Lauric acid is cytotoxic and antimicrobial against oral pathogens

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Abstract

Lauric acid is an omega-9 fatty acid found in coconut oil. It has antibacterial properties and kills cancer cells. It kills bacteria without damaging human cells. This makes lauric acid a good additive for making skin creams. In this present study, lauric acid was prepared, characterized using FT-IR and tested for its antimicrobial activity against five oral pathogens. The antimicrobial results showed that there is a dose-dependent increase in the zone of inhibition. Among five oral pathogens, *S.mutans, S. aureus, Lactobacillus* showed higher sensitivity to lauric acid. The biocompatibility was checked by Brine shrimp lethality assay, in which even at higher concentration such as 25µL showed less toxicity. Both the study results revealed lauric acid to be a less toxic potent antimicrobial agent.

Keywords: Lauric acid, Antimicrobial activity, Biocompatibility, Zone of inhibition.

Introduction

Lauric Acid is a monounsaturated fatty acid that is naturally present in coconut oil (Leyton et al., 1987, Sharma et al., 2021). It is used in skin care products as well as cosmetics. It helps to moisturize the skin and prevent dryness. It is also known to help reduce acne breakouts. It is a colorless crystalline solid with a melting point of 50 °C (122 °F). Lauric acid is odorless and possesses a mild coconut scent. Lauric acid is commonly used in perfumes, cosmetics, soaps, detergents, shampoos and moisturizers. It is also an ingredient in many foods including butter, milk, cheese, eggs, and meats (Kinderlerer, 1994).

The synthesis of lauric acid begins with the hydrolysis of palm kernel oil. Palm kernel oil is composed mainly of triglycerides, but it contains some amount of free fatty acids. To produce lauric acid from palm kernel oil, first the triglyceride bonds are broken down into glycerol and fatty acids using enzymes called lipases (Nainggolan et al., 2021). Then, the fatty acids are converted into their corresponding alcohols through a reaction catalyzed by dehydratase enzymes. Finally, the alcohols are oxidized into carboxylic acids using iron oxide.

In addition to being a natural preservative, lauric acid is widely used in organic chemistry as well. Researchers use this chemical for synthesizing various chemicals such as esters, amines, detergents, surfactants, polymers, and others (Ganesan et al., 2019). Lauric acid

is often combined with another fatty acid called myristoleic acid to form a mixture called monostearin. The resulting substance is much easier to process and can be used in the production of plasticizers, emulsifiers, and surfactants. It is also commonly used as a feedstock for making biodiesel, which is a renewable alternative fuel source (Inoue et al., 2004, He et al., 2016).

Palm kernel oil is the most common source of lauric acid. Other sources include coconut oil, beef tallow, wool grease, and fish oils.Lauric acid is known as a natural antibacterial substance produced from coconut oil (Lee et al., 2021). Lauric acid is used as an ingredient in many foods and cosmetic products. Besides its antibacterial properties, lauric acid possesses antifungal, antiviral, antiparasitic, anti-inflammatory, and immune-modulating benefits (Singer et al., 2008, Intahphuak et al., 2010, Rajeshkumar et al., 2018). When applied topically, lauric acid provides protection against microbial infection.

Streptococcus mutans is a common microbe found in the mouth, particularly in children. As a result, it is responsible for dental caries (Borrelli et al., 2021). This bacterium lives in a biofilm community, which means it is surrounded by many other bacterial species. The previous studies showed that lauric acid had an antibacterial effect against *S. mutans, S. aureus* (Nakatsuji et al., 2009, Tangwatcharin&Khopaibool, 2012, Rajeshkumar et al., 2020).

Lauric acid (LA) is a saturated fatty acid naturally occurring in coconut oil. LA is used as a surfactant, emulsifier, stabilizer, and solubilizing agent. The most common applications of LA are in cosmetics, pharmaceuticals, foods, and nutritional supplements (Lieberman et al., 2006). LA is being investigated as a potential treatment for various diseases including cancer, diabetes, multiple sclerosis, Alzheimer's disease, and cardiovascular disorders (Dayrit et al., 2015).

Lauric acid is nontoxic and non-irritating to human skin. However, lauric acid does cause irritation to the eyes and respiratory tract. Lauric acid has been shown to be effective against acne vulgaris and rosacea (Huang et al., 2014). In addition, lauric acid may be able to reduce inflammation associated with psoriasis and eczema (Silva et al., 2015). Larger doses of lauric acid were found to be beneficial in treating hyperglycemia in diabetic rats (Saat et al., 2013).

Lauric Acid is considered safe for topical application to the skin and mucous membrane as well as ingestion. When ingested orally, there is little risk of toxicity; however, large doses should be avoided due to possible liver damage. There is minimal absorption when applied topically, but absorption increases when taken by mouth (Liu et al., 2022).The recommended dose of lauric acid is 10% to 20%. The optimal concentration depends on the type of formulation. For example, a cream containing 1% lauric acid would require less than 2% for a gel.

In this present research work, lauric acid was prepared, characterized and tested for its efficacy in antimicrobial and cytotoxic activity.

Materials and Methods

Preparation of Lauric acid:

0.5gm of lauric acid was weighed and added to 10 mL ethanol. The lauric acid solution was uniformly mixed by using a

vortex mixer for 15 minutes and kept in an orbital shaker for overnight at 110 rpm.

Fig 1: Preparation of Lauric acid

Characterization of lauric acid:

The lauric acid was characterized by using Fourier Transform Infra-red spectroscopy. The main aim of the characterization study is to identify its functional compounds. Bruker alpha ll FT-IR instrument was used for this study.

Antimicrobial activity

The inhibition of growth of microorganisms is otherwise called antimicrobial activity. The antimicrobial efficacy of lauric acid is tested by adopting agar well diffusion technique.The five oral pathogens used to test the lauric acid are *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Candida albicans, Pseudomonas aeruginosa.* Mueller Hinton Agar agar was utilized for this activity to determine the zone of inhibition. Muller hinton agar was prepared and sterilized for 15 minutes at 121 degree celsius. Media was poured into the sterilized plates and let it stable for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed using sterile cotton swabs.The Lauric acid with different concentrations such as (25 µL, 50 µL,100µL)was loaded on three respective wells measured.

The standard drug amoxyrite loaded on the fourth well was used for *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Pseudomonas aeruginosa. For C. albicans,* Rose bengal agar base was used as the medium and Flucanazole was used as the standard drug. And the bacterial plates were incubated for 24 hours at 37 °C and the *Candida albicans* plates were incubated at 28° C for 48-72hours. After the incubation time the zone of inhibition was measured. . After the incubation time and the zone of inhibition were measured.

Cytotoxic effect

BRINE SHRIMP LETHALITY ASSAY:

2g of iodine free salt was weighed and dissolved in 200ml of distilled water. Six well ELISA plates were taken and 10-12 ml of saline water was filled. To that 10 nauplii were slowly added to each well (5µL,10 µL,20 µL.40 µL,80 µL, control). Then the lauric acid was added according to the concentration level except to the control well. The plates were incubated for 24 hours. After 24 hours, the ELISA plate were observed and noted for number of live nauplii present and calculated by using following formula,

Number of dead nauplii/number of dead nauplii+number of live nauplii×100

Result and discussion

Fourier Transform Infra-red spectroscopy (FT-IR)

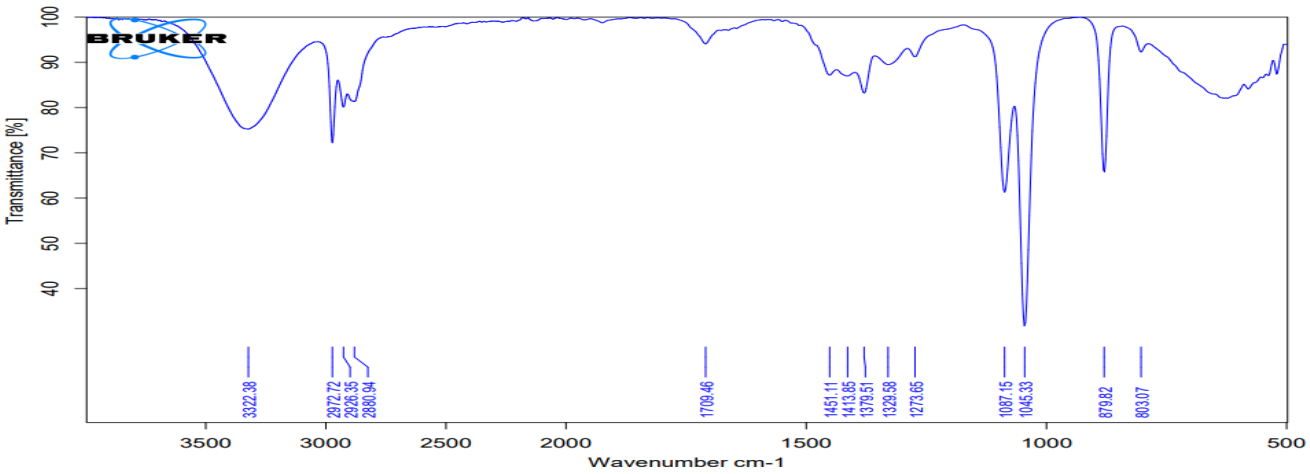

Fig 2: FT-IR spectra of Lauric acid

The Fourier transform Infra-red spectroscopy was done to analyze the organic contents including chemical bonds of lauric acid. The major peak at 3322 cm-1 corresponds to the presence of alcohol and hydroxy compound which is a single bonded OH stretch. The narrow peak at 2972.72 and 2880.94 cm-1 indicates the presence of an alkene group denoting methylene single bonded C-H asymmetric stretch. The broad peak at 1709.46 cm-1 indicates the presence of ketone groups. The other peaks at the fingerprint region such as 1451.11 cm-1 correspond to the presence of common inorganic ions such as carbonate ions and at 1379.51 cm-1 denotes the presence of aliphatic nitro compounds. The peak at 1273.65 cm-1 indicates the existence of aromatic amino compounds such as aromatic primary amine CN stretch. The peak at 879.82 cm-1 indicates the presence of ether and oxy compounds like peroxides, C-O-O stretch. The FT-IR results of lauric acid found to be similar to the FT-IR results of dodecanoic acid in previous studies (Peres et al., 2014).

Antimicrobial activity

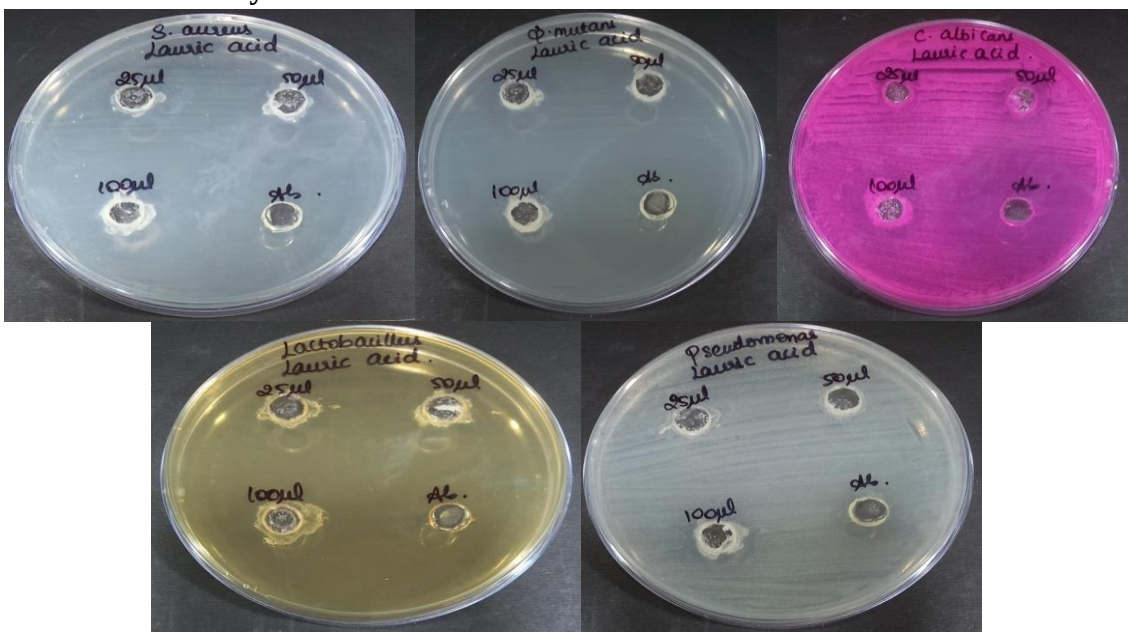

Fig 3: Antimicrobial activity of Lauric acid against oral pathogens.

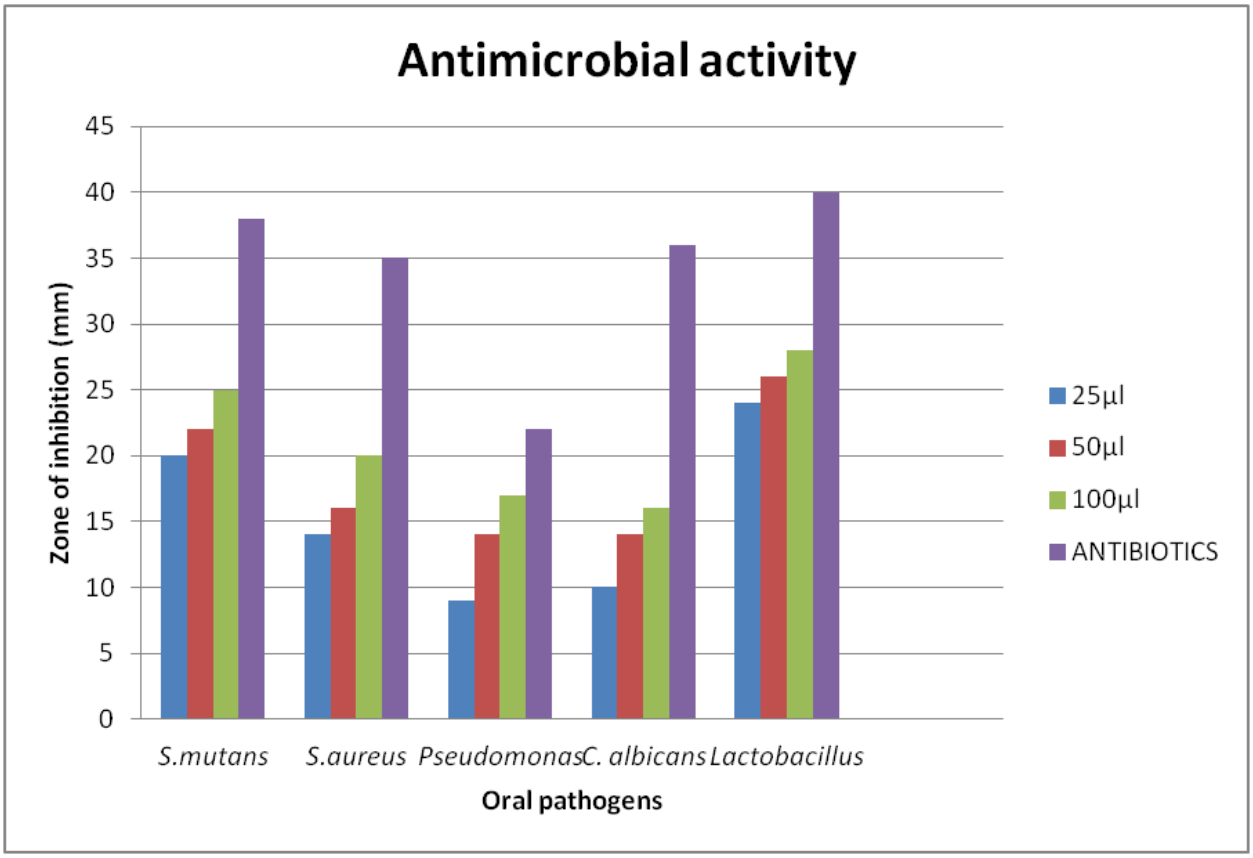

Fig 4: Histogram of antimicrobial activity of Lauric acid

The antimicrobial activity of lauric acid was tested against five oral pathogens such as *Streptococcus mutans, Staphylococcus aureus, Lactobacillus acidophilus, Candida albicans, Pseudomonas aeruginosa* which was depicted in fig 3. Three different concentrations like 25µL, 50µL, 100µL were used for lauric acid. The major zone of inhibition was observed against *S. aureus, S.mutans, Lactobacillus* which indicates the high sensitivity of oral pathogens to lauric acid. The minimum zone of inhibition was observed against *Pseudomonas aeruginosa* followed by *Candida albicans* which indicates the resistance nature of the pathogens to lauricacid.The higher zone of inhibition of lauric acid against oral pathogens resides more or less equal to the standard drugs (amoxyrite and fluconazole) at 100 µL concentration. As the concentration increases, the zone of inhibition values of lauric acid also increases in a dose dependent manner (Rajeshkumar and Malarkodi, 2017, Anandhi et al., 2022) which was depicted in fig 4. In existing research work, lauric acid, chlorhexidine, non- functional fatty acid, and saline was tested for its antimicrobial activity against *Streptococcus mutans* biofilm which was grown over human dentin skin and performed by biofilm viability assay. The results revealed that lauric acid exhibited decreased biomass of *S.mutans* on dentin skin than chlorhexidine (Lee et al., 2016). Currently, lauric acid was combined with chitosan and studied for prevention of bacterial spoilage and discolouration in aerobically packaged beef steaks during refrigerated storage. A beef steak around 2cm thickness was used for this study which was wrapped with different

concentrations of lauric acid + chitosan combination such as 1 mM lauric acid + 2 % chitosan, 1mM lauric acid + 2% chitosan and 3 mM lauric acid in 1 % chitosan.The results showed that the 1-3 mM lauric acid prevented the formation of bacterial spoilage forming volatile compounds and discoloration of the beef steak in the refrigerator after 21 days storage (Hoa et al., 2022, Malarkodi et al., 2017).

Cytotoxic effect

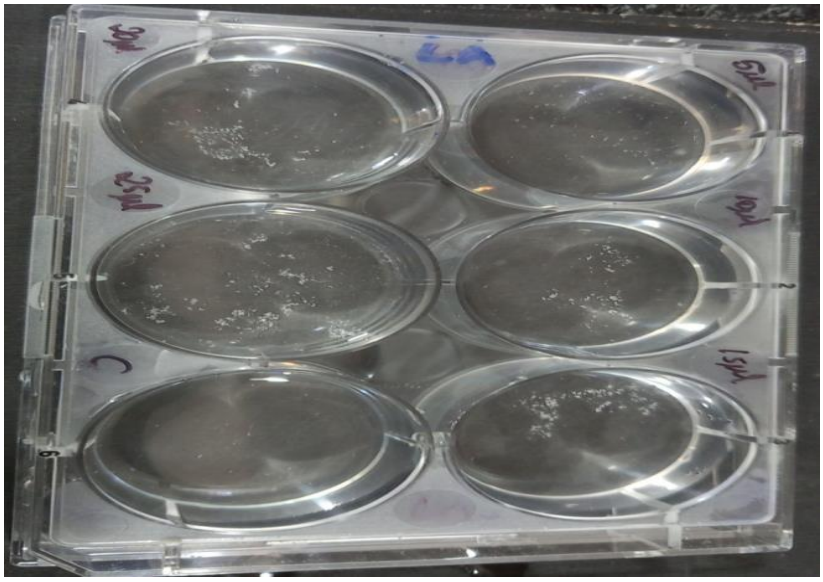

Fig 5: Brine shrimp lethality assay setup of Lauric acid

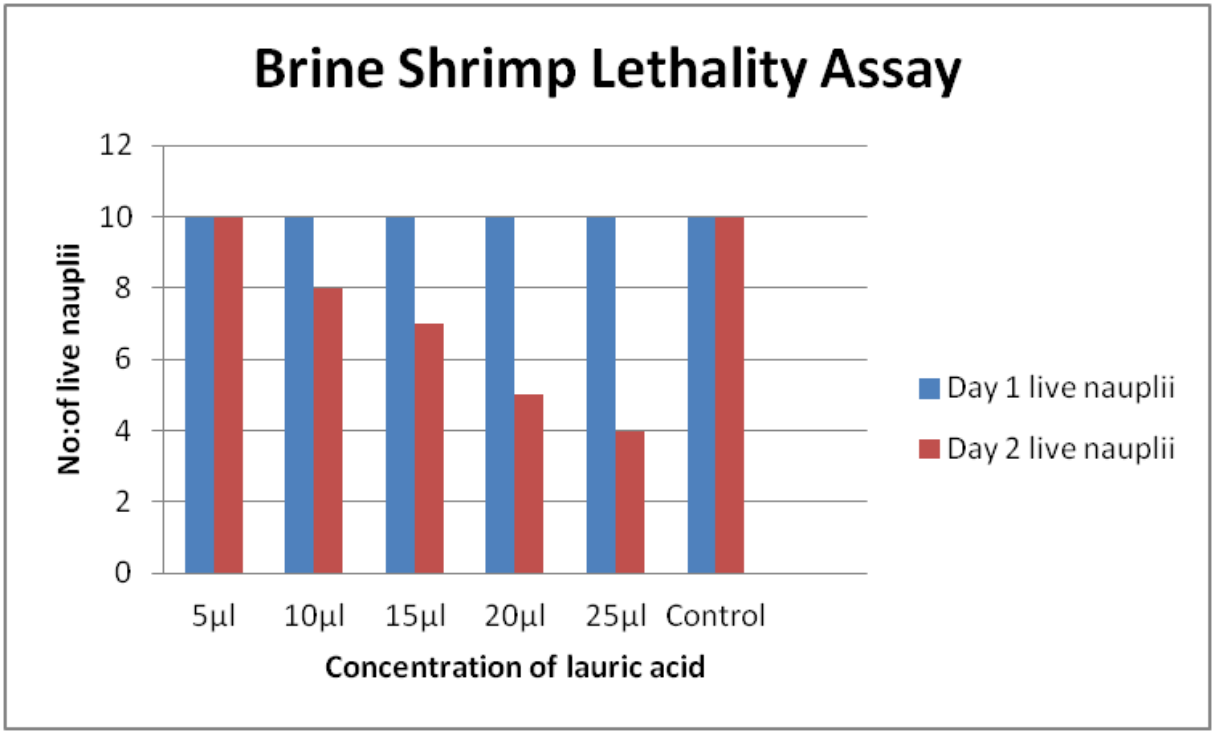

Fig 6: Histogram of Brine shrimp lethality assay - Lauric acid

Figure 5 depicts the brine shrimp lethality assay setup in which *Artemia salina* nauplii were used to test the biocompatibility of lauric acid. Fig 6 represents diverse concentrations (5µL, 10µL,15µL, 20µL, 25µL) of lauric acid

used in testing Brine shrimp lethality assay. A control well was kept devoid of drug lauric acid to compare with the different concentration of lauric acid. In minimum concentration such as 5 µL showed 100 % live nauplii and at 10 µL concentration showed 80% live nauplii count. It has to be noted that there is no previous study done on brine shrimp lethality of lauric acid. In existing research works, brine shrimp lethality assay was performed for various plant extracts, nanoparticles, essential oils etc (Anjana et al., 2020 & Roy et al., 2020).The results of brine shrimp lethality assay represents the non-toxic nature of lauric acid even at higher concentration like 25 µL it showed 40% live nauplii count.

Conclusion

Lauric Acid is a monounsaturated fatty acid found naturally in coconut oil. Coconut oil contains lauric acid, which may help reduce cholesterol levels. Lauric acid is used in many commercial applications including cosmetics, pharmaceuticals, and food processing. Lauric acid may act as an antifungal agent and antibacterial agent, and this is due to its ability to disrupt membrane function through detergent action. This disruption causes leakage of cellular contents and ultimately cell death. The current study results proved lauric acid to be an excellent antimicrobial agent and its less toxic nature was proven by brine shrimp lethality assay. The FT-IR results of lauric acid also confirmed the presence of lauric acid derivatives.

References

1. Anjana, G., Roy, A., Rajeshkumar, S., &Ezhilarasan, D. (2020). Cassia oleoresin Mediated Synthesis of Magnesium Oxide Nanoparticles and Brine Shrimp Lethality Assay.
2. P. Anandhi, M. Tharani, S. Rajeshkumar*, T. Lakshmi Antibacterial activity of cinnamon and clove oil against wound pathogens J PopulTher Clin Pharmacol Vol 28(2):e41–e46; DOI: 10.47750/jptcp.2022.871
3. Borrelli, L., Varriale, L., Dipineto, L., Pace, A., Menna, L. F., &Fioretti, A. (2021). Insect derived lauric acid as a promising alternative strategy to antibiotics in the antimicrobial resistance scenario. *Frontiers in Microbiology*, *12*, 620798.
4. Dayrit, F. M. (2015). The properties of lauric acid and their significance in coconut oil. *Journal of the American Oil Chemists' Society*, *92*(1), 1-15.
5. Ganesan, S., Nadarajah, S., Khairuddean, M., &Teh, G. B. (2019). Studies on lauric acid conversion to methyl ester via catalytic esterification using ammonium ferric sulfate. *Renewable Energy*, *140*, 9-16.
6. He, Y., Zhang, X., Zhang, Y., Song, Q., & Liao, X. (2016). Utilization of lauric acid-myristic acid/expanded graphite phase change materials to improve thermal properties of cement mortar. *Energy and Buildings*, *133*, 547-558.
7. Hoa, V. B., Song, D. H., Seol, K. H., Kang, S. M., Kim, H. W., Kim, J. H., & Cho, S. H. (2022). Coating with chitosan containing lauric acid (C12: 0) significantly extends the shelf-life of aerobically–Packaged beef steaks during refrigerated storage. *Meat Science*, *184*, 108696.
8. Huang, W. C., Tsai, T. H., Chuang, L. T., Li, Y. Y., Zouboulis, C. C., & Tsai, P. J. (2014). Anti-bacterial and anti-

inflammatory properties of capric acid against Propionibacterium acnes: a comparative study with lauric acid. *Journal of dermatological science*, *73*(3), 232-240.

9. Inoue, T., Hisatsugu, Y., Ishikawa, R., & Suzuki, M. (2004). Solid–liquid phase behavior of binary fatty acid mixtures: 2. Mixtures of oleic acid with lauric acid, myristic acid, and palmitic acid. *Chemistry and physics of lipids*, *127*(2), 161-173.
10. Intahphuak, S., Khonsung, P., &Panthong, A. (2010). Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. *Pharmaceutical biology*, *48*(2), 151-157.
11. Kinderlerer, J. L. (1994). Degradation of the lauric acid oils. *International biodeterioration & biodegradation*, *33*(4), 345-354.
12. Lee, J. H., & Jo, Y. W. (2016). Antimicrobial effect of a lauric acid on Streptococcus mutans biofilm. *Annals of International Medical and Dental Research*, *2*(4), 60-5.
13. Lee, J. H., Kim, Y. G., Khadke, S. K., & Lee, J. (2021). Antibiofilm and antifungal activities of medium-chain fatty acids against Candida albicans via mimicking of the quorum-sensing molecule farnesol. *Microbial biotechnology*, *14*(4), 1353-1366.
14. Leyton, J., Drury, P. J., & Crawford, M. A. (1987). Differential oxidation of saturated and unsaturated fatty acids in vivo in the rat. *British Journal of Nutrition*, *57*(3), 383-393.
15. Lieberman, S., Enig, M. G., & Preuss, H. G. (2006). A review of monolaurin and lauric acid: natural virucidal and bactericidal agents. *Alternative & Complementary Therapies*, *12*(6), 310-314.
16. Liu, M., Chen, S., Zhang, Z., Li, H., Sun, G., Yin, N., & Wen, J. (2022). Anti-ageing peptides and proteins for topical applications: a review. *Pharmaceutical Development and Technology*, *27*(1), 108-125.
17. C Malarkodi, S Rajeshkumarand G Annadurai Detection of environmentally hazardous pesticide in fruit and vegetable samples using Gold nanoparticles Food control (2017) http://dx.doi.org/10.1016/j.foodcont.2017.04.023
18. Nainggolan, M., &Sinaga, A. G. S. (2021). Characteristics of fatty acid composition and minor constituents of red palm olein and palm kernel oil combination. *Journal of Advanced Pharmaceutical Technology & Research*, *12*(1), 22.
19. Nakatsuji, T., Kao, M. C., Fang, J. Y., Zouboulis, C. C., Zhang, L., Gallo, R. L., & Huang, C. M. (2009). Antimicrobial property of lauric acid against Propionibacterium acnes: its therapeutic potential for inflammatory acne vulgaris. *Journal of investigative dermatology*, *129*(10), 2480-2488.
20. Peres, R. S., Baldissera, A. F., Armelin, E., Alemán, C., & Ferreira, C. A. (2014). Marine-friendly antifouling coating based on the use of a fatty acid derivative as a pigment. *Materials Research*, *17*, 720-727.
21. Roy, A., Shanmugam, R., &Warrier, D. (2020). Preparation and Characterization of Cinnamon Oil Mediated Gold Nanoparticles and Evaluation of Its Cytotoxicity Using Brine Shrimp Lethality Assay. *Journal*

of Evolution of Medical and Dental Sciences, *9*(39), 2894-2898.

22. S Rajeshkumar and C Malarkodi Optimization of *Serratia nematodiphila*using Response surface methodology to silver nanoparticles synthesis for aquatic pathogen control IOP Conf. Series: Materials Science and Engineering263 (2017) 022041 doi:10.1088/1757-899X/263/2/022041.
23. Rajeshkumar SKarpagambigai S, Jacquline Rosy P and Venkat Kumar S (2017) Controlling of Disease Causing Pathogens Using Silver Nanoparticles Synthesized by One Step Green Procedure Journal of Applied Pharmaceutical Science (2018) 8 (01); 142-146.
24. Rajeshkumar S , Tharani M, Sivaperumal P, Lakshmi T (2020) Synthesis of Antimicrobial Silver Nanoparticles by Using Flower of Calotropis Gigantea . *J Complement Med Res*, 11 (5), 8-16.
25. Saat, A. Z. M. A. H., Rosli, R. O. Z. I. T. A., &Syakroni, N. O. R. M. A. W. A. T. I. (2013). Potential hypoglycemic property of Albizia myriophylla and virgin coconut oil in streptozotocin induced diabetic rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *5*(4), 199-202.
26. Sharma, N., Verma, M., & Sharma, P. (2021, April). Influence of Lauric acid on mechanical properties of Portland cement. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1116, No. 1, p. 012153). IOP Publishing.
27. Silva, E. L., Carneiro, G., De Araújo, L. A., de Jesus, M., Trindade, V., Yoshida, M. I., ... & Ferreira, L. A. M. (2015). Solid lipid nanoparticles loaded with retinoic acid and lauric acid as an alternative for topical treatment of acne vulgaris. *Journal of Nanoscience and Nanotechnology*, *15*(1), 792-799.
28. Singer, P., Shapiro, H., Theilla, M., Anbar, R., Singer, J., & Cohen, J. (2008). Anti-inflammatory properties of omega-3 fatty acids in critical illness: novel mechanisms and an integrative perspective. *Intensive care medicine*, *34*(9), 1580-1592.
29. Tangwatcharin, P., &Khopaibool, P. (2012). Activity of virgin coconut oil, lauric acid or monolaurin in combination with lactic acid against Staphylococcus aureus. *Southeast Asian Journal of Tropical Medicine & Public Health*, *43*(4), 969-985.