AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

# Evaluation of antioxydant and antibacterial activities of squalene produced by *Saccharomyces cerevisiae* strain under aerobic and anaerobic conditions

Benlounissi Aïcha[1*], Bouldjadj Doria Imene[1]

Bioengineering laboratory, Higher National School of Biotechnology Taoufik Khaznadar Constantine, Algeria[1]

Corresponding author: 1*

**Keywords:**
Fermentation, production, *Saccharomyces cerevisiae*, squalene.

**ABSTRACT**

The study focuses on the production of squalene from *Saccharomyces cerevisiae* for cosmetic or medical applications. Monitoring the growth kinetics of the yeast highlighted its metabolism under both aerobic and anaerobic conditions. The sugar-rich culture, along with the extraction techniques, demonstrated their efficiency following squalene analysis using HPLC and FT-IR. The antioxydante and antibacterial activities were tested.



## 1. Introduction

The global demand for bioactive ingredients has significantly increased in recent decades due to the cosmetic and pharmaceutical industries, which are major consumers of biomolecules [1]. These industries must constantly seek to improve both standard and innovative formulations. This is where microbial biotechnology comes into play, used for generating new industrial, pharmaceutical, and medical compounds. It is employed as a tool to aid in the development of more efficient systems for commercial production processes [2].

Squalene, a polyunsaturated triterpene, is known for its various biological roles as an antioxidant, anti-cancer agent, chemopreventive agent, antibacterial agent, and vaccine adjuvant. It has become a preferred target for pharmaceutical, dietary supplement, and cosmetic industries due to its numerous positive effects observed on human skin [3]. Traditionally, squalene has been extracted from shark liver illegally and inhumanely. Subsequently, it has been produced from plant sources such as olive oil or amaranth, but with very low yields [4].

The global squalene market is projected to reach a value of $241.9 million USD in 2022, primarily driven by skincare and beauty products. However, with the current pandemic situation and the urgent need to rapidly produce anti-SARS-CoV-2 vaccines to immunize the global population, there is a growing demand for this triterpene for extensive industrial use. Therefore, it has become necessary to develop new alternative biotechnological systems that lead to high yields using ethical sources [5], [6].

Microbial production of squalene has recently attracted researchers' attention due to its sustainability, environmental friendliness, and affordability compared to exploited methods [7]. Among these new resources, the yeast Saccharomyces cerevisiae stands out as it is easy to genetically and physiologically manipulate, readily available, and rich in squalene as it naturally synthesizes it during its metabolism [8]. This yeast has been widely used by humans for thousands of years and is undoubtedly one of the most important microbial species in human history [9].

For all these reasons, this study has focused on producing squalene from the yeast *Saccharomyces cerevisiae*, cultivated in a medium rich in carbon sources. The goal is to quantify the produced molecule and highlight its antioxidant and antibacterial activities, preparing it for potential use in cosmetics.

## 2. Methods

### *2.1 Preparation of the S. cerevisiae strain*

The strain used, S. cerevisiae, is a commercial strain: "Saf instant" brewer's yeast, which consists of an agglomeration of microscopic living cells and is in the form of a water-soluble powder. The strain was reactivated in sterile distilled water at a ratio of 1g per 10 ml and incubated at 30°C in an oven (Memmert) for 24-48 hours. Subsequently, 10µl of the suspension was plated on nutrient agar and incubated for 48 hours at 37°C. Microscopic and macroscopic observations were made.

### *2.2 Cell counting*

Cell counting was performed using a Malassez cell (MARIENFELD) and an optical microscope (Optika Microscopy-Italy) with a 40x objective lens.

### *2.3 Aerobic culture of the microorganism*

The S. cerevisiae strain was cultured in 50 mL of modified YPD culture medium in 250mL Erlenmeyer flasks. After cooling, the flasks containing the medium autoclaved at 120°C for 20 minutes were inoculated with 2.2*10^8 cells/ml of the yeast suspension and incubated in a rotary incubator (Edmund Buhler GmbH) at 44g at 30°C for 48 hours.

### *2.4 Anaerobic culture of the microorganism*

After 48 hours of culture, 5% of the aerobic culture was inoculated onto fresh medium onto which a 1cm thick layer of paraffin oil was deposited to maintain anaerobic conditions. The culture was incubated under the same conditions as mentioned above.

### *2.5 Sugars analysis*

Sugars were quantified using the colorimetric method described by [10], based on the use of two reagents: sulfuric acid ($H_2SO_4$) and phenol. Concentrated sulfuric acid causes dehydration of sugars, leading to the formation of hydroxymethylfurfural (HMF) in the case of hexoses and pentoses. These substances then condense with phenol to form colored compounds (yellow-orange). The intensity of the coloration is proportional to the concentration of sugars. Absorbance was measured at 490 nm.

### *2.6 Lipids analysis*

The fat content was determined using the Röse-Gottlieb method [11], known as a reference method for lipid analysis in dairy products. This method involves treatment with ammonia and ethanol to denature proteins, and petroleum ether to remove water for accurate lipid measurement. Centrifugation followed by processing was used to determine the fat content in grams per 100mL of sample.

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

### *2.7 Biomass quantification*

To determine the biomass content, the culture was collected in Falcon tubes, then centrifuged (SIGMA centrifuge) at 20,000g for 20 minutes. The supernatant was removed, and the lower phase was dried in an oven (Memmert) at 105°C overnight. Weighing was performed after this period, and then the samples were placed in a desiccator. Weighing (KERN PLS balance) was done every 15 minutes until the weight stabilized.

### *2.8 Squalene production*

Squalene production was carried out in 400ml of culture medium in 1L Erlenmeyer flasks. Cultures were done in triplicate. In the case of anaerobic culture, 400ml of culture medium was inoculated with 16ml of *S. cerevisiae* suspension, and the culture was incubated at 30°C for 48 hours in a rotary incubator (Edmund Buhler GmbH) at 44g. The subsequent steps were identical to those mentioned earlier.

#### *2.8.1 Squalene extraction by centrifugation*

Squalene was extracted from the culture medium by centrifuging it at 10,000g for 20 minutes at +4°C to collect the cell mass. This mass was frozen at -20°C overnight and then lyophilized at -50°C under vacuum for 8 hours (Cryotec vacuum). The resulting powder was dispersed in 80 ml of chloroform-methanol solution (2:1, v/v) [12]. The solution underwent sonication (Nahita Model 610/22) for 5 minutes under cold conditions. Squalene extraction was carried out overnight at 30°C, 180 rpm in agitated flasks (Ibx Instruments), while the volume of the organic solvent mixture chloroforms: methanol was increased to 120 ml. Subsequently, filtration on Whatman No. 1 paper was done, followed by flash evaporation under vacuum of the solvents in round-bottom flasks at 50°C and 300 psi.

#### *2.8.2 Squalene extraction by saponification*

According to Adams and Parks (1968) method, squalene and sterols were quantified after alkaline saponification (60% KOH, 45°C) in the presence of phenol [13]. The cell mass was collected by centrifugation at 10,000g for 20 minutes, after treatments the dried samples were re-suspended in 1 mL of chloroform and stored at -20°C until use.

### *2.9 Squalene content analysis*

#### *2.9.1 High-Performance Liquid Chromatography analysis (HPLC)*

HPLC analysis was performed using an ultraviolet detector (Shimadzu) and a C18 column. HPLC-grade acetonitrile was used as the mobile phase at a flow rate of 1 ml/min at room temperature. Squalene was detected at a wavelength of 190 nm [14].

#### *2.9.2 Fourier-transform infrared spectroscopy analysis (FTIR)*

Fourier-transform infrared spectroscopy (FT-IR) (Shimadzu) was performed using chloroform ($CHCl_3$) as the solvent.

### *2.10 Antioxidant activity test*

The determination of antioxidant activity using the DPPH test was carried out according to the slightly modified method [15]. This test is based on a redox reaction with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, used to determine the antioxidant capacity of squalene. The radical has a violet color due to the unpaired nitrogen electron, and after reacting with the oxygen atom of a radical scavenger, DPPH-H (2,2-diphenyl-1-picrylhydrazine) is formed, which turns yellow.

The DPPH radical is expressed as the corresponding 50% inhibitory concentration (IC50), which is the

concentration of polyphenols needed to inhibit or neutralize 50% of the initial concentration of DPPH. Absorbance is measured at 517 nm.

### *2.11 Antibacterial activity test*

The antibacterial activity of the extracted squalene was determined against five strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus*. Bacterial suspensions were streaked on MH medium and incubated at 37°C for 18-24 hours. From the cultures, seeding was done in sterile physiological water, and OD was measured at 650nm. A second seeding by swabbing bacterial suspensions on solid MH medium in Petri dishes was performed. Sterile discs soaked in the solution containing diluted squalene in chloroform (10µl), as well as a negative control disc, were placed in each Petri dish. A first incubation at 4°C for 1 hour was done to allow the solution to diffuse into the agar while temporarily stopping bacterial growth, followed by a second incubation at 37°C for 24 hours [16].

## 3. Results

### *3.1 Cell counting*

The cell count of the *S. cerevisiae* strain using a Malassez cell and an optical microscope (Optika Microscopy-Italy) (40x objective) yielded a concentration of 1.1 * 10^8 CFU/ml.

### *3.2 S. cerevisiae growth*

S. cerevisiae growth was monitored for 4 days in the presence and absence of oxygen. Daily samples were taken and results are reported on Figures 1 and 2.

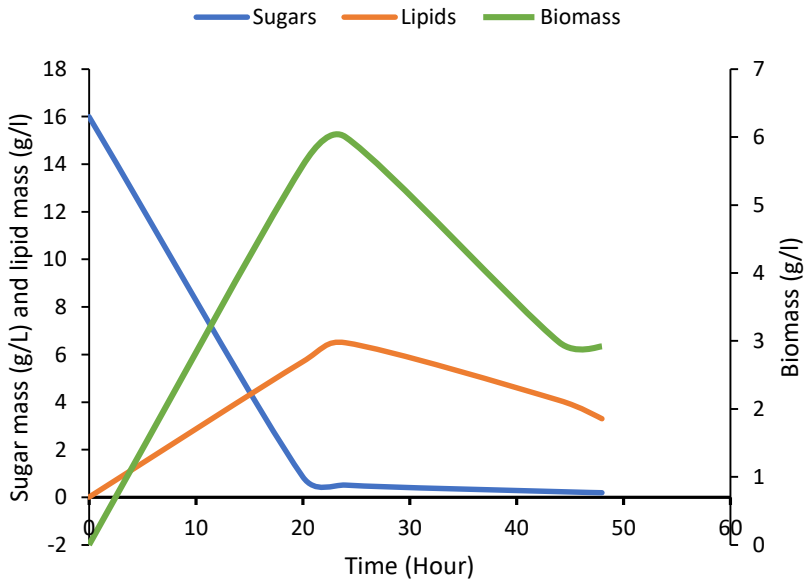


**Figure 1.** Growth curve of Saccharomyces cerevisiae under aerobic conditions

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

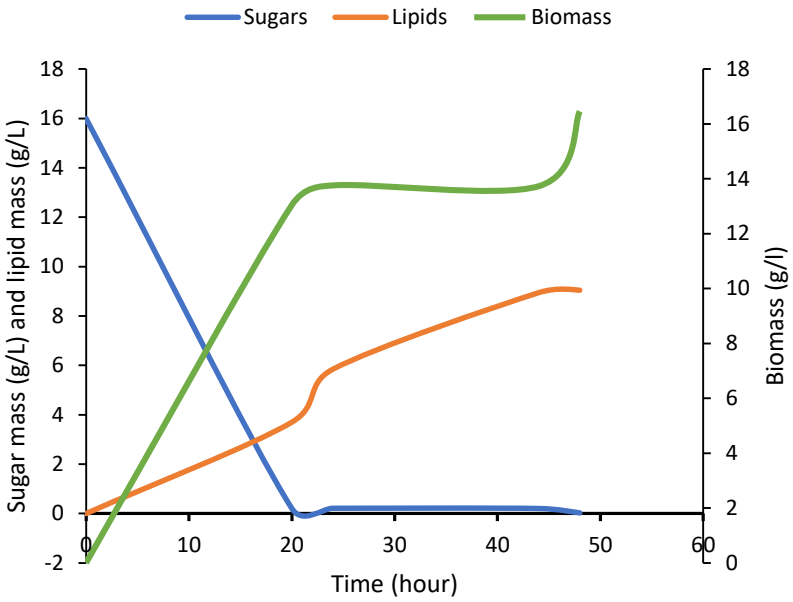


**Figure 2.** Growth curve of Saccharomyces cerevisiae under aerobic conditions

### *3.3 Squalene extraction*

The product obtained after the extraction processes described earlier has an oily consistency, is colorless, and odorless, thus perfectly matching the characteristics of the hydrocarbon studied [17].

### *3.4 High-Performance Liquid Chromatography (HPLC)*

The figure 3 shows multiple elution peaks, indicating that the extract obtained is not pure. The elution peak of squalene, as shown in Figure 4, has a retention time (RT) of 12.977 min.

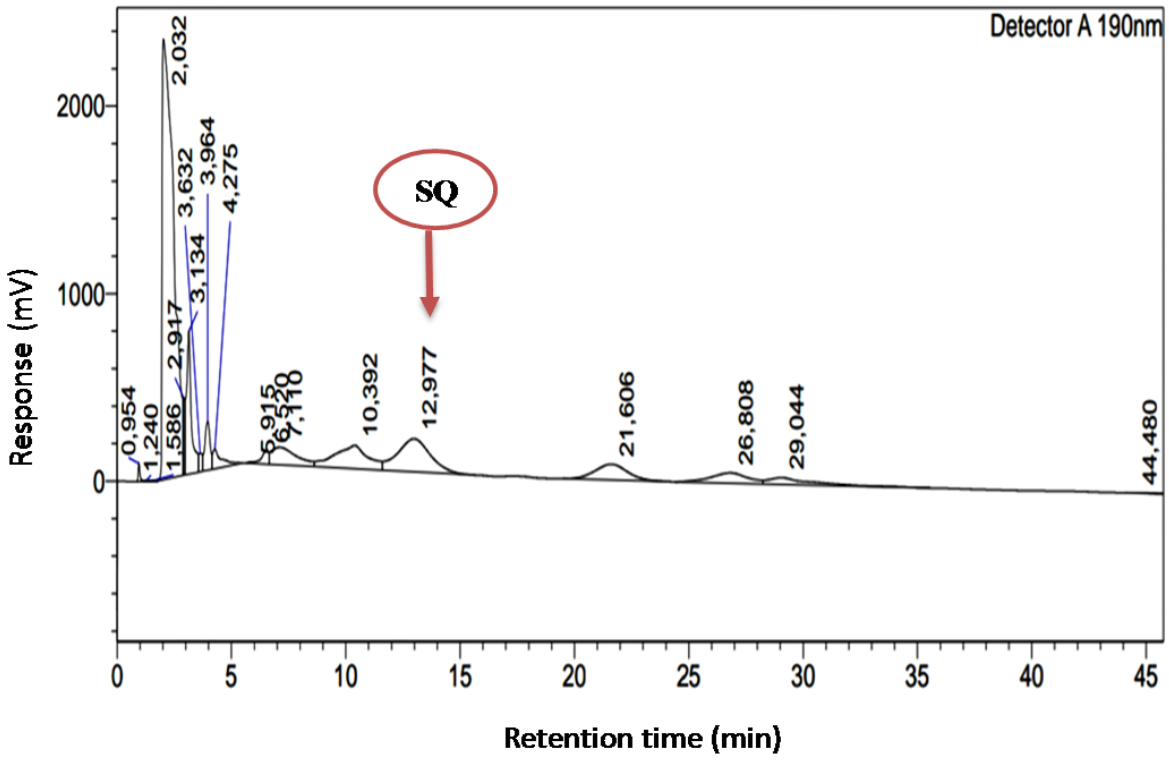

**Figure 3.** Result of the HPLC analysis of squalene

### *3.5 Fourier-transform infrared spectroscopy (FTIR)*

The results of the analysis by Fourier-transform infrared spectroscopy (FTIR) for squalene are represented in Figure 4. The spectrum shows peaks of high intensity at 2922.16 $cm^{-1}$, 2852.72 $cm^{-1}$, and 759.95 $cm^{-1}$, and other less intense peaks at 1458.18 $cm^{-1}$ and 1209.21 $cm^{-1}$.

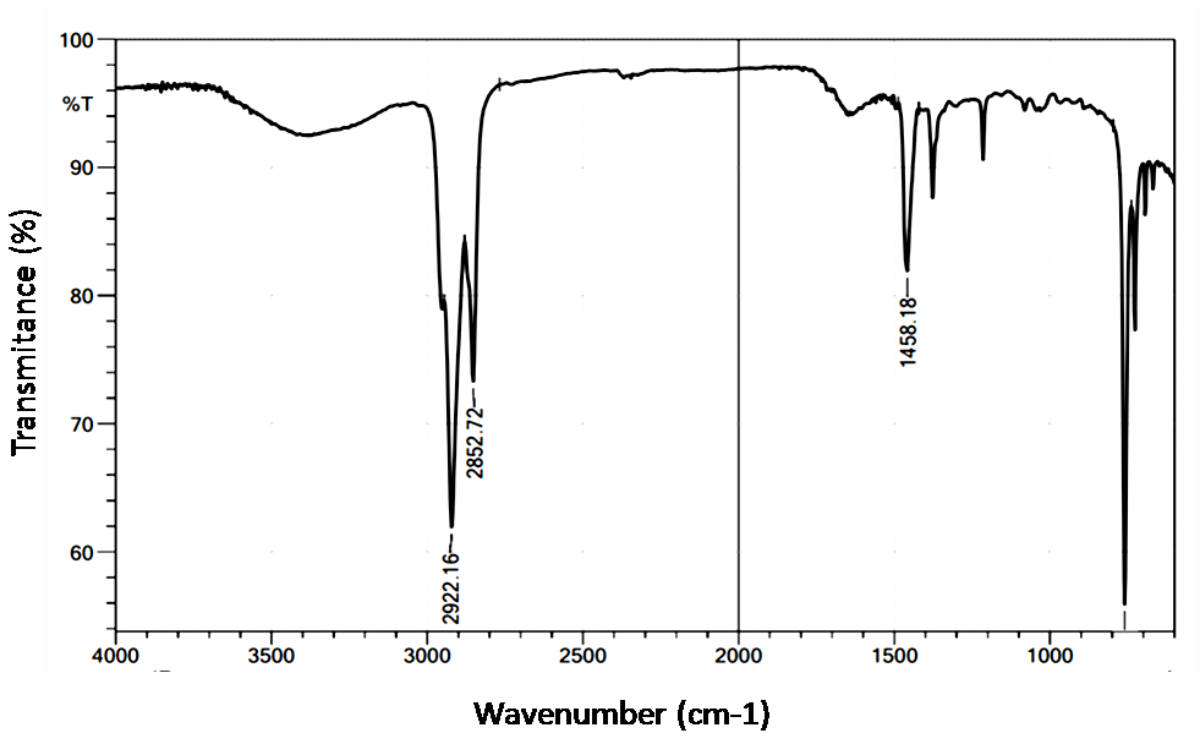


**Figure 4.** Result of the squalene analysis by FT-IR

### *3.6 Antioxydant activity*

The antioxidant activity of squalene was tested by the DPPH free radical scavenging method, the results are expressed as percentages of IP inhibition (%). The inhibition percentages (IP) calculated for the molecule as well as for ascorbic acid used as a standard are presented in Tables 1 and 2.

**Table 1.** Inhibition percentages of squalene (SQ)

| Concentration (µg/ml) | 7.81 | 15.62 | 31.25 | 62.5 | 125 | 250 | 500 |
|---|---|---|---|---|---|---|---|
| IP% (SQ) | 17.46 ± 4.508 | 19.5 ± 1.931 | 25.066 ± 4.637 | 34.1 ± 8.860 | 37.7 ± 9.364 | 44.4 ± 7.778 | 52.13 ± 2.571 |

**Table 2.** Inhibition percentages of vitamin C

| Concentration (µg/ml) | 3.91 | 7.81 | 15.62 | 31.25 | 62.5 | 125 | 250 | IC50 |
|---|---|---|---|---|---|---|---|---|
| IP% (Vit C) | 20.37 ± 3.288 | 33.9 ± 8.244 | 46.12 ± 1.463 | 84.62 ± 3.068 | 90.34 ± 3.726 | 97.41 ± 1.463 | 98.96 ± 0.148 | 2.80 ± 0.028 |

These indicated values were used to plot the graphs (Figures 5 and 6) of I (%) = f(C) for each of the extracts used. The curve of the percentage inhibition (IP) of ascorbic acid (Figure 5) shows an increase in IP correlated with an increase in concentration; indeed, it is used as a reference and inhibited the DPPH radical

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

by 84.62 ± 3.068 % at 31.25 µg/mL. Then, its activity has stabilized until reaching a very high percentage of 98.96 ± 0.148% at 250 µg/mL.

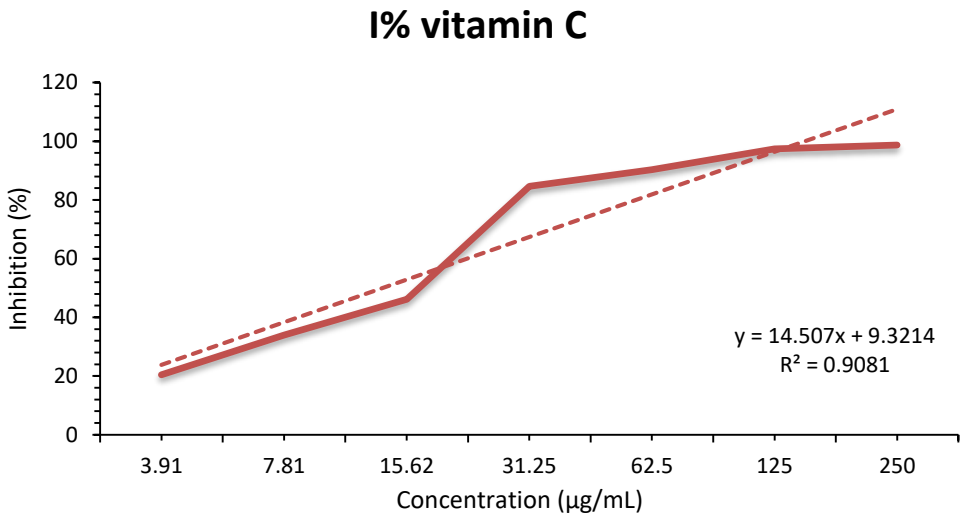


**Figure 5.** The inhibition percentages (PI) of ascorbic acid

The PI curve of squalene illustrated in Figure 6 shows an increase in PI correlated with the concentration of the extract. It reaches a IP exceeding 50% when reaching 500 µg/mL.

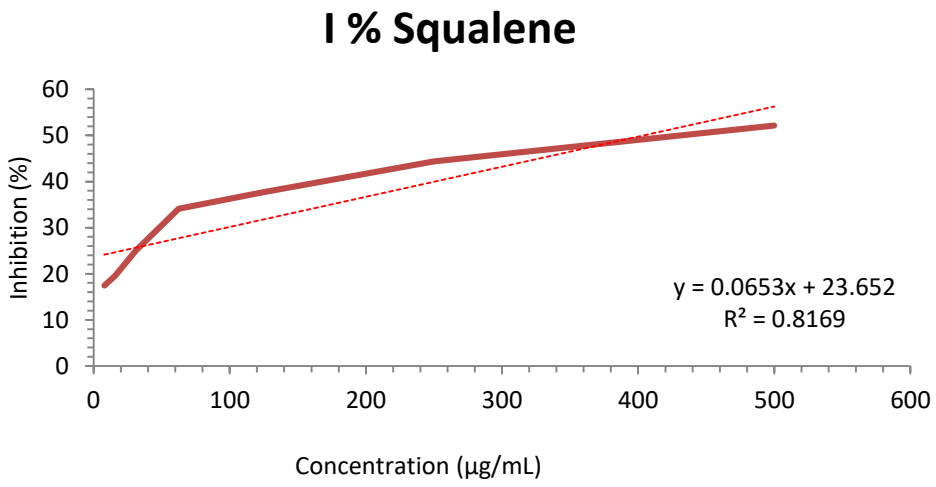


**Figure 6.** The inhibition percentages (IP) of squalene

The linear shape of the curves representing the inhibition percentages (IP) of each extract allowed us to calculate the IC50 values from the linear part of the squalene and Vitamin C curves, presented in the form of a histogram (Figure 7).

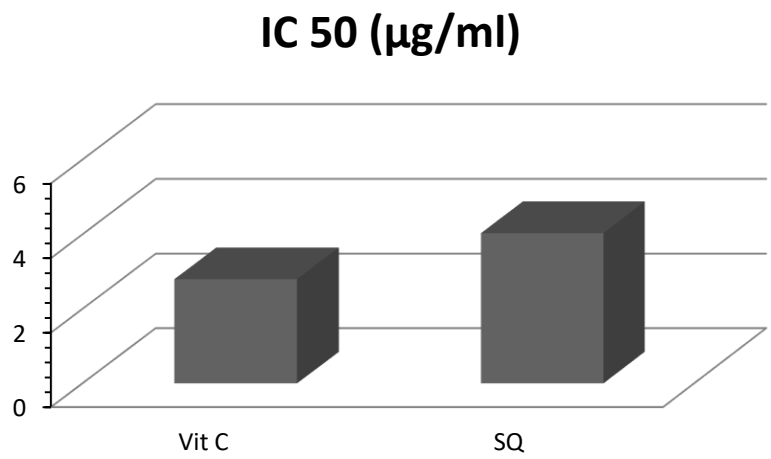


**Figure 7.** IC50 of Vitamin C and Squalene.

The results obtained have shown the effectiveness of squalene in trapping free radicals, although the percentage remains low, it represents antioxidant activity with an inhibition percentage of 52.13 ± 2.571% at a concentration of 500 µg/mL of extract. The results indicate that Vitamin C has a lower IC50 than squalene, estimated at 2.80 µg/mL and 4.034 µg/ml respectively. This means that Vitamin C has a better and higher antioxidant activity than squalene.

***3.7 Antibacterial activity***

The antibacterial activity of squalene was tested against five strains: *E. coli*, *P. aeruginosa* (Gram-negative), *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus* spp. (Gram-positive). This was observed after 24 hours of incubation (Figure 8). This figure allowed us to visualize the zones of inhibition of the molecule of interest and calculate the inhibition diameters.

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

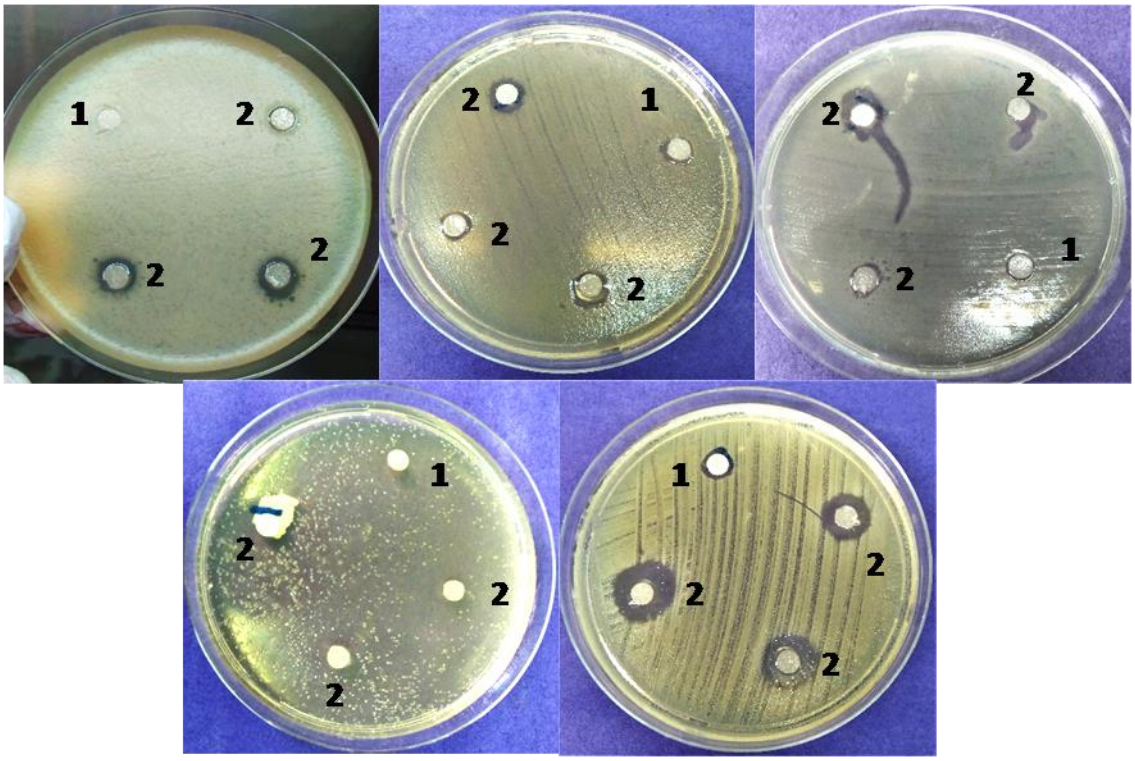


**Figure 8.** Antibacterial activity of squalene against strains (a): Bacillus cereus; (b): Escherichia coli; (c): Enterococcus spp.; (d): Pseudomonas aeruginosa; (e): Staphylococcus aureus; (1): Control and (2): Squalene.

**4. Discussion**

The *S. cerevisiae* strain is known for its ability to grow in anaerobic and aerobic conditions [18]. Figure 1 depicts the growth of the yeast strain in the presence of oxygen, which lasts approximately 48 hours. Upon observing the graph, no lag phase is evident, possibly due to a rapid adaptation of *S. cerevisiae* to the culture medium. This observation was also noted by another study with five strains of *S. cerevisiae* [19].

However, the exponential phase appears in the early hours of culture, during which the biomass reached a value of 6g/L in 23 hours. The lipids level closely followed microbial growth, while the sugar level decreased considerably, tending towards zero, confirming the use of these molecules as the primary carbon source for its respiratory metabolism. Similar results were observed by [20], during this phase, the biomass reached 6 g/L, lipids 6.5 g/L, and sugars 514 mg/L in 23 hours. After that, a very short stationary phase of about sixty minutes set in, indicating nutrient depletion and waste accumulation in the environment, leading to cell death and the onset of the declining phase characterized by a 56% loss in biomass, which was in perfect correlation with the lipid level obtained by [21], at this point, the sugar level tended towards zero, particularly when the strain was cultivated at high sugars levels, primarily glucose exceeding 1g/L.

In the presence of oxygen, *S. cerevisiae* exhibits a Crabtree effect, consuming less glucose and producing more ethanol, which serves as a substrate later [22]. This results in a decrease in biomass production and oxygen consumption, along with ethanol accumulation [23]. After 48 hours of growth in the presence of oxygen, incubating the strain under anaerobic conditions allowed it to enter directly into an extended exponential phase (Figure 2), longer than that observed in aerobic conditions. During this phase, the biomass reached a maximum of 13.76g/L in just 20 hours. Throughout this phase, the lipid level was in correlation with biomass, and the sugar level tended towards zero, indicating that the lack of oxygen

directed the strain towards a fermentative process that mobilized all catabolic pathways to enable the microorganism to survive in the culture medium. Other researchers have reported that yeast cells under growth conditions mobilize all their triglycerides and sterols for membrane biogenesis, and all released fatty acids are directed towards phospholipids biosynthesis [24].

Figure 2 reveals a very brief slowdown phase that transition into the stationary phase, during which yeast growth stabilizes due to nutrient depletion and waste accumulation, while the lipid level increases considerably. It was also demonstrated that yeast cells, at the end of the exponential phase or the beginning of the stationary phase, convert diacylglycerols into triacylglycerols capable of leaving the endoplasmic reticulum to support metabolism and returning to it when their level is sufficiently reduced [25]. However, this is not the case here since growth has stopped.

The increase in biomass following the stationary phase can be explained by the utilization of newly secreted molecules during the stationary phase, which served as nutrients for the strain a second time, thus allowing the reactivation of its energy metabolism and enabling it to resume growth.

According to another study, the formation of lipid bodies, the main lipid reserves in yeasts, depends on the synthesis of triglycerides, neutral lipids, and sterol esters, but other neutral lipids such as squalene may also be present [26]. Based on these observations, it is possible to explain the increase in lipid levels during the stationary phase of *S. cerevisiae* by the strain's inability to maintain the pace of lipid metabolism. This includes the synthesis of lipid bodies, the main cellular lipid reserve, and the synthesis of membrane phospholipids, which play a crucial role in cellular permeability and exchange. This imbalance in internal lipid molecule proportions facilitated the release of certain lipid molecules, mainly those with low molecular weights, from the intracellular environment to the external environment through various mechanisms. Another study reported that squalene accumulates in cells cultured under anaerobic conditions because, like most eukaryotes, the yeast *S. cerevisiae* synthesizes squalene in the isoprenoid (mevalonate) pathway as the primary precursor dedicated to ergosterol synthesis [27]. Under normal conditions, yeast cells quickly use squalene for sterol formation; therefore, squalene levels in aerobically growing or resting cultures are generally very low [28].

After extraction using the first chloroform-methanol solvent method, the amount of material obtained after lyophilization and drying was 2.194g, with a squalene quantity of 1.352g. However, the amount of dry matter obtained by the second KOH-based extraction method was 0.986g, with a squalene content of 400mg. Therefore, the production of squalene by *S. cerevisiae* in a sugar-rich medium resulted in a yield of 551mg/g DCW (Dry Cell Weight).

It has been reported that several factors impact the increase in squalene yield and the selectivity of bioprocesses, including yeast strain, aeration strategy, inoculum size, and bioprocess parameter control strategy [29].

Additionally, it has been claimed that squalene extraction using chloroform or a hypothetical chloroform-methanol solvent increases lipid yield by 2.5 times compared to other solvents [12]. This was explained by the fact that chloroform, being a less polar solvent than methanol, when mixed, increases cell wall damage, thus aiding in squalene release from cells. Conventional extraction using pyrogallol and saponification with 60% KOH at 45°C also increases squalene yield [30], [31].

HPLC results were consistent with that of researchers who analyzed squalene and cholesterol obtained from

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

oil recovered from different species of freshwater and marine fish, olive oil, Amaranth, and rice bran [32]. The figure 3 shows multiple elution peaks, indicating that the extract obtained is not pure. The elution peak of squalene, as shown in figure, has a retention time (RT) of 12.977 min. This result is close to that obtained by analyzed various compounds containing squalene, including pure squalene capsules, olive oil, lipids, and algal cells, and found an RT of approximately 14 min for all tested samples [33].

The same authors reported that the differences in retention times can indeed be attributed to the difference in C18 column types, minimal impurities and residues remaining in the sample that are complex and may contain appreciable amounts of lipids such as triacylglycerols, sterols, etc., and consequently, the squalene peak overlaps with other interferences, explaining the presence of multiple peaks with different retention times. The extraction technique also differs and could influence the result, as previous study noted, a C18 column with core-shell technology yielded better results due to its porosity, leading to more distinct peak separations [32]. This analysis technique remains sensitive for squalene detection; however, the detection limits are low, and analysis of the product should be conducted in the presence of standards.

Results obtained by Fourier-transform infrared spectroscopy (FTIR) can be compared with another study where peaks of high intensity were observed at 2965, 2913, and 2852 $cm^{-1}$ in the case of squalene derived from shark liver, along with three less significant peaks around 1458.18 $cm^{-1}$ and 1209.21 $cm^{-}1$, indicating the presence of $CH_2$ and $CH_3$ alkanes as well as trans-distributed alkenes [34]. On the other hand, another study reported appearance of peaks during the identique squalene analysis at 1446, 1224, and 722.1 $cm^{-1}$ [35].

In the case of antioxydantes activity the results obtained were very close to those related by a study which said that squalene is not very sensitive to peroxidation and protecting human skin surfaces from UV lights responsible of lipid peroxidation [36]. The low antioxydant activity observed for squalene could be due to its chemical structure that lacks aromatic nuclei, its low concentration obtained from yeast which does not allow a good demonstration of antioxidant activity, and its interaction with other fatty acids, which can reduces oxidation rates due to competitiveness [37].

The antiradical activity by the DPPH method is influenced by the solvent used [38], and in current study, the hydrocarbon is preserved in chloroform, which could affect the obtained result. Studies have highlighted antioxidant activity using this method on a Squalene isolated from the plant, *Canthiam coromendelicum*, for medicinal applications, IP values obtained were greater than 80% for concentrations ranging from 10 to 50 µg/mL [39]. On the other hand, it was demonstrated demonstrated that in the case of a squalene-rich critical oil extracted from shark liver, there was no difference between the radical trapping and HCO and SCO radical reduction activities evaluated by DPPH, due to their similar and high squalene content, unlike CCO whose squalene content was 10 and 8 times lower than the SCO and HCO concentrations respectively, and consequently, the antioxidant activities of CCO were significantly weaker [40]. Another experiment involving squalene extraction from palm fatty acid distillate (PFAD) showed higher activity on the order of 0.0073 mg. [41] Squalene extracted from *Terminalia catappa* showed that senescent abscised leaves had a high content of 12.29%, while seeds had 0% after DPPH assay. The seeds did not show trapping activity (≤ 10%), whereas the senescent leaves rich in squalene had significant activity > 80% for 500 µg/ml of extract [42].

These important properties of squalene offers several advantages in cosmetic use, considered an excellent skin protector against solar and UV rays, its antioxidant power can be used in formulations for sunscreens, anti-aging creams, brightening products, and this effect will also play a role in stabilizing and improving the

shelf life of products while preventing auto-oxidation [43].

The antibacterial activity was only observed in *S. aureus*, with halo diameters of approximately 15 mm, indicating that squalene possesses antibacterial activity against this strain, which corroborates the findings which reported that this compound has the ability to render *S. aureus* avirulent, and its effect is significant on staphyloxanthin, the golden pigment contributing to the pathogenicity of this bacterium [44]. Another study on squalene CPC nanosystems showed antibacterial activity of this molecule against drug-resistant *S. aureus* [45]. However, according to [40] squalene-rich fish oil has antibacterial activity against *B. cereus* and *E. coli*, despite most antimicrobial agents being unable to act against Gram-negative species due to the complex structure of their cell wall.

Considering that human skin is characterized by a skin microbiota with a microbial diversity greater than that of the intestinal microbiota, the potential effect of squalene allows it to be used in cosmetic preparations against these potential pathogens to treat skin disorders [46], but also against acne, psoriasis, skin lesions related to xerosis, seborrheic dermatitis, and atopic dermatitis [3]. However, there are very few studies on the relationship between squalene and microorganisms, which is probably correlated with the fact that squalene does not possess described antimicrobial properties and its effects on *staphylococci* remain poorly understood [47].

## 5. Conclusion

This study focused on the production of squalene, which has numerous beneficial effects in the pharmaceutical and cosmetic industries, from the yeast *Saccharomyces cerevisiae*. This yeast demonstrated its ability to produce squalene with significant yield in a medium and under conditions favorable to it. The molecule was analyzed by HPLC and FT-IR, showing reducing power against free radicals with an inhibition percentage (IP) of 52.13 ± 2.571% at a concentration of 500 µg/ml, as well as antibacterial activity against *Staphylococcus aureus*.

The results obtained in this study prove the efficacy of *S. cerevisiae* as a promising alternative biotechnological source for the production of this molecule of interest. Although the quantities obtained from microorganisms remain low compared to those from animal sources, the potential for improvement of these species and their fermentation is still interesting. Given ethical and social concerns and its high global demand that continues to grow, the likelihood that sharks should, or could, remain the main source of squalene risks reducing.

The future of squalene holds new applications waiting to be discovered, and it would be beneficial to increase microbial yields for its production through rapid and remarkable advancements in genetic engineering.

Funding: This research received no external funding.
Data Availability: Data will be made available on request.
Conflicts of Interest: The authors declare no conflicts of interest.

## 6. References

[1] Mendes A, Azevedo-Silva J and Fernandes J C 2022 From Sharks to Yeasts: Squalene in the Development of Vaccine Adjuvants Pharmaceuticals (Basel) 15 265

[2] Hull C M, Loveridge E J, Rolley N J, Donnison I S, Kelly S L and Kelly D E 2014 Co-production

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

of ethanol and squalene using a Saccharomyces cerevisiae ERG1 (squalene epoxidase) mutant and agro-industrial feedstock Biotechnol Biofuels 7 133

[3] Gohil N, Bhattacharjee G, Khambhati K, Braddick D and Singh V 2019 Engineering Strategies in Microorganisms for the Enhanced Production of Squalene: Advances, Challenges and Opportunities Front Bioeng Biotechnol 7 50

[4] Micera M, Botto A, Geddo F, Antoniotti S, Bertea C M, Levi R, Gallo M P and Querio G 2020 Squalene: More than a Step toward Sterols Antioxidants (Basel) 9 688

[5] Spanova M, Zweytick D, Lohner K, Klug L, Leitner E, Hermetter A and Daum G 2012 Influence of squalene on lipid particle/droplet and membrane organization in the yeast Saccharomyces cerevisiae Biochim Biophys Acta 1821 647–53

[6] Baumgartner H, De Backer J, Babu-Narayan S V, Budts W, Chessa M, Diller G-P, Lung B, Kluin J, Lang I M, Meijboom F, Moons P, Mulder B J M, Oechslin E, Roos-Hesselink J W, Schwerzmann M, Sondergaard L, Zeppenfeld K, and ESC Scientific Document Group 2021 2020 ESC Guidelines for the management of adult congenital heart disease Eur Heart J 42 563–645

[7] Li T, Liu G-S, Zhou W, Jiang M, Ren Y-H, Tao X-Y, Liu M, Zhao M, Wang F-Q, Gao B and Wei D-Z 2020 Metabolic Engineering of Saccharomyces cerevisiae To Overproduce Squalene J Agric Food Chem 68 2132–8

[8] Garaiová M, Zambojová V, Simová Z, Griač P and Hapala I 2014 Squalene epoxidase as a target for manipulation of squalene levels in the yeast Saccharomyces cerevisiae FEMS Yeast Res 14 310–23

[9] Goddard M R and Greig D 2015 Saccharomyces cerevisiae: a nomadic yeast with no niche? FEMS Yeast Research 15 fov009

[10] Dubois M, Gilles K, Hamilton J K, Rebers P A and Smith F 1951 A colorimetric method for the determination of sugars Nature 168 167

[11] Laiterie F I de 1958 La détermination de la matière grasse dans le lait liquide normal par la méthode gravimétrique de Röse-Gottlieb (Standard International). Lait 38 380–3

[12] Paramasivan K, Rajagopal K and Mutturi S 2019 Studies on Squalene Biosynthesis and the Standardization of Its Extraction Methodology from Saccharomyces cerevisiae Appl Biochem Biotechnol 187 691–707

[13] Adams B G and Parks L W 1968 Isolation from yeast of a metabolically active water-soluble form of ergosterol J Lipid Res 9 8–11

[14] Thompson A, Kwak S and Jin Y-S 2014 Squalene Production using Saccharomyces cerevisiae i-ACES 1 57–63

[15] Molyneux P 2004 The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant Songklanakarin Journal of Science and Technology (SJST) 26 211–9

[16] Balouiri M, Sadiki M and Ibnsouda S K 2016 Methods for in vitro evaluating antimicrobial activity: A review J Pharm Anal 6 71–9

[17] Mckee R H, Adenuga M D and Carrillo J-C 2015 Characterization of the toxicological hazards of hydrocarbon solvents Critical Reviews in Toxicology 45 273–365

[18] Salari R and Salari R 2017 Investigation of the Best Saccharomyces cerevisiae Growth Condition Electron Physician 9 3592–7

[19] Nehme N 2008 Etude des interactions entre Saccharomyces cerevisiae et Oenococcus oeni : impact sur la réalisation de la fermentation malolactique en cultures séquentielles et mixtes phd

[20] Käppeli O 1987 Regulation of Carbon Metabolism in Saccharomyces cerevisiae and Related Yeasts Advances in Microbial Physiology vol 28, ed A H Rose and D W Tempest (Academic Press) pp 181–209

[21] Linskens H F and Jackson J F 1988 Wine Analysis Modern Methods of Plant Analysis vol 6 p I–XV

[22] Hussenet C 2017 Instrumentation, modélisation et automatisation de fermenteurs levuriers à destination oenologique phdthesis (Université Paris Saclay (COmUE))

[23] Hencke S 2000 Utilisation alimentaire des levures other (UHP - Université Henri Poincaré)

[24] Zanghellini J, Natter K, Jungreuthmayer C, Thalhammer A, Kurat C F, Gogg-Fassolter G, Kohlwein S D and Von Grünberg H 2008 Quantitative modeling of triacylglycerol homeostasis in yeast – metabolic requirement for lipolysis to promote membrane lipid synthesis and cellular growth The FEBS Journal 275 5552–63

[25] Markgraf D F, Klemm R W, Junker M, Hannibal-Bach H K, Ejsing C S and Rapoport T A 2014 An ER protein functionally couples neutral lipid metabolism on lipid droplets to membrane lipid synthesis in the ER Cell Rep 6 44–55

[26] Sandager L, Gustavsson M H, Ståhl U, Dahlqvist A, Wiberg E, Banas A, Lenman M, Ronne H and Stymne S 2002 Storage lipid synthesis is non-essential in yeast J Biol Chem 277 6478–82

[27] Spanova M, Zweytick D, Lohner K, Klug L, Leitner E, Hermetter A and Daum G 2012 Influence of squalene on lipid particle/droplet and membrane organization in the yeast Saccharomyces cerevisiae Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids 1821 647–53

[28] Wiersma S J, Mooiman C, Giera M and Pronk J T 2020 Squalene-Tetrahymanol Cyclase Expression Enables Sterol-Independent Growth of Saccharomyces cerevisiae Appl Environ Microbiol 86 e00672-20

[29] Mantzouridou F and Tsimidou M Z 2010 Observations on squalene accumulation in Saccharomyces cerevisiae due to the manipulation of HMG2 and ERG6 FEMS Yeast Res 10 699–707

[30] Shin G-H, Veen M, Stahl U and Lang C 2012 Overexpression of genes of the fatty acid

ISSN: 0005-2523
Volume 64, Issue 04, April, 2024

biosynthetic pathway leads to accumulation of sterols in Saccharomyces cerevisiae Yeast 29 371–83

[31] Tokuhiro K, Muramatsu M, Ohto C, Kawaguchi T, Obata S, Muramoto N, Hirai M, Takahashi H, Kondo A, Sakuradani E and Shimizu S 2009 Overproduction of geranylgeraniol by metabolically engineered Saccharomyces cerevisiae Appl Environ Microbiol 75 5536–43

[32] Bavisetty S C B and Narayan B 2015 An improved RP-HPLC method for simultaneous analyses of squalene and cholesterol especially in aquatic foods J Food Sci Technol 52 6083–9

[33] Lu H-T, Jiang Y and Chen F 2004 Determination of Squalene Using High-Performance Liquid Chromatography with Diode Array Detection Chromatographia 59 367–71

[34] Hall D W, Marshall S N, Gordon K C and Killeen D P 2016 Rapid Quantitative Determination of Squalene in Shark Liver Oils by Raman and IR Spectroscopy Lipids 51 139–47

[35] Bhattacharjee P, Shukla V B, Singhal R S and Kulkarni P R 2001 Studies on fermentative production of squalene World Journal of Microbiology and Biotechnology 17 811–6

[36] Dieng S I M, Fall A D, Diatta-Badji K, Sarr A, Sene M, Sene M, Mbaye A, Diatta W and Bassene E 2017 Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de Piliostigma thonningii Schumach. International Journal of Biological and Chemical Sciences 11 768–76

[37] Lozano-Grande M A, Gorinstein S, Espitia-Rangel E, Dávila-Ortiz G and Martínez-Ayala A L 2018 Plant Sources, Extraction Methods, and Uses of Squalene International Journal of Agronomy 2018 1–13

[38] Bourgou S, Beji R S, Medini F, Ksouri R, Zaouchi Y, Rezgui S, Jeddi F B, Labidi S and Bettaieb T 2016 Effet du solvant et de la méthode d'extraction sur la teneur en composés phénoliques et les potentialités antioxydantes d'Euphorbia helioscopia Journal of New Sciences 28

[39] Arulprakash. P Sasidharan P Jayachitra A 2017 Isolation and structural elucidation of biological and pharmacological activities compounds from Canthiam coromendjelicum

[40] Lee S-C, Nkurunziza D, Kim S-Y, Surendhiran D, Singh A A and Chun B-S 2022 Supercritical carbon dioxide extraction of squalene rich cod liver oil: Optimization, characterization and functional properties The Journal of Supercritical Fluids 188 105693

[41] Yusuf N R K, Legowo E H and Sutanto H 2015 Utilization of Crude Palm Oil Waste to Produce Squalene

[42] Ko T-F, Weng Y-M and Chiou R Y-Y 2002 Squalene content and antioxidant activity of Terminalia catappa leaves and seeds J Agric Food Chem 50 5343–8

[43] Pandey P, Ramegowda V and Senthil-Kumar M 2015 Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms Front Plant Sci 6 723

[44] Sri Charan Bindu B, Mishra D P and Narayan B 2015 Inhibition of virulence of Staphylococcus aureus – a food borne pathogen – by squalene, a functional lipid Journal of Functional Foods 18 224–34

[45] Fang J-Y, Lin Y-K, Wang P-W, Alalaiwe A, Yang Y-C and Yang S-C 2019 The Droplet-Size Effect Of Squalene@cetylpyridinium Chloride Nanoemulsions On Antimicrobial Potency Against Planktonic And Biofilm MRSA Int J Nanomedicine 14 8133–47

[46] Grice E A, Kong H H, Conlan S, Deming C B, Davis J, Young A C, Program N C S, Bouffard G G, Blakesley R W, Murray P R, Green E D, Turner M L and Segre J A 2009 Topographical and Temporal Diversity of the Human Skin Microbiome Science 324 1190–2

[47] Zhao X 2017 Staphylococcal Responses to Skin Surface Lipid Squalene phd (University of Liverpool)