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Review: Exploration of Squalene from Natural Materials as its Potential in Health and Food Fields

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ABSTRACT

Squalene, a natural isoprenoid, plays a critical role in sterol biosynthesis and exhibits various health benefits, including antioxidant, anti-inflammatory, and antitumor properties. Found in sources such as shark liver oil, amaranth, olive oil, and microbial species, its demand has surged across pharmaceutical, cosmetic, and food industries. However, traditional extraction methods, predominantly from shark liver, raise sustainability concerns. This study explores alternative sources and production strategies, focusing on plant-based and microbial synthesis of squalene. Amaranth seeds and olive oil were highlighted as promising renewable sources due to their high squalene content. Advances in synthetic biology and metabolic engineering have enabled microbial platforms, such as genetically modified yeasts and microalgae, to produce pharmaceutical-grade squalene sustainably. Moreover, the development of innovative extraction techniques, such as supercritical CO_2 extraction, enhances yield and purity while minimizing environmental impact. The research further emphasizes squalene's potential as a precursor for cholesterol, hormones, and vitamins, alongside its role in improving human health through applications in vaccines, cardiovascular protection, and cancer prevention. Future work should prioritize optimizing extraction methods, exploring untapped natural sources, and scaling microbial production to address the growing global demand sustainably.

1. Introduction

The Squalene is a sterol biosynthesis intermediate metabolite found everywhere in plants and animals. Squalene is known for its nutritional benefits and antioxidant properties. Squalene is contained in rice bran oil and has gained attention for its potential health benefits, including the prevention of cancer and cardiovascular disease. However, squalene is susceptible to oxidation, which can lead to a decrease in its beneficial properties and the formation of off-flavor compounds in edible oils [1]. Squalene is an isoprenoid that serves as an intermediate in the biosynthesis of phytosterols and terpenes in plants and animals, and has antioxidant, anti-inflammatory, anti-atherosclerotic, and tumor-protective properties.[2]. All squalene is converted into cholesterol, but can be stored in fatty tissues [1]. Squalene synthase activity, as measured by serum squalene levels, correlates with serum cholesterol fraction, especially in type 2 diabetics [3].

Squalene is mainly extracted from shark liver, which is still the cheapest option. However, its extraction is not sustainable, and methods to obtain non-animal squalene are being developed and improved to meet commercial needs [4]. Squalene is a naturally occurring compound found in various biological sources such as shark liver oil, olive oil, amaranth seeds, and microorganisms like microalgae and yeast. Due to conservation concerns, there is a push to find renewable sources of squalene. With the declining shark population, there is an urgent need to develop sustainable extraction methods and explore alternative squalene sources that can be commercially exploited [5]. Squalene is a naturally occurring hydrocarbon compound that serves as a key intermediate in sterol and hopanoid biosynthesis, which is found in both eukaryotic and prokaryotic organisms [6]. In addition, squalene can also be released as a volatile compound from vegetative tissues of plants damaged by insects or from flowers [6].

In particular medicinal plants can be a viable alternative for squalene production, especially as traditional sources such as shark liver oil face environmental and health concerns [7]. Alternatives such as phytosqualene from plants and synthetic production using genetically modified microbes are explored. This article emphasizes the potential of engineered microbes to provide a sustainable and economical source of pharmaceutical-quality squalene, which can meet the growing global demand for vaccines while minimizing environmental damage [8]. Despite advances in biotechnology for the production of squalene from modified yeast, natural sources remain preferred in the market [9]. Squalene can modulate pro- and anti-inflammatory mediators to control excessive activity of neutrophils, monocytes, and macrophages. In addition, squalene helps improve steroid metabolism and reduce cardiovascular and cerebrovascular diseases. Due to its hydrophobic structure, however, it cannot be used in humans [10].

Squalene acts as a protective agent in microorganisms, plants, and animals. It plays an important role in plant growth, development, and stress tolerance [11]. Squalene can enhance immunity and disease resistance and also has a high antioxidant capacity, which can reduce lipid peroxidation damage and improve the immune status of the organism [12]. Squalene is an important component used in cosmetics for its photoprotective role and as a lubricant. It is also associated with a reduced risk of colon cancer, which is attributed to its chemopreventive properties [13]. Squalene is used directly in the food, cosmetic, and pharmaceutical industries due to its anti-cancer and antioxidant properties, as well as the ability to maintain cell membrane integrity and provide protection and moisture. Another function of squalene is as a biosynthetic precursor of cholesterol, hormones, and vitamins, which are essential for regulating cell division and growth [14]. Squalene has a wide range of health benefits, including the ability to reduce skin damage from UV radiation, lower LDL cholesterol levels, and has anticancer properties. Nonetheless, natural sources of Squalene are limited, found mainly in marine animals and some plants, and challenges in efficient extraction methods hinder its commercialization [9].

Recent developments in synthetic biology and metabolic engineering have made microbial squalene a promising alternative to traditional sources, contributing to sustainable development goals [15]. In the pharmaceutical world, squalene is used as an adjuvant in many vaccines. Squalene protects against oxidative DNA damage in human mammary epithelial cells MCF10A, although not in human breast cancer cells MCF7 and MDA-MB-231, Squalene has an effect on the tissue defense system in isoproterenol-induced myocardial infarction in mice and plays a role in cholesterol biosynthesis as a precursor of the sterol ring [16].

Squalene production can be enhanced through various strategies such as promoter engineering, compartmentalization, organelle engineering, and adaptive laboratory evolution [17]. The aim of this study is to analyze the physicochemical characteristics and stability of squalene under various conditions, in order to understand its bioactive effectiveness that plays an important role in various applications. In addition, this study aims to identify potential natural sources of squalene, including more environmentally friendly alternatives, to meet industrial needs.

2. Research Methodology

The method used in this research is a literature study or literature review. Literature review involves collecting data related to a particular theme from various sources, including research, books, official websites and other references. The inclusion criteria in this study were scientific journals that have been published without time limitation, which discuss squalene from natural ingredients for commercial production. Meanwhile, the exclusion criteria included scientific journals, both national and international, published after 2015 and did not discuss squalene from natural materials.

2.1. Literature Review

The stages in the literature review are important steps in the research. These steps identified natural materials that have the ability to produce squalene. In addition, the review investigates how squalene can be produced through natural materials. This data includes relevant journals, abstracts and scientific information. The main purpose of this literature review is to find out the studies that have been done previously related to the problem to be studied.

2.2. Jurnal Tracking Tracking

Based on journal searches through sites such as Google Scholar and several journal databases such as Garuda Portal, PubMed, and ScienceDirect, researchers found 80 journals that were relevant to the keywords used. After that, the researchers screened 75 journals, and of these journals, 54 full text journals were used for the literature review, which met the inclusion and exclusion criteria.

3. Results and Discussion

After conducting a literature study from journal sources, natural extracts that can produce squalene and effective squalene production methods are known. In this article, there are two discussions that will be presented, namely the source of squalene from plants and Squalene Production Methods.

3.1. Plants Sources of Squalene

Plant sources for squalene include olive oil, soybean oil, rice, grape seed oil, peanut, corn, and amaranth. Among these species, amaranth has the highest reported squalene content of 5942 mg/100 g. The structure of squalene can be seen in Figure 1 although olive oil is the only one used for commercial squalene extraction [9].

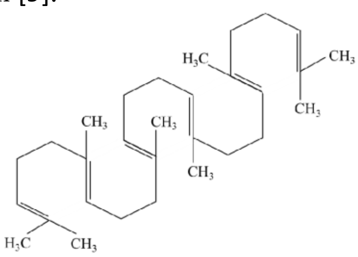

Fig.1 Structure Squalene

One important source of squalene from vegetable oils is fragrance distillates, and spinach oil has the highest squalene concentration of all kinds of vegetable oils. The present invention shows that by different methods, squalene can be extracted from various vegetable sources with high purity [18]. Research by Soetjipto (2021) showed that wild amaranth seed oil (Amaranthus dubius Mart) has a high squalene content of 94.9%, which makes it a valuable source of this compound. The oil also showed good quality after refining, with an acid value of less than 0.6 mg KOH/g and a saponification value of 208.9 mg KOH/g [19]. In a study by Park, et. Al (2016) which focused on the isolation and characterization of squalene synthase (SQS) gene from amaranth seeds (Amaranthus cruentus). The SQS gene shows high sequence identity that supports the potential of amaranth as a natural source of squalene for applications in plant biology and health [20].

Research by Maleki, et. Al (2023) investigated the presence of squalene in various medicinal plants using thin-layer chromatography (TLC). Oils from several plants-clove, opium poppy, nigella, and purslane-were extracted in this study, and squalene was found in five of them. Cloves had the highest squalene concentration, but nigella and opium poppy had higher oil yields. In addition, another study found that cockscomb and wild spinach also had high squalene content; oils from amaranthus and oil palm also had high levels of squalene [7]. In the study by Kim, et. Al. (2010) investigated squalene in panax ginseng plants. It was found that panax ginseng has several SQS genes possibly due to its tetraploid nature [21]. In the squalene analysis of the Gymnema sylvestre plant, squalene was found. This plant is known for its triterpenoid content and its use in traditional medicine [22].

In the study by Gitea, et. Al. (2023) showed that grape seed oil contains a number of beneficial compounds, such as fatty acids, sterols, squalene, phenolics, and tocopherols. Supercritical carbon dioxide extraction, for example, can produce high concentrations of these ingredients. Grape Seed Oil is known for its health benefits, such as its antioxidant and anti-inflammatory properties, as well as their ability to be used in pharmaceutical and cosmetic products. This oil is also considered a great dermatological product and dietary supplement [23]. Squalene extraction and variation from tea (Camellia sinensis) leaves based on cultivar and leaf maturity. Squalene, a triterpene that has many bioactivities, is more abundant in older tea leaves. Once optimized, the sample preparation method showed that saponification was not necessary for squalene extraction. Among the 30 tea cultivars tested, “Pingyun” cultivar had the highest squalene content, reaching 0.289-3.682 mg/g in old leaves. The results suggest that aged tea leaves can be used as a better source of natural squalene extraction [14].

In the study by Suhendra., et. al. (2021) showed that the potential of thraustochytrids microalgae found in Indonesian mangroves as an environmentally friendly source of squalene [24]. Research was conducted on olives, in the study squalene biosynthesis in olives (Olea Europaea L.). In the study Chi, et. al., (2024) found in olive plants there is squalene, after being examined when plants experience drought stress, squalene production in leaves and roots increases significantly. The purpose of the study was to find the best reference gene (RG) that could be used to analyze genes related to squalene production in olive plants (Olea europaea L.) when facing drought. One of the important genes in squalene biosynthesis is squalene synthase (SQS), whose expression is higher in leaves, especially when plants face drought. SQS gene expression can be measured to understand how much a plant or organism produces squalene. In this study SQS was found to have the highest expression in olive leaves, especially when facing drought stress, suggesting that leaves are the main organ for squalene production in olive [11].

Squalene is also found in Virgin Olive Oil or VOO. Research by Beltran, et. al (2016) analyzed squalene concentrations in virgin olive oil (VOO) from 28 olive cultivars, showing significant genetic variability in squalene content, which ranged from 110 to 839 mg/100 g, with an average of 502 mg/100 g [25]. In a study by Kumar, et. al. (2020) showed the presence of squalene content in Basella rubra L. seed oil. squalene contained in it contributes to the activity of carotenoids and flavonoids which are high antioxidants that are significant [13]. Research by Ali (2016) identified the squalene synthase (SQS) gene in Artemisia annua L., which competes with amorpha-4, 11-diene synthase (ADS) in the artemisinin biosynthetic pathway, and showed the highest expression of SQS in callus tissue. Molecular analysis revealed that the SQS enzyme has moderate stability, high thermal stability, and close evolutionary relationship with other plants [26].

In a study by Qian, et. al. (2019) which analyzed squalene synthase (SQS) proteins in the Cucurbitaceae family. The study identified 45 positively selected sites in SQS, with particular emphasis on the 196S and 407S sites associated with the regulation of triterpenoid biosynthesis. Converted domains, transmembrane domains, and phosphorylation sites were found that could affect SQS enzyme activity. From the study, it is necessary to develop the regulation of triterpenoid biosynthesis and molecular evolution of SQS in the Cucurbitaceae family [27]. Extraction and purification of squalene from Amaranthus seeds as a plant-based alternative to marine sources. The squalene content in these eleven genotypes of four species ranged from 3.6% to 6.1% of total lipids, and the purification method increased the purity to 98%. The results suggest that Amaranthus has the potential to be a valuable source of squalene for pharmaceutical, cosmetic and health applications, while reducing environmental effects [28].

Research by Shinozaki, et. al (2008) explored the biosynthetic pathway of triterpenes and sterols in the fern Adiantum capillus-veneris. Squalene cyclase (SC) and oxidosqualene cyclase (OSC) genes were successfully cloned using homology-based PCR method. The resulting products of this biosynthesis were then analyzed using GC-MS method, allowing identification of the compounds produced in the biosynthetic pathway. This study provides insight into the metabolic potential of ferns to produce important bioactive compounds, one of which is squalene [29]. Squalene production using marine microalgae Shizochytrium sp. To increase squalene yield by fermentation [30]. Thraustochytrids, have great potential as a sustainable alternative source for the production of bioactive compounds such as squalene and docosahexaenoic acid (DHA). [31].

Squalene has an important role in influencing plant growth and defense mechanisms. Research by lindo, et. al (2020) tested squalene in tomato and Arabidopsis thaliana. The combination of ergosterol and squalene showed synergistic effects on jasmonic acid (JA) and ethylene (ET) related genes. An appropriate ergosterol/squalene ratio can influence plant growth and plant-fungal interactions, allowing plants to distinguish between beneficial and pathogenic fungi [32]. Targeted genetic manipulation of the ERG1 gene could be an effective strategy to increase squalene production, which has a wide range of biotechnological applications [33]. Targeted genetic manipulation of the ERG1 gene could be an effective strategy to increase squalene production, which has a wide range of biotechnological applications [34]. Squalene can be obtained from existing plant sources as shown in Table 1, but it is not enough to meet industrial demand, so microorganisms are the main focus for sustainable squalene production.

Table 1. Sources and Content of Squalene in Natural Ingredients

Sources	Material Type	Squalene Content (mg/100g)
Virgin Olive Oil	Vegetable oil	502 (average)
Amaranth	Grains	110 – 839
Shark Liver Oil	Animal Oil	3.600 – 6.100
Panax Ginseng	Medicinal Plants	-
Nigella Sativa (Black Cumin)	Vegetable Oil	1.500 – 2.300
Palm Oil	Oil Refining Distillate	200 - 400
Microalgae Thraustochytrids	Microorganisms	15.000 – 20.000
The Old Leaf	Plants Leaves	289 – 3.682
Grape Seed Oil (GSO)	Vegetable Oil	-

In a study by Patel, et. al (2022) Thraustochytrids have great potential for squalene production, the main challenges in their genetic modification include multinucleate cells which complicate the process of genetic editing and selection [4]. Squalene was also found in the fungal isolate Talaromyces Pinophilus CJ15 from soil samples in Dandeli, Karnataka. Squalene, a secondary metabolite produced by this isolate, showed cytotoxicity against human leukemia cells with an IC_{50} of 26.22 g/ml, which can be seen in figure 2 indicating potential as an anticancer agent. Significant antimicrobial and anticancer activities have been demonstrated by this isolate [35].

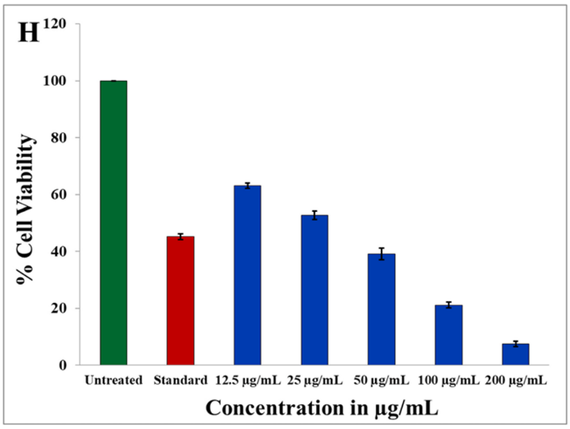

Fig.2 Graph of Cell Squalene Viability Against Leukimia Cells

Squalene can be produced through genetic engineering in the fungus Yarrowia lipolytica. The genetic transformation process produced an engineered strain named Polg17, which was then fermented using used cooking oil (WCO) as a carbon source. This strain was able to achieve the highest scualene production up to 2549.1 mg/L. This study illustrates how the combination of metabolic engineering with the utilization of waste as raw material can create a sustainable method to produce high-value metabolites such as scualene [14]. Squalene can be produced through genetic engineering in the fungus Yarrowia lipolytica. The genetic transformation process produced an engineered strain named Polg17, which was then fermented using used cooking oil (WCO) as a carbon source. This strain was able to achieve the highest squalene production up to 2549.1 mg/L. This study illustrates how the combination of metabolic engineering with the utilization of waste as feedstock can create a sustainable method to produce high-value metabolites such as squalene [12].

In a study by Marcos, et. al. (2023) squalene has complex effects on liver health, particularly in the context of non-alcoholic steatohepatitis (NASH). Squalene can reduce the accumulation of certain lipids in the liver, such as triglycerides and lipid droplet area, and modify the plasma lipid profile [36]. Squalene has potential health benefits, including a protective role against cancer and oxidative damage, as well as its role in lipid metabolism. Various extraction methods have been developed to obtain squalene, including supercritical fluid extraction (SCFE), Soxhlet extraction, and cold pressing. SCFE is considered promising due to its high purity and safety, despite its high cost [5].

3.2. Squalene Isolation and Production Method

Squalene can be extracted and separated from various sources. These include palm oil distillate through supercritical fluid extraction and fractionation with compressed carbon dioxide; soybean oil deodorization distillate through separation and purification technology; and using short-pass molecular distillation, squalene can also be separated from olive oil deodorization distillate [37]. Response surface method (RSM) was effective in maximizing the enzymatic hydrolysis of palm fatty acid distillate (PFAD). Water content of 61.40% w/w, reaction time of 7.05 h, and enzyme concentration of 2.23% w/w were the ideal conditions found. The model showed a satisfactory coefficient of determination (R2 = 0.8448) and significant impacts of water content and enzyme concentration on squalene yield [38].

An optimized green micro-saponification method for the extraction of sterols and squalene from cyanobacterial biomass shows effective and efficient results. Using an experimental design, this method can optimize important factors such as saponification time and temperature, as well as gas chromatography injection conditions. The results showed that longer saponification time and moderate temperature enhanced the recovery of stigmasterol and β-sitosterol. With the use of fewer solvents and reagents and lower energy consumption, this method is also considered environmentally friendly [39]. Squalene, which is synthesized by the enzyme squalene synthase (ERG9), is a key step in the triterpene and ergosterol biosynthetic pathway in yeast [40].

A method for rapid quantification of squalene in vegetable oils using NMR (Nuclear Magnetic Resonance) spectroscopy without the need for reference standards. The method utilizes the characteristic signal of the terminal methyl group at 1.67 ppm, allowing accurate quantification even in complex oil matrices [41]. In research by Potijun, et.al. (2021) showed that strains from green microalgae foundations. This study concluded that resistance to terbinafine can be used to screen strains with increased sterol or squalene levels in green microalgae without disrupting growth [42].

Research by Bibik, et. al. (2022) focuses on metabolic engineering to increase squalene production in plants, which is a high-value triterpene with various industrial applications. In table 2, the methods used for squalene production are classified. This study optimized the squalene biosynthetic pathway by targeting plastids and using synthetic scaffolding on lipid droplets to increase production yield. Several enzyme variants were screened, and the biosynthetic pathway was optimized, resulting in an increase in squalene yield of up to 0.58 mg/gFW, which was significantly higher than the traditional method. The results from the study suggest that engineered plants can be an effective platform for producing squalene and its derivatives, potentially benefiting a wide range of industries [43].

Table 2. Comparison of Extraction Techniques

Extraction Method	Eficiency (%)	Purity (%)	Advantages	Shortage
Soxhlet	65.3	85.7	Low cost, easy to do	High solvent consumption
Supercrticial Fluide extraction	78.5	94. 2	High purity, environmentally friendly	High equipment cost
Cold Press	54.1	81.4	Environmentally friendly, no solvents	Low Efficiency

Squalene improved growth and antioxidant status of piglets under oxidative stress, increased antioxidant enzyme activities (SOD and GSH-Px), and improved gut integrity by reducing jejunum damage and epithelial cell apoptosis. SQ shows potential as a feed supplement to support growth and health of piglets under oxidative stress conditions [37]. In the study of Unland, et. al (2018) who investigated molecular cloning in the rubber-producing plant Taraxacum koksaghyz. Squalene is a key intermediate in the biosynthesis of triterpenes and sterols. It is synthesized by the enzyme squalene synthase (SQS), which catalyzes the conversion of two molecules of farnesyl diphosphate (FPP) into squalene through a two-step process. This process involves the formation of presqualene diphosphate followed by its reduction to squalene, which requires NADPH and a divalent cation. Squalene is then converted to 2,3-oxidosqualene by squalene epoxidase (SQE), which is the first oxidation step in the triterpene biosynthetic pathway [44]. Research by Yu, et. al (2020) shows the potential of developing squalene and its derivatives into health products to address CoQ10 deficiency in mouse plasma, with significant economic and social implications [45].

Research by Drozdikova, et. al (2015) conducted a biotechnology platform research to produce squalene. Kluyveromyces lactis has great potential as a biotechnology platform for squalene production. Kluyveromyces lactis can collect large amounts of squalene, especially in lactose-based media, with a terbinafine concentration of 7.5 g/ml. This microorganism showed growth sensitivity to terbinafine comparable to Saccharomyces cerevisiae, suggesting a similar sterol biosynthetic mechanism [46]. Metabolic engineering of the cyanobacterium Synechocystis sp. PCC 6803 can also be used to increase squalene production [47]. Microalgae such as Botryococcus braunii and Chlamydomonas reinhardtii have also been studied for squalene production. Genetic engineering on these organisms has shown the potential to improve squalene synthesis [15]. The cyanobacterium P. autumnale, for example, can produce bioactive compounds such as squalene and sterols. Cultivation of cyanobacteria using complex exogenous carbon sources such as agro-industrial waste, which is also part of industrial waste treatment, can increase squalene production [39].

Research by Singh, et. al. (2015) explored the function of squalene synthase (SQS) gene in withanolide biosynthesis and plant defense mechanism of Withania somnifera (ashwagandha) through Virus-Induced Gene Silencing (VIGS) approach. The results showed that inhibition of SQS gene expression significantly decreased the levels of withanolide and phytosterols, which are key components in plant protection mechanisms against biotic stress. Plants with repressed SQS genes had decreased expression of defense-related genes, making them more susceptible to pathogen and herbivore attack. This study confirms the important role of SQS in the withanolide biosynthetic pathway and the plant defense system, while underscoring the potential of VIGS as a tool for gene function analysis in this species [48].

Research by Gao, et. al (2021) showed that the SQS gene was successfully cloned and functionally characterized from Paris polyphylla var. yunnanensis. The recombinant enzyme PpSQS1 was shown to be able to catalyze the conversion of farnesyl diphosphate (FDP) to squalene in an In vitro enzymatic assay [49]. Penelitian oleh Gao, et. al (2021) menunjukkan bahwa gen SQS berhasil dikloning dan dikarakterisasi secara fungsional dari Paris polyphylla var. yunnanensis. Enzim rekombinan PpSQS1 terbukti mampu mengkatalisis konversi farnesyl diphosphate (FDP) menjadi squalene dalam uji enzimatik in vitro [50]. Squalene synthase inhibitors have been developed as a substitute for statins to reduce cholesterol levels, and have been shown to reduce total cholesterol and LDL-C levels in plasma [3]. Squalene plays an important role in the biosynthesis of phytosterols and brassinosteroids (BRs) that support growth, development and stress tolerance in plants [51].

Squalene is an important ingredient in vaccine manufacturing due to its stability-enhancing and biocompatibility properties. These include MF59, AS03, and AF03, which are used in influenza vaccines and are being considered for COVID-19 vaccines [8]. Research shows that squalene, not other lipids such as triacylglycerol (TAG) or sterol esters (SE), correlates strongly with LD droplet (LD) clustering in yeast and mammalian cells [52]. Tellurium nanoparticles (Te NPs) can inhibit squalene monooxygenase activity, leading to squalene accumulation in Candida albicans cells [53]. Overexpression of the squalene synthase (SQS) gene in plants such as Panax ginseng, Glycine max, and transgenic tomato increases the accumulation of squalene, sterols, and ginsenosides. In addition, squalene functions as a singlet oxygen quencher, free radical scavenger, and has potential as a chemopreventive agent [54].

4. Conclusion

Squalene is a bioactive compound that plays an important role in various applications, including the pharmaceutical, cosmetic and food industries, thanks to its antioxidant, anti-inflammatory and chemopreventive properties. The main natural sources of squalene include vegetable oils such as olive oil and grape seed oil, microorganisms, and shark liver oil, although extraction from vegetable sources and microorganisms is more environmentally friendly and sustainable. Extraction methods such as Supercritical Fluid Extraction (SFE) show high efficiency and purity, although the operational costs are quite high. Further research needs to be conducted to develop more efficient, environmentally friendly and economical squalene production methods to meet the growing demand of the global industry.

References

[1] N. Shimizu, J. Ito, S. Kato, T. Eitsuka, T. Miyazawa, and K. Nakagawa, "Significance of squalene in rice bran oil and perspectives on squalene oxidation," *Journal of Nutritional Science and Vitaminology*, vol. 65, pp. S62–S66, 2019, doi: 10.3177/jnsv.65.S62.

[2] J. Sánchez-Marco *et al.*, "Thioredoxin domain containing 5 is involved in the hepatic storage of squalene into lipid droplets in a sex-specific way," *Journal of Nutritional Biochemistry*, vol. 124, 2024, doi: 10.1016/j.jnutbio.2023.109503.

[3] R. Do, R. S. Kiss, D. Gaudet, and J. C. Engert, "Squalene synthase: A critical enzyme in the cholesterol biosynthesis pathway," *Clinical Genetics*, vol. 75, no. 1, pp. 19–29, 2009, doi: 10.1111/j.1399-0004.2008.01099.x.

[4] M. Bettiga, U. Rova, P. Christakopoulos, and L. Matsakas, "Microbial genetic engineering approach to replace shark livering for squalene," vol. 40, no. 10, pp. 1261–1273, 2022.

[5] T. Rosales-Garcia, C. Jimenez-Martinez, and G. Davila-Ortiz, "Squalene Extraction: Biological Sources and Extraction Methods," *International Journal of Environment, Agriculture and Biotechnology*, vol. 2, no. 4, pp. 1662–1670, 2017, doi: 10.22161/ijeab/2.4.26.

[6] Y. Jiang *et al.*, "Volatile squalene from a nonseed plant Selaginella moellendorffii: Emission and biosynthesis," *Plant Physiology and Biochemistry*, vol. 96, pp. 1–8, 2015, doi: 10.1016/j.plaphy.2015.07.010.

[7] M. Maleki, O. N. Dehbakri, D. D. Zeidabadi, and S. Shakeri, "Reaserch Paper," vol. 3, no. 1, pp. 24–30, 2023.

[8] A. Mendes, J. Azevedo-Silva, and J. C. Fernandes, "From Sharks to Yeasts: Squalene in the Development of Vaccine Adjuvants," *Pharmaceuticals*, vol. 15, no. 3, 2022, doi: 10.3390/ph15030265.

[9] M. A. Lozano-grande, S. Gorinstein, E. Espitia-rangel, D. Gloria, and A. L. Mart, "Plant Sources , Extraction Methods , and Uses of Squalene," vol. 2018, 2018.

[10] Y. Cheng *et al.*, "A comparative transcriptomics analysis reveals ethylene glycol derivatives of squalene ameliorate excessive lipogenesis and inflammatory response in 3T3-L1 preadipocytes," *Heliyon*, vol. 10, no. 5, p. e26867, 2024, doi: 10.1016/j.heliyon.2024.e26867.

[11] H. Chi *et al.*, "Plant Stress Identification of reference genes via real-time quantitative PCR for investigation of the transcriptomic basis of the squalene biosynthesis in different tissues on olives under drought stress," vol. 14, no. March, 2024.

[12] X. Yan, S. Pan, T. Li, W. Huang, and M. Zhou, "Squalene activates Wnt / β -Catenin signaling pathway to mediate NF- κ B pathway to regulate inflammatory response and disease resistance in hybrid grouper (♀ Epinephelus fuscoguttatus × ♂ E . lanceolatu)," vol. 33, no. October, 2023.

[13] S. S. Kumar, V. Manasa, A. W. Tumaney, B. B. K., S. R. Chaudhari, and P. Giridhar, "Chemical composition, nutraceuticals characterization, NMR confirmation of squalene and antioxidant activities of: Basella rubra L. seed oil," *RSC Advances*, vol. 10, no. 53, pp. 31863–31873, 2020, doi: 10.1039/d0ra06048h.

[14] S. Wang *et al.*, "Sustainable biosynthesis of squalene from waste cooking oil by the yeast Yarrowia lipolytica," *Metabolic Engineering Communications*, vol. 18, no. June, 2024, doi: 10.1016/j.mec.2024.e00240.

[15] A. Patel, M. Bettiga, U. Rova, P. Christakopoulos, and L. Matsakas, "Microbial genetic engineering approach to replace shark livering for squalene," *Trends in Biotechnology*, vol. 40, no. 10, pp. 1261–1273, 2022, doi: 10.1016/j.tibtech.2022.03.008.

[16] D. M. Pham, B. Boussouira, D. Moyal, and Q. L. Nguyen, "Oxidization of squalene, a human skin lipid: a new and reliable marker of environmental pollution studies," *International Journal of Cosmetic Science*, vol. 37, no. 4, pp. 357–365, 2015, doi: 10.1111/ics.12208.

[17] K. Paramasivan and S. Mutturi, "Recent advances in the microbial production of squalene," *World Journal of Microbiology and Biotechnology*, vol. 38, no. 5, pp. 1–21, 2022, doi: 10.1007/s11274-022-03273-w.

[18] O. Popa, N. E. Bəbeanu, I. Popa, S. Niţə, and C. E. Dinu-Pârvu, "Methods for obtaining and determination of squalene from natural sources," *BioMed Research International*, vol. 2015, 2015, doi: 10.1155/2015/367202.

[19] H. Soetjipto, M. Krisdayanti, and N. R. Aminu, "Composition of fatty acids and squalene content of wild spinach (Amaranthus dubius mart) seed oil," *Makara Journal of Science*, vol. 25, no. 2, pp. 85–90, 2021, doi: 10.7454/mss.v25i2.1192.

[20] Y. J. Park *et al.*, "Molecular cloning, expression and characterization of a squalene synthase gene from grain amaranth (Amaranthus cruentus L.)," *Japan Agricultural Research Quarterly*, vol. 50, no. 4, pp. 307–317, 2016, doi: 10.6090/jarq.50.307.

[21] T. D. Kim, J. Y. Han, G. H. Huh, and Y. E. Choi, "Expression and functional characterization of three squalene synthase genes associated with saponin biosynthesis in panax ginseng," *Plant and Cell Physiology*, vol. 52, no. 1, pp. 125–137, 2011, doi: 10.1093/pcp/pcq179.

[22] K. A. Kalariya, R. P. Meena, L. Poojara, D. Shahi, and S. Patel, "Characterization of squalene synthase gene from Gymnema sylvestre R. Br.," *Beni-Suef University Journal of Basic and Applied Sciences*, vol. 10, no. 1, pp. 0–10, 2021, doi: 10.1186/s43088-020-00094-4.

[23] M. A. Gitea, S. G. Bungau, D. Gitea, B. M. Pasca, A. L. Purza, and A. F. Radu, "Evaluation of the Phytochemistry–Therapeutic Activity Relationship for Grape Seeds Oil," *Life*, vol. 13, no. 1, pp. 1–32, 2023, doi: 10.3390/life13010178.

[24] S. Suhendra, T. Pantoiyo, S. Fazlia, E. Sulistiawati, and R. T. Evitasari, "Bioprocess Potentials of Squalene from Thraustochytrids Microalgae for Nutraceuticals in New Normal Era Isolated from Indonesian Mangroves: A Review," *CHEMICA: Jurnal Teknik Kimia*, vol. 8, no. 1, p. 18, 2021, doi: 10.26555/chemica.v8i1.19121.

[25] G. Beltrán, M. E. Bucheli, M. P. Aguilera, A. Belaj, and A. Jimenez, "Squalene in virgin olive oil: Screening of variability in olive cultivars," *European Journal of Lipid Science and Technology*, vol. 118, no. 8, pp. 1250–1253, 2016, doi: 10.1002/ejlt.201500295.

[26] A. Ali *et al.*, "Computational characterization and in vivo expression of squalene synthase gene in different tissues of Artemisia annua L. plants," *South African Journal of Botany*, vol. 105, pp. 169–177, 2016, doi: 10.1016/j.sajb.2016.03.009.

[27] J. Qian *et al.*, "Positive Selection of Squalene Synthase in Cucurbitaceae Plants," vol. 2019, 2019.

[28] H. He, Y. I. Cai, M. ei Sun, and H. A. Corke, "Journal of Agricultural and Food Chemistry Volume 50 issue 2 2002 [doi 10.1021%2Fjf010918p] He, Han-Ping; Cai, Yizhong; Sun, Mei; Corke, Harold -- Extraction and Purification of Squalene from Amaranth.pdf," pp. 368–372, 2002.

[29] J. Shinozaki, M. Shibuya, K. Masuda, and Y. Ebizuka, "Squalene cyclase and oxidosqualene cyclase from a fern," *FEBS Letters*, vol. 582, no. 2, pp. 310–318, 2008, doi: 10.1016/j.febslet.2007.12.023.

[30] Q. Yang *et al.*, "Genetic regulation and fermentation strategy for squalene production in Schizochytrium sp.," *Applied Microbiology and Biotechnology*, vol. 106, no. 7, pp. 2415–2431, 2022, doi: 10.1007/s00253-022-11887-1.

[31] Ç. Yarkent and S. S. Oncel, "Recent Progress in Microalgal Squalene Production and Its Cosmetic Application," *Biotechnology and Bioprocess Engineering*, vol. 27, no. 3, pp. 295–305, 2022, doi: 10.1007/s12257-021-0355-z.

[32] L. Lindo, R. E. Cardoza, A. Lorenzana, P. A. Casquero, and S. Gutiérrez, "Identification of plant genes putatively involved in the perception of fungal ergosterol-squalene," *Journal of Integrative Plant Biology*, vol. 62, no. 7, pp. 927–947, 2020, doi: 10.1111/jipb.12862.

[33] V. Zambojova, S. Zuzana, P. Griac, and I. Hapala, "Squalene epoxidase as a target for manipulation of squalene levels in the yeast Saccharomyces cerevisiae," 2013, doi: 10.1111/1567-1364.12107.

[34] L. J. Wei *et al.*, "Improved squalene production through increasing lipid contents in Saccharomyces cerevisiae," *Biotechnology and Bioengineering*, vol. 115, no. 7, pp. 1793–1800, 2018, doi: 10.1002/bit.26595.

[35] M. P. Bhat *et al.*, "In-vitro investigation on the biological activities of squalene derived from the soil fungus Talaromyces pinophilus," *Heliyon*, vol. 9, no. 11, 2023, doi: 10.1016/j.heliyon.2023.e21461.

[36] L. V. Herrera-Marcos *et al.*, "Dietary squalene supplementation decreases triglyceride species and modifies phospholipid lipidomic profile in the liver of a porcine model of non-alcoholic steatohepatitis," *Journal of Nutritional Biochemistry*, vol. 112, 2023, doi: 10.1016/j.jnutbio.2022.109207.

[37] J. Tian *et al.*, "Plant-derived squalene supplementation improves growth performance and alleviates acute oxidative stress-induced growth retardation and intestinal damage in piglets," *Animal Nutrition*, vol. 15, pp. 386–398, 2023, doi: 10.1016/j.aninu.2023.09.001.

[38] M. Jawad, R. Schoop, A. Suter, P. Klein, and R. Eccles, "Perfil de eficacia y seguridad de Echinacea purpurea en la prevención de episodios de resfriado común: Estudio clínico

aleatorizado, doble ciego y controlado con placebo," *Revista de Fitoterapia*, vol. 13, no. 2, pp. 125–135, 2013, doi: 10.1002/jsfa.

[39] M. B. Fagundes *et al.*, "Green microsaponification-based method for gas chromatography determination of sterol and squalene in cyanobacterial biomass," *Talanta*, vol. 224, no. October 2020, 2021, doi: 10.1016/j.talanta.2020.121793.

[40] J. Cheng *et al.*, "Development of a green Komagataella phaffii cell factory for sustainable production of plant-derived sesquiterpene (–)-α-bisabolol," *Synthetic and Systems Biotechnology*, vol. 10, no. 1, pp. 120–126, 2025, doi: 10.1016/j.synbio.2024.09.006.

[41] Amit, S. Kumari, R. Jamwal, P. Suman, and D. K. Singh, "Expeditious and accurate detection of palm oil adulteration in virgin coconut oil by utilizing ATR-FTIR spectroscopy along with chemometrics and regression models," *Food Chemistry Advances*, vol. 3, no. November 2022, p. 100377, 2023, doi: 10.1016/j.focha.2023.100377.

[42] S. Potijun, S. Jaingam, N. Sanevas, S. Vajrodaya, and A. Sirikhachornkit, "Green microalgae strain improvement for the production of sterols and squalene," *Plants*, vol. 10, no. 8, pp. 1–13, 2021, doi: 10.3390/plants10081673.

[43] J. D. Bibik *et al.*, "Pathway Engineering, Re-targeting, and Synthetic Sca ff olding Improve the Production of Squalene in Plants," 2022, doi: 10.1021/acssynbio.2c00051.

[44] K. Unland *et al.*, "Functional characterization of squalene synthase and squalene epoxidase in Taraxacum koksaghyz," *Plant Direct*, vol. 2, no. 6, pp. 1–15, 2018, doi: 10.1002/pld3.63.

[45] W. Yu *et al.*, "Investigation of the Effects of Squalene and Squalene Epoxides on the Homeostasis of Coenzyme Q10 in Rats by UPLC-Orbitrap MS," *Chemistry and Biodiversity*, vol. 17, no. 8, 2020, doi: 10.1002/cbdv.202000243.

[46] E. Drozdíková, M. Garaiová, Z. Csáky, M. Obernauerová, and I. Hapala, "Production of squalene by lactose-fermenting yeast Kluyveromyces lactis with reduced squalene epoxidase activity," *Letters in Applied Microbiology*, vol. 61, no. 1, pp. 77–84, 2015, doi: 10.1111/lam.12425.

[47] B. Pattanaik, E. Englund, N. Nolte, and P. Lindberg, "Introduction of a green algal squalene synthase enhances squalene accumulation in a strain of Synechocystis sp. PCC 6803," *Metabolic Engineering Communications*, vol. 10, no. February, p. e00125, 2020, doi: 10.1016/j.mec.2020.e00125.

[48] A. K. Singh *et al.*, "Virus-induced gene silencing of Withania somnifera squalene synthase negatively regulates sterol and defence-related genes resulting in reduced withanolides and biotic stress tolerance," *Plant Biotechnology Journal*, vol. 13, no. 9, pp. 1287–1299, 2015, doi: 10.1111/pbi.12347.

[49] J. X. Gao, Y. G. Chen, D. Sen Li, L. Lin, Y. Liu, and S. H. Li, "Cloning and Functional Characterization of a Squalene Synthase from Paris polyphylla var. yunnanensis," *Chemistry and Biodiversity*, vol. 18, no. 7, pp. 1–10, 2021, doi: 10.1002/cbdv.202100342.

[50] P. Peltola *et al.*, "Visceral obesity is associated with high levels of serum squalene," *Obesity*, vol. 14, no. 7, pp. 1155–1163, 2006, doi: 10.1038/oby.2006.132.

[51] C. Vriet, R. Eugenia, and C. Reuzeau, "From squalene to brassinolide: The steroid metabolic and signaling pathways across the plant kingdom," *Molecular Plant*, vol. 6, no. 6, pp. 1738–1757, 2013, doi: 10.1093/mp/sst096.

[52] M. T. Ta *et al.*, "Accumulation of squalene is associated with the clustering of lipid droplets," *FEBS Journal*, vol. 279, no. 22, pp. 4231–4244, 2012, doi: 10.1111/febs.12015.

[53] B. Zare, Z. Sepehrizadeh, M. A. Faramarzi, M. Soltany-Rezaee-Rad, S. Rezaie, and A. R. Shahverdi, "Antifungal activity of biogenic tellurium nanoparticles against Candida albicans and its effects on squalene monooxygenase gene expression," *Biotechnology and Applied Biochemistry*, vol. 61, no. 4, pp. 395–400, 2014, doi: 10.1002/bab.1180.

[54] P. Liao, X. Chen, M. Wang, T. J. Bach, and M. L. Chye, "Improved fruit α-tocopherol, Carotenoid, Squalene And Phytosterol Contents Through Manipulation Of Brassica Juncea 3-Hydroxy-3-Methylglutaryl-Coa Synthase1 in transgenic tomato," *Plant Biotechnology Journal*, vol. 16, no. 3, pp. 784–796, 2018, doi: 10.1111/pbi.12828.