissue is shown to be in central neutral lipid droplet and remaining 20% is bound to the microsomal membranes which remains metabolically active. Freshly synthesized squalene (90%) is stored in lipid droplet and the rest is converted to cholesterol (Tilvis et al., 1982)

Toxicity, Side-Effects, and Dosage

Information on toxicity and side effects regarding supplementation of squalene in humans is limited because the beneficial effects of this biomolecule are not well studied yet. Different experimental studies on animal models (rats and dogs) demonstrated that there are no significant side-effects and toxic signs found in serum biochemical and liver functional analysis of squalene supplemented animals. Dietary supplementation of squalene is safe for continuous use in humans as it is an isolate of natural origin and commonly used as a dietary supplement. Years back, fishermen all over the world exploited the oils extracted from the liver oil of sharks which used to cure a wide range of conditions. Similarly olive oil, another natural source of squalene is used widely in the daily diet of Mediterranean people. Strandberg et al., (1990) reported that dietary supplementation of squalene for at least one week is sufficient for increasing the feacal squalene output (from 2 to 359 mg day^{-1}) and intestinal absorption of squalene is correctly take place without disturbing the cholesterol absorption efficiency by squalene feeding. Daily dosage recommendations differ considerably depending upon the way of its application. Generalizing from the existing literature data, 2-5 g day^{-1} is usually recommended for cancer treatment (Kelly GS, 1999).

Applications of squalene

Food

Dietary supplementation of squalene has been associated with many health benefits as it is widely accepted as nontoxic compound and there is enough proof for it. Several squalene formulations are available in market as nutraceuticals. It is essential to use naturally extracted and purified squalene obtained from a standard firm. In fact it is recommended to use squalene under the guidance of an experienced doctor.

Cosmetics

Squalene is an important component of human sebum, provides normal lubrication for hairy and non-hairy skin. Squalene is able to protect the skin from UV radiation, also takes part in repairing the impaired skin and regenerating the old skin. Squalene, one of the greatest natural emollients effectively absorbed into the skin, repairing its natural suppleness and flexibility. Squalene administration does not have oil residues on applyed in skin that makes this compound an essential constituent in cosmetic preparations like moisturizing creams, makeup, hair, lip and nail care products. Properties like odourless and colourless, high spreadability, light consistency, non-greasy texture, rapid transdermal absorption, antibacterial properties is an added advantage for squalene as an ingredient in cosmetic products. Squalene plays a vital role in caring skin from free radical oxidative damage and also used in healing eczema. It is an excellent skin protector against anti-aging and wrinkle formation (Popa et al., 2015)

Squalene in cancer treatment

It is well documented that shark liver oil is the rich source for squalene accounts more than 40%. Such squalene level is associated with the absence of cancer in sharks. In addition rare occurrence of malignacies in Mediterranean people is partially associated with their daily diet rich in olive oil. Incresed dietary squalene intake may increase the level of exogenous squalene and thus reducing the level of farnesyl pyrophosphate leading to the reduction in tumor growth. Newmark (1997) and Rao et al., 1998 reported that squalene an active element in olive oil partially liable for its chemoprotective effect. Nakagawa et al. (1985) reported that squalene has increased protective effect on cytotoxicity of certain neoplastic drugs such as adriamycin, 5-fluorouracil, bleomycin, and cisplatin. Senthilkumar et al., 2006 reported that oral administration of squalene (0.4 ml/day/rat) effectively protects the toxicity developed in liver, kidney and heart tissue induced by cyclophosphamide. Similar studies conducted by Das et al., (2003) demonstrated that squalene selectively protected normal bone marrow cells against cisplatin and other anticancer drugs induced toxicity. Considering the reports of recent studies it was suggested that squalene is an effective chemotherapeutic agent mainly for brest cancer, pancreatic tumor, colon

carcinomas, and other similar tumors. These findings indicated that squalene has potential application in cancer treatment (Alvaro & Eduardo, 2013)

Squalene as a protective agent against other diseases

Squalene is believed to protect the skin from UV radiation by its high concentration in sebum (12%). Similarly, Hashim et al. (2005) reported the radio protective effect of squalene in animal model. Furthermore it has been reported that squalene and phenolic compounds found in olive oils are obviously responsible for the protection against coronary heart diseases and aging. A study conducted by Farvin et al. (2005) demonstrated that dietary supplementation of squalene exhibits antioxidant effect against isoproterenol induced myocardial infarction by inhibiting lipid peroxidation. Buddan et al. (2007) reported that dietary squalene supplementation at 2% level is effective for improving the mitochondrial function in liverduring aging and reduce age-associated ailments. Obulesu et al. (2005) reported that dietary supplementation of squalene and n-3 PUFA is effective for improving the age associated illnesses in brain tissue. Different aches and pains developed in the human body were reduced by squalene supplementation and also help in proper functioning of the various body organs. In addition squalene helps in the shrinkage of hemorrhoids and control obesity. Squalene also acts as relaxant, generates hair and smoothens skin (Hema & Velayutham, 2018). Gopakumar, 2002 reported that squalene provides penetrating action while applying on skin and helps to prevent several types of disease and enhanced healing process.

Squalene and cholesterol metabolism

Available evidences suggest orally administered squalene is absorbed well in human body and a small fraction is turns into cholesterol. Dietary squalene supplementation causes 50% increase in cholesterol synthesis which is not directly associated with constant increase of serum cholesterol levels, but maybe with the increase in fecal elimination. A study conducted by Strandberg et al., (1990) reported that dietary squalene supplementation (900 mg day=-1 for 7-30 days) in humans has no significant change in serum cholesterol and triglyceride concentrations but17-fold increase in serum squalene level.

Detoxification of xenobiotics

Squalene is a nonpolar compound thus it shows high affinity to nonionized substances. Squalene helps in eliminating the xenobiotics which have high lipophilic property from the animal body (Kamimura et al., 1992). Squalene is capable for stimulating detoxification enzymes (p450 enzyme system) existing in liver. Some toxins that are effectively detoxified by squalene include dibenzofurans, hexachlorobifenyl, hexachlorobenzene, 12-o-tetradekanoilforbol-13acetate, and 4 (methylnitrozamino)-l-(3-pyridyl)-l(butanone) (Murakoshi et al., 1992). Richter et al., (1982a) found that squalene can be used as ansubstitute to paraffin for the removal of hexachlorobenzene and revealed dietary squalene supplementation by 8% is successful for the better feacal elimination of hexachlorobenzene. Dietary supplementation of squalene in animals at a period of three weeks has increased the fecal elimination of hexachlorobenzene by three times and reduced halflife (Richter et al., 1982a). Animal studies demonstrated that squalene increases xenobiotics elimination and accelerates the same by stimulating bile secretion. Kamimura et al., (1992) demonstrated that squalene is effective in detoxifying the various compounds such as hexachlorobiphenyl, hexachlorobenzene, theophylline, phenobarbital, strychnine and its elimation from body. Smith et al. (1998) reported that squalene has a protective activity against numerous carcinogens, including azoxymethane and nitrosaminoketone-(NMK), a derivative of nicotine induced colon, lung cancer respectively. Induction of sister chromatid exchanges and micronuclei by sodium arsenite was inhibited by squalene supplementation in Chinese hamster ovary K cells (Fan et al., 1996). Squalene supplementation at 2% level was successful for attenuating the oxidative damage affected in the heart tissue of experimental rats induced by sodium arsenite (Rajesh & lakshmanan, 2008). Farvin et al., 2009 reported that squalene showed cardiopreotective activity by improving the oxidation process against isoprenaline-induced myocardial infarction in male albino rats.

Squalene and immune system

Experimental studies showed that squalene is an optimizing factor for the immune system performance and also improves macrophage function (Sotiroudis et al., 2008). Studies suggested that biomembranes of immune cell is protected by squalene by resisting the oxidative stress developed

during phagocytosis (Owen et al., 2000). Dietary supplementation of squalene at 1% level reduced azoxymethane induced abnormal cell division and proliferation in colon by 46% and indicated that squalene have protective activity in colon cancers. Katdare et al., 1997 reported that squalene effectively inhibited abnormal high rate of cell proliferation by rapid division. Squalene appears to have an important role in inhibiting ornithine decarboxylase enzyme, which takes place in endogenous synthesis of polyamines (Murakoshi et al., 1992). An experimental study conducted in sarcoma in mice, squalene has been reported to enhance function of reticuloendothelial system, increase amount of IgM in particular, and prolong survival (Ohkuma et al., 1983).

Squalene emulsion-based adjuvants and vaccine delivery

Recently squalene emulsion – based adjuvants is approved for human use owing to its safety and effectiveness. Squalene in water emulsion stabilized with polysorbate 80 has recognized as an efficient adjuvant. An influenza vaccine containing squalene concentration of about 10 mg dose^{-1} is considered to be safe for use and has approved in European countries 1997 (Reddy & Couvreur 2009). In addition a. squalene emulsion preparation based on polysorbate 80 and glycerol is used for obtaining high antibody responses (Hjorth, 1998).

Squalene as a drug carrier

Drug carriers have several advantages in therapeutics as it provides improved half-life, toxicity reduction and enhanced drug targeting. Squalene has identified as a biocompatible material for drug delivery in either emulsion formulations or squalene based drug conjugates. Lipid nanoparticles have gained considerable attention in recent years as drug carriers for topical use. Fang et al. (2008) reported that a nanostructured lipid carrier made of squalene and precirol displayed improved permeation and controlled release of an encapsulated anti-psoriatic medicine namely psoralen. Nanoparticles based on squalene derivative of gemcitabine (SQGem) act with lipoproteins (LPs), ultimately supporting the targeting of cancer cells with high LP receptors expression. Several lipid drug conjugates wereinphase I/II clinical trials for their use in drug delivery.

Oral delivery of therapeutic substances using squalene

Dietary squalene is well absorbed in humans. Intestinal synthesized and orally administrated squalene is transported by chylomicrons into systemic circulations and is rapidly converted into bile acids and sterols in the liver. This property makes squalene an important compound to deliver orally administrated therapeutic molecules. Recently squalene is attached to four amino group of gemcitabine to get a nanoassemblies for drug delivery. Reddy et al. (2008) reported that oral administration of squalenoyl gemcitabine formulations showed comparatively slow absorption than free squalenoyl gemcitabine. In addition squalenoyl gemcitabine showed relatively improved plasma half-life and mean resident time after 24 h of treatment when compared with free squalenoyl gemcitabine. Similarly this necleoside analogue of squalene displays improved tissue distribution and better anticancer activity compared to free gemcitabine.

Squalene in gene delivery applications

Emulsions based on squalene extensively is used in gene delivery applications. Such emulsion formulations offer efficient DNA protection in serum and also identified highest gene transfection activity. Kwon et al. (2008) reported that cationic squalene emulsion/DNA complex showed prolonged gene expression mainly in liver, spleen and lung of experimental animal when injected intravenously in comparison with other liposomal DNA complexes.

Induction of antibodies by squalene

Asa et al. (2000) reported that antigenic epitopes present in squalene possess immunostimulant properties. Matyas et al. (2000) demonstrated that antibodies that are capable for binding with squalene can be produced after immunization of mice with liposomes containing squalene and lipid A. The study demonstrated that patients suffering from symptoms of Gulf war syndrome but not healthy gulf war veterans possessed natural antibodies to squalene and further proposed that it was due to the intense multiple vaccination schedule as a part of preparation for the gulf war. Some controversies arise demanding the clarification for the induction of anti-squalene antibodies following vaccination using squalene based formulations. Thus it is important to take most attention when developing squalene based emulsions for use

Physiological properties of squalene

- Cell invigorating properties

  Squalene revitalizes injured body cells and aids regeneration of cells. Its principal protective action is the prevention of cell oxidation. About 6 billion oxygen reliant cells were present in human body. Research study by Gregory & Kelly, 1999 reported that oxygenation to the healthier cells stimulates more strength to the life.

- Blood purifying properties

  The natural triterpene has a promising role in eliminating, purifying and detoxifying the body fluids from various contaminants and facilitates its circulation. It helps in proper cleaning and functioning of the gastrointestinal tract as well as kidneys which results in the healthier bowel movement also the urination. Blood purification is necessary in curing numerous diseases and such problems could be solved by supplementing squalene (Gregory & Kelly, 1999).

- Anti-aging property

  Squalene prevents cellular damage by inhibiting the production of reactive oxygen species and produces normal cells which are connected together with lipoproteins thus forming an anti-aging material (lipofuscin). This substance inhibits its production of lipid peroxides which are responsible for destroying the useful vitamins (Passi et al., 2002).

- Oxygen control property

  Squalene combined with hydrogen ions from water releases 3 unbounded oxygen molecules leading to the saturated form of squalene which results in oxygen reaching cell, causing enhancement in function of organs like kidney and liver. Squalene provides certain improvements in cellular metabolism, preventing the acidotic cell syndrome which is occuring due to the lack of oxygen. Human health and total oxygen utilization by the body cells are directly related. Thus squalene becomes a valuable source of oxygen for human body in this polluted environment (Yokota, 1995).

- Sterilizing property

  Squalene's terpene exhibites a sterilizing effect, combating the growth of various microorganisms such as dysentery bacilli, hemolytic streptococcus, *Micrococcus pyocynanel*, coliform bacilli, *Staphylococcus*, and *Candida albicans* (Masuda et al., 1982).

- Generation of body hormones

  Squalene is found to naturally increase male potency and vitality. Rukmini & Raghuram, 1989 reported that squalene aids to normalize the female menstrual cycle and improves irregular and abnormal cycles.

Conclusion

Squalene is an interesting natural biomolecule, with wide-range of applications in food and cosmetics industry and can play a key role in the prevention and management of various disorders. Current research has proved that squalene is a highly promising compound capable to offer immense functions in nutraceutical and pharmaceutical industries. Interesting applications of squalene in drug and gene delivery systems indicates the future demand for this natural triterpene. More research works are necessary to explore and estimate the possible health beneficial role of squalene in humans.

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