



Analysis of the Effective Components of *Cocos nucifera L.* Oil on Atopic Dermatitis Skin Disease and *Staphylococcus Aureus Bacteria*

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ABSTRACT

The major objective of the research is to investigate the in vitro antibacterial susceptibility to fatty acids contained in coconut oil on a collection of clinical isolates. especially, the chronic skin condition Atopic dermatitis (Atopic eczema). caused by gram-positive bacteria (*Staphylococcus aureus*).

Clinical isolates are collected from Shar Hospitals, and the organisms pass through a typical biochemical examination evolution. While coconut oil is obtained using the centrifuge method to extract coconut oil (CE VCO). The sensitivity evaluation is conducted using the disc diffusion method. Lastly, use a measuring tool to estimate the inhibitory zones' diameter. In another hand, after analysis of the oil by GC-MASS, Coconut oil contained numerous chemical components and the highest amount is Oleic acid 54.62%, while the smallest amount was N-Hexadecanoic acid, N-Hexadecanoic acid, (Myristic acid), % 3.34. Sequentially, Heneicosane, 9-Octadecenoic acid (Z)- % 13.78, with 9,12-Octadecadienoic acid 11.23%, and Nonadecane and his isomers 5.77% percent. Coconut oil doesn't show any sensitivity and a high potential for antibacterial activity due to the high contents of medium and long-chain saturated and unsaturated fatty acids so This study recommends further studies should be done on the oil and its derivative both in vitro and in vivo for showing its mechanisms of actions.

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Keywords: *Cocos nucifera L.* oil, GC-MS/MS, *Staphylococcus aureus*, and Atopic Dermatitis.

1. Introduction

The coconut palm tree (*Cocos nucifera L.*), a member of the *Palmacea* family, is the source of coconut oil, a type of vegetable oil^[1]. Additionally, oil can be produced by processing copra from coconut trees in a variety of ways. which at ambient temperature is a soft, almost wax-like substance that is semi-solid^[2]. One obvious downside of coconut oil is the lack of necessary fatty acids. particularly linoleic acid, as a result, natural plant compounds, particularly those from medicinal plants, can be studied in an effort to discover potential alternatives to the various antimicrobials. Numerous phytochemicals found in plants have significant therapeutic benefits that can be employed to cure diseases^[3]. Natural and modern antibiotics have eradicated a variety of illnesses, but Pathogens have evolved as a result of their indiscriminate use^[4]. However, coconut oil's antimicrobial characteristics are just one of its remarkable qualities. By treating and preventing skin infections, skin care products can also have a positive clinical impact^[5, 6]. According

to^[5, 7], Lauric acid, Caprylic acid, and Capric acid have antibacterial effects on bacteria with thick peptidoglycan layers, such as *Staphylococcus* species, but not on bacteria with thin peptidoglycan layers.

Because it is a rich source of healthy fatty acids, or lipids, notably lauric acid, coconut oil can help lessen the symptoms of atopic dermatitis by calming the skin. It can also help reduce inflammation, lower the risk of infection, and improve itching. By swiftly and effectively permeating the skin and enhancing moisture and skin suppleness, this fatty acid is vital for lowering the presence of bacteria and fungi on the skin, which helps to decrease the risk of infection caused by scratching itchy or rash skin^[8].

Because it is a vital organ, the skin's general health can be determined by its condition. The skin is the most widespread and diversified human organ, and it is sensitive to disorders caused by water loss or a diminished ability to retain water in the skin. One such condition is Atopic dermatitis (eczema), characterized by red, dry, itchy skin^[9]. Additionally, it has a tendency to flare up from time to time and is brought on by frequent bacterial infections like *Staphylococcus aureus*. Another reason why

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moisturizers should be used on the skin is that the therapy of atopic dermatitis should take a phased approach, in which the severity of the disease-related treatments is taken into account. since symptoms are frequently manageable^[10]. On the other hand, as the skin is the body's interface tissue, it serves as a main tissue and may be accessed by various microbiome species.

In addition to colonizing the skin and producing toxins and other proteins that contribute to increased inflammation, *Staphylococcus aureus* can lead to skin infections on the surface. This increased inflammation leads to persistence and the worsening of skin infections. due to human keratinocytes' capacity to ingest it^[11]. *Staphylococcus aureus* is the most common occasional cause of skin disorders among microorganisms, including impetigo, atopic dermatitis, and scalded skin syndrome. On the other hand, it can cause life-threatening situations including septicemia^[12].

The overall goal of this study is to investigate the chemical makeup of the essential oils derived from (*Cocos nucifera* L.), as well as to ascertain how patients with mild to moderate atopic dermatitis are significantly influenced by coconut oil. by measuring the percentage of phytochemicals present in the oil and how those results affected the skin by assessing the oil's antimicrobial activity. by employing the tests for disc diffusion. As far as we have been aware, this study in Sulaimani, Iraq, is the first to be done on this coconut oil (*Cocos nucifera* L.).

2. Method and Material

2.1 Patients and study design

Based on descriptive research, adult patients between the ages of 18 and 25 are selected for in-vivo experiments between December 15, 2021, and May 1, 2022, to investigate the effects of topical *Cocos nucifera* L. oil skin capacitance in mild to moderate adult *atopic dermatitis*. Every patient has just had a diagnosis. The modified *Hanifin* primary criteria of morphological course, frequent relationship with a family history of *Atopic dermatitis*, and skin disease capacitance ranging from mild to moderate are used to make the diagnosis of *Atopic dermatitis*^[13, 48].

2.2 Categorizing atopic dermatitis

According to the Conference on Atopic Dermatitis Number 11^[13], the severity of atopic dermatitis was divided into three categories: mild, moderate, and severe. Mild atopic dermatitis is initially characterized by areas of dry skin with infrequent itching. It may also have minor areas of redness. While moderate is shown by the appearance of dry skin patches that itch and get red frequently. Last but not least, severe, which is characterized by vast areas of dry skin, either cracked or not, bleeding, excoriation, significant skin thickness, and persistent redness and itching. Only participants in this study had mild to moderate atopic dermatitis^[14].

2.3 Collection and preparation of coconut oil material.

Collecting and preparing coconut oil from coconut fruits without the shell at a nearby market in the Iraqi province of

Sulaymaniyah. were acquired between 15 December 2021 and 1 May 2022. It is utilized as a delicious fruit that can be eaten^[15, 16].

2.4 Preparation of Plant oils

By weighing (500 g) of grated coconut meat and combining it with water (1:1), the centrifugation method (CE VCO) was used to prepare coconut oil (Model 80-2, Table type centrifuge, Volume 12 to 20ml). The oil-water emulsion is centrifuged twice for 30 min each at a speed of 1372 g (3500 rpm) to destabilize it for 30 min at room temperature. first centrifugation to get the cream, followed by a second to divide the cream into (oil, cream, and aqueous). Before usage, the top oil layer is decanted, weighed, and kept in dark-brown bottles at 4 °C^[16].

2.5 Chemical compounds identification and detection by GC-MS/MS

Instrumentation: Waters Alliance 2695 HPLC - Micromass Quattro microAPI mass spectrometer (Waters Corporation, Milford, MA, USA). HPLC column: Atlantis T3-C18 3 μ , 2.1 \times 100 mm (Waters, USA), column temperature of 35 °C, mobile phase: a mixture of 50% solvent A (acetonitrile + 0.1% formic acid) and 50% solvent B (H₂O + 0.1 % formic acid), flow rate of 0.2 ml/min for 10 min, mass spectrometers parameters: Mode: ESI+, cone volt: 25 V, capillary volt: 3 kV, extractor: 2 V, RF lens: 0.2 V, collision energy: 30 eV, gas nebulizer: N₂ (grade 5), flow gas: 200 L/h, source temperature: 150 °C, desolvation temperature: 350 °C. The GC-MS instrument model (QP 2010 Plus SHIMADZU),^[17].

2.6 Bacteria Isolation

For the purpose of isolating and identifying *Staphylococcus aureus*, samples from 10 clinical skin patients (*Atopic dermatitis* patients) are obtained from Shar Hospitals. The samples are obtained, cultured on nutrient agar, and then the plates underwent conventional microbiological procedures for growth and identification for 24-36 hours at 35-37°C^[18]. For *Staphylococcus aureus* identification, first, a Gram stain technique is used to identify *S. aureus*, resulting in the observation of gram-positive cocci bacteria. Next, a catalase test is carried out to distinguish *S. aureus* from other *Staphylococcus species*^[19], Belatedly, selective and differential media, such as mannitol salt agar (MSA), are used to identify *S. aureus*^[19]; Also a coagulase test to detect *S. aureus* from other *Staphylococcus species* is performed^[20]. Finally, the *vitek* device is employed to highlight the outcome. In the end, the *McFarland* standard is used to obtain sub-culturing from diluted solution^[19, 20].

2.7 Disk diffusion method

The antibacterial efficacy of the various dilution oils is tested using the disk diffusion method. Following the transplantation of bacterial colonies from both media—mannitol salt agar and MacConkey agar—to broth nutrient agar for 12–18 hours, Mueller Hinton agar plate culture is performed using the pour plate technique. 0.9 ml of nutrient broth is cultured in the plate and dispersed in an L-shape before adding 4 infected disks to the Mueller Hinton agar surface. The oils are then poured into the disks in 0.5 μ l increments until the disks are completely saturated.

The plates are incubated for 24 hours at 37° after the oils were absorbed by the filter paper^[18, 20].

For measuring the zone of inhibition (ZOI) of bacterial growth in millimetres, the sensitivity test of bacteria is estimated. The National Committee for Clinical Laboratory Standards is used to compare the result (NCCLS, 2012) sensitivity to antimicrobials^[20, 21].

3. Results and Discussion:

In the present study and through the statistical analysis of (*Cocos Nucifera L.*) coconut oils by Gas chromatography, the results showed the identification of 9 compounds (Fatty acid compositions) with isomerism (Table 1 and Fig.1), with a % 54.62 (9-Octadecenoic acid), % 13.78 Linoelaidic acid and (9,12-Octadecadienoic acid (Z, Z)-), % 11.26 Palmitic acid (n-Hexadecanoic acid) and Myristic acid, % 11.23 (9,12-Octadecadienoic acid (Z, Z)-), % 5.77 Nonadecane and % 3.34 Heneicosane and 9-Octadecenoic acid (Z)- constitute the highest percentage of essential oil.

Atopic dermatitis, skin infections, and other infectious problems are all significantly exacerbated by *Staphylococcus aureus*. The severity of *Atopic Dermatitis* is correlated with the amount of *Staphylococcus aureus* present on the skin. The abuse of antibiotics and prior hospitalization are also strongly linked to *MRSA* colonization. The cause of this connection is believed to be *S. aureus*'s release of poisons, proinflammatory, and proteases, which impact keratinocytes and various immune cells in atopic dermatitis skin and disrupt skin homeostasis.

Atopic dermatitis (eczema), is an itchy inflammatory skin disease that follows a chronic remitting course. mold and moderate eczema have a significant impact on the quality of life because of itching and scratching.

Atopic Dermatitis patients colonized by *Staphylococcus aureus*, SC lipids, including free fatty acid, ceramides, and sphingosine, play critical

roles in the maintenance of skin barrier integrity and in preventing pathologic infections such as *S. aureus*.

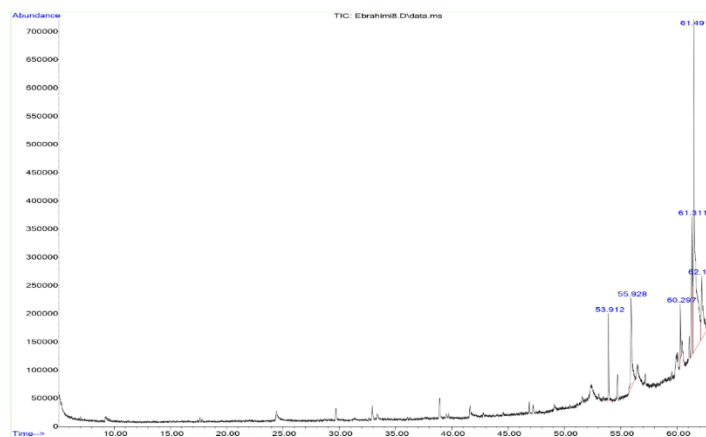


Figure 1: General GC-MS/MS Analysis for all chemical compounds.

Table 1: *Cocos nucifera L.* fatty acid obtained by the GC-MS/MS Analysis.

N	Name	Chemical formula	Structure	RT	Percentage
1.	Nonadecane	CH ₃ (CH ₂) ₁₇ CH ₃		53.91	5.76%
2.	Palmitic acid (n-Hexadecanoic acid)	C ₁₆ H ₃₂ O ₂		55.93	11.26%
3.	Myristic acid	CH ₃ (CH ₂) ₁₂ COOH		55.93	11.26%
4.	Heneicosane	CH ₃ (CH ₂) ₁₉ CH ₃		60.29	3.34%
5.	9-Octadecenoic acid (Z)-	C ₃₉ H ₇₂ O ₅		60.29	3.34%
6.	Linoelaidic acid	C ₁₈ H ₃₂ O ₂		61.49	54.62%
7.	9-Octadecenoic acid, (E)-	C ₁₈ H ₃₄ O ₂		61.49	54.62%
8.	Oleic Acid	C ₁₈ H ₃₄ O ₂		62.2	11.23%
9.	9,12-Octadecadienoic acid (Z, Z)-	C ₁₈ H ₃₂ O ₂		62.2	11.23%

The GC-MS/MS analysis results can be obtained from different chemical compounds by separating the mixtures of chemicals into identifying components, especially phenols, terpenes, and other phytochemicals. the results show that peaks appearing after 53.91 mint identified Nonadecane and his isomers amount 5.77% percent. (Figure 2). While, from (Figure 3) the analysis shows

different retention times for (n-Hexadecanoic acid, n-Hexadecanoic acid, and Myristic acid at 55.93 mint.

The oils from Coconut can be observed in (Figures 4 and 5), that oil is comprised of a mixture of chemical compounds in small amounts, or most of them are identified in small quantities like Heneicosane, 9Octadecenoic acid (Z)-, 9,12-Octadecadienoic acid (Z, Z)-, and Linoelaidic acid (% 3.34 and % 13.78).

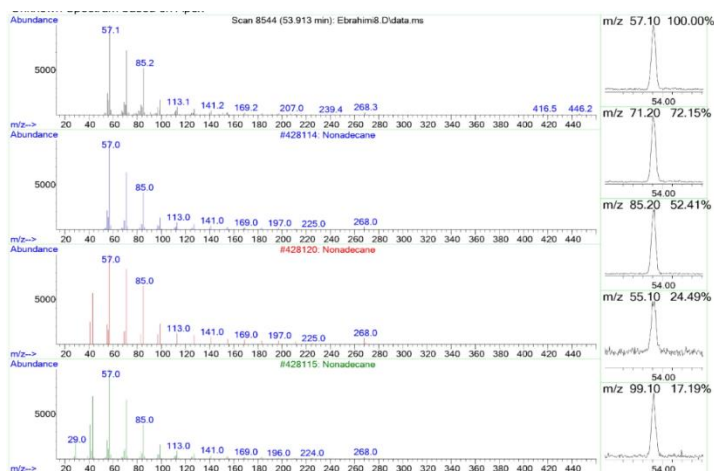


Figure 2: The GC-MS/MS Analysis for the chemical compounds Nonadecane and his isomers.

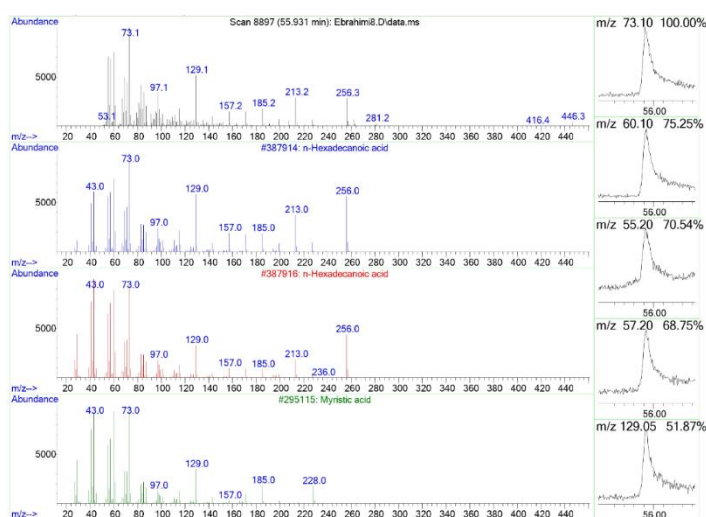


Figure 3: The GC-MS/MS Analysis for the chemical components N-Hexadecanoic acid, N-Hexadecanoic acid, and Myristic acid.

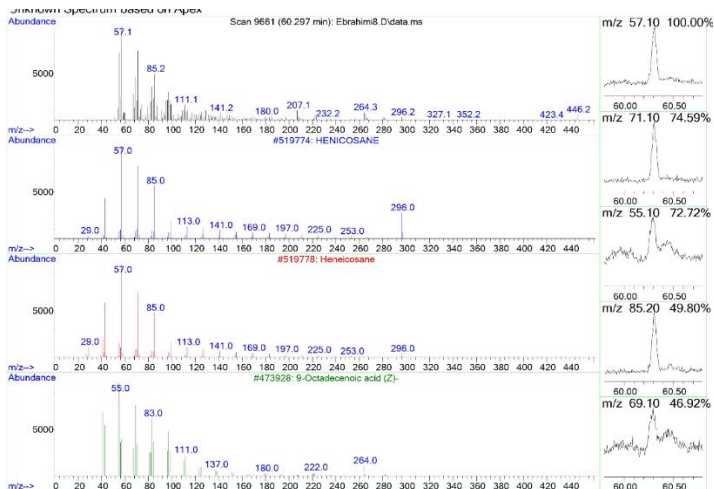


Figure 4: The GC-MS/MS Analysis for the chemical components Heneicosane, 9-Octadecenoic acid (Z)-, and 9,12-Octadecadienoic acid (Z,Z).

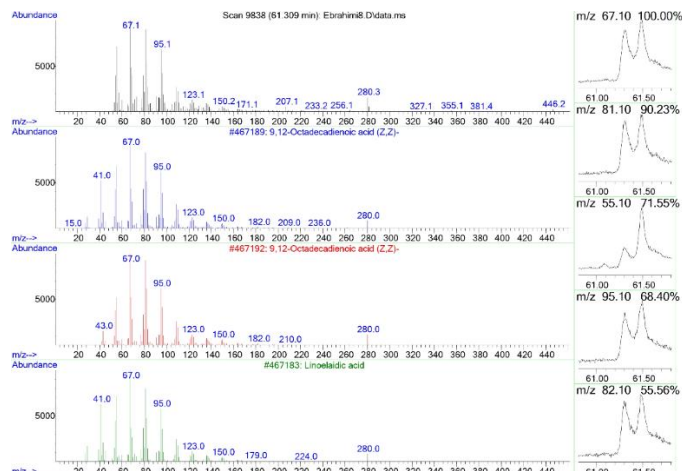


Figure 5: The GC-MS/MS Analysis for the chemical components 9-Octadecenoic acid (Z)-, 9,12-Octadecadienoic acid (Z, Z)-, and Linoelaidic acid.

Finally, Oleic acid and isomers from (Figure 6), explain that the oil is comprised of a mixture of chemical compounds in the highest amount of 54.62%, it identified as important quantities like 9-Octadecenoic acid, (E)-, 9-Octadecenoic acid, and Oleic acid. Also (from Figure7) the same thing except for more isomers about Oleic acid amount around 11.23% (9-Octadecenoic acid (Z)-, 9-Octadecenoic acid (Z-Z), and Oleic Acid).

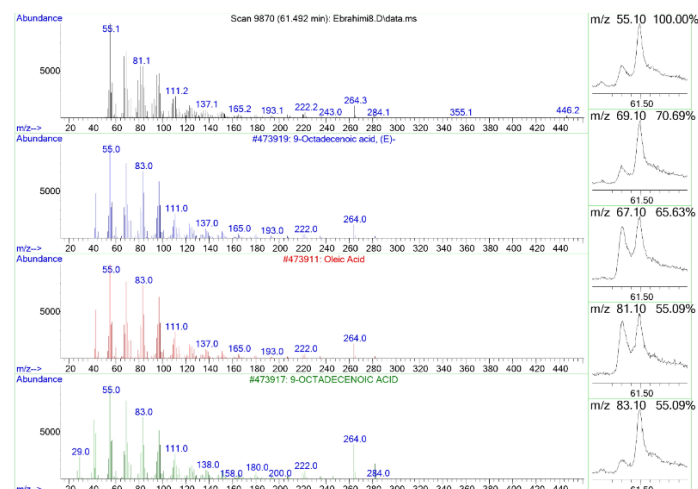


Figure 6: The GC-MS/MS Analysis for the chemical components 9-Octadecenoic acid, (E)-, 9-Octadecenoic acid, and Oleic acid.

Atopic dermatitis has no specific diagnostic procedures, People with AD have skin that lacks ceramides and antimicrobial peptides. Therefore, this infectious pathogen that causes AD most frequently is *S. aureus*, which also colonizes the skin and generates proteins, toxins, and enzymes that contribute to inflammation in atopic dermatitis by causing the skin cell to produce antimicrobial peptides like commensal bacteria, particularly *Staphylococcus species* which grow on the skin^[22, 23]. These outcomes are consistent with the findings of our tests. So, the different antimicrobial resistances are the main cause of spread of many infectious diseases. And efforts are needed to overcome the pathological effects of etiological agents of infectious disease^[24, 25] Therefore, finding medications made from plants is the simplest way to achieve the aim of physiochemists and microbiologists^[26].

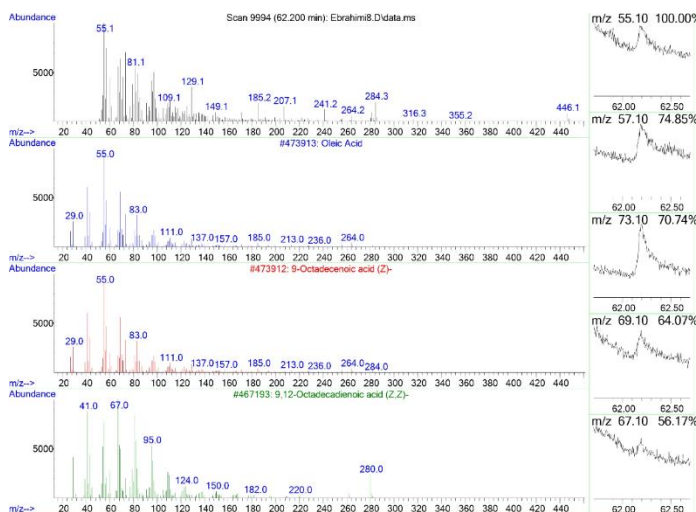


Figure 7: The GC-MS/MS Analysis for the chemical components 9-Octadecenoic acid (Z)-, 9-Octadecenoic acid (Z-Z), and Oleic Acid.

Since they have few side effects and can be used to create novel pharmaceuticals to treat a variety of conditions, medicines, herbs, and medicinal plants have become more significant in recent years^[27]. In this study, the antimicrobial potential of Coconut oil fractions against some pathogenic microbes is tested by analyzing the chemical composition determined by GC-MS. The results show that Nonadecane, N-Hexadecenoic acid, and Myristic acid (C19, C16, and C14). as the most common saturated and unsaturated fatty acids founds in coconut oils that give antibacterial activity, but in our study have no effect, this result doesn't agree with others result^[28]. So, their preparations have more applications as a natural anti-aging on the skin as a natural therapy, and medicine^[29, 30]. This may be due to lower of fatty acid from the fruit that used for extraction.

by the analysis, the result shows that all isomers from C18 fatty acid haven't any important antibacterial activity including 9,12-Octadecadienoic acid (Z, Z)-, Linolenic acid and 9- Octadecenoic acid, and Oleic acid. (C18, C18, C18, and C18) from different research, most of the fatty acids in coconut oil and C1–C5 were saturated, Lauric acid (C12), is the most prevalent saturated fatty acid in coconut oil. but in our study, polyunsaturated linoleic acid (C18), and monounsaturated oleic acid (C18) were identified^[31] These outcomes contradict each other. The majority of the Gram-positive bacterial species tested with the minimum inhibitory concentration (MIC) on the skin were, however, inhibited from growing by the antibacterial activity guided by oleic acids and linoelaidic acid, and it had no effect in contrast to other findings that were noted by the researchers^[32, 33]. but in the nutrition part several study mention it can be beneficial because the limited essential fatty acid content of edible Coconut oil allows for blending with other edible oils, such as olive, soybean, and sunflower oil, which are higher in polyunsaturated fatty acids and healthier for external use^[34].

On the other hand, Rabail, mentioned combining flaxseed oil will increase the quantities of polyunsaturated fatty acids, which will increase antioxidant enzymes this more effective for anti-aging on the skin^[35].

On the other side, different studies have proved that Coconut oil is widely used as an edible and therapeutic oil in different

countries around the world. It differs in composition from other oils because of the high content of small and medium-chain fatty acids especially a rich source of lauric acid which contains 92% saturated fatty acids being the main constituent. Hence, Lauric acid has antibacterial and anti-inflammatory properties^[36]. in the chemical analysis, we got different fatty acid chains (long-chain C14-C24 and very-long-chain C24) that have little antimicrobial activity, especially against (gram-positive), and have fewer activities when compared to (short and medium) chains that have antimicrobial activity. this is proved by Huang et al 2010,^[37].

Several studies mention, Coconut oil is a rich source of beneficial medium-chain fatty acids, particularly, lauric acid, capric acid, and caproic acid, that gave it antimicrobial activity, While, others mention Coconut oil is mainly known as a rich source of triglycerides, which are anti peroxidation it contains more phenolic substances. Additionally, other research demonstrated that coconut oil has a high saponification value (a rich source of small fatty acids) but the results from our investigation are rich for long chain fatty acids and agree with Huang,201^[37-39]. This is in line with a study proved by Hierholzer and Kabara, Which has shown that Coconut oil can have different antibacterial activity, such as (*Staphylococcus aureus* and *Streptococcus mutans*) gram-positive, and (*Escherichia vulneris* and *Enterococcus spp*) gram-negative^[40]. According to the findings of S.S. et al, the study's dangerous organisms were inhibited by coconut oil by utilizing the disk diffusion method. This is referred to as the saponification number since a higher saponification number of results in smaller molecules and vice versa. As a result, the study finds coconut oil has a very low saponification number and the results got medium and long-chain fatty acids and it doesn't contain lauric acid (C6) chain, therefore, the coconut oil doesn't show any antibacterial activity^[41, 42]. this agrees with the CLSI stander test of antibacterial activity (CLSI)^[43].

Due to a wide range of phytochemical constituents and their bioactive components, natural oils from several medicinal plants have great biological potential. As a result, the composition of the sample polyunsaturated fatty acids was kept in mind during determining the antibacterial activity of coconut oil in various lab experiments. since the presence of these chemicals is what causes the antibacterial action^[44]. against gram-positive bacteria^[45].

De Azevedo, et al. 2020, Concluded in a study that Neither Gram-negative nor Gram-positive strains were significantly reduced. The results might occur from structural variations in the germ cell membrane^[46]. Also, according to Meng et al.2020, the synergistic effect and bacterial integrity by chemical composition effects that may permeate through the cell wall and hinder the cellular respiration process are really explanations of the antibacterial activity of medicinal plant oils. but this finding is opposite to ours^[41]. Coconut oil and its fatty acid (lauric acid) are studied in 2015 by Abbas for their potential antibacterial effects on *Staphylococcus aureus*, *Streptococcus*, *Escherichia coli*, and *Lactobacillus species* are among the organisms isolated. In contrast to the work by Ogbolu et al. (2007), which reported the antibacterial efficacy of coconut oil on fungal organisms, both species showed resistance to coconut oil at various dilution concentrations. Ogbolu et al (2007).s approach is different from this study's because coconut oil was diluted with 1% ethanol, and

prior information has shown us that all classes of alcohol that contain ethanol have antibacterial capabilities. The inhibiting impact shown in the study may have been caused by the diluent^[47]. This agrees with the achieved results.

Conclusion

Due to physicochemical characterization, the chemical composition of coconut oils investigated in the latest study had diverse composition profiles by fatty acid-containing concentration and percentage. Coconut oil doesn't show any sensitivity and high potential for antibacterial activity due to the high contents of medium and long-chain saturated and unsaturated fatty acids such as Nonadecane, Heneicosane, Oleic acid, myristic, and palmitic acids, which influenced the saponification number and decreased. but regarding coconut oils, Skin-promoting products, Satisfactory results it is containing a high concentration of medium and long-chain poly saturated and unsaturated fatty acids by its phenolic compounds present as an alternative source of industry products. Further, it is predicted that continuing use and purification of the oil will enhance the properties and stability of the result.

Conflict of interests

None

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