Comparative In vitro study of antibacterial and antifungal activity of Tea Tree (*Melaleuca alternifolia*) essential oil, Lemon grass (*Cymbopogon citratus*) essential oil, and Peppermint (*Menthae piperitae aetheroleum*) essential oil

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***Abstract* - Essential oil is a naturally occurring substance that has demonstrated a variety of antibacterial and antifungal actions. As a result of this ability, it finds extensive use in the pharmaceutical and cosmetics sectors. Three significant essential oils—Peppermint (*Menthae piperitae aetheroleum*), Lemon Grass (*Cymbopogon citratus*), and Tea Tree (*Melaleuca alternifolia*)—have been examined for their antibacterial and antifungal properties in this study. This study also compares the lowest bacterial concentration range and minimum inhibitory concentration of these three essential oils. Two types of assays were used to demonstrate the activities of essential oils with four bacteria, namely, *E. coli* strain DH5α, *Bacillus cereus, Staphylococcus aureus,* and *Klebsiella pneumoniae.* Similarly, antifungal activities of all the three oils were analyzed using fungal cultures of *Aspergillus flavus, Aspergillus niger,* and yeast *Candida albicans.* All the oils showed effective antimicrobial activities.**

Index Terms* - Essential oils, Antimicrobial activities, *E. coli strain DH5α, Bacillus cereus, Staphylococcus aureus, Klebsiella pneumoniae, Aspergillus flavus, Aspergillus niger, Candida albicans.

1.INTRODUCTION

Due to the changing climate, there are many bacteria and fungi that are emerging as opportunistic pathogens, that need to be under control(Chahal, 2021). Studies are being conducted to deal with this situation naturally using natural agents like medicinal plants, essential oils, and extracts(“Drug Repurposing and Traditional Drug Discovery: An Overview,” 2021). Essential oils that are derived from various plant parts have long been known to have antibacterial properties(Prachi S & Reena G., 2018). Because they exhibit inhibition against bacteria, fungus, and yeasts, higher and aromatic plants have long been employed in folk medicine to lengthen the shelf life of foods. Essential oils produced by their secondary metabolism are mostly responsible for their characteristics (Pegard, 2015a). Gram-negative and Gram-positive bacteria, as well as those linked to skin, dental cavities, and food spoilage, can be controlled by using essential oils and extracts from a variety of plant species (Adaszynska-Skwirzynska & Dzieciol, 2018). In this study, the antimicrobial effects of three essential oils, including tea tree, peppermint, and lemongrass, were examined. The comparison of the minimum bacterial concentration and minimum inhibitory concentration range of these three essential oils in the aforementioned microorganisms is also shown in this study.

1.1. Tea Tree Oil

The leaves of the tea tree, *Melaleuca alternifolia*, are the source of the essential oil known as tea tree oil (TTO). *M. alternifolia* is a native of Australia. TTO is applied externally as a folk or traditional therapy for a variety of ailments, such as lice, oral candidiasis (thrush), cold sores, dandruff, skin lesions, athlete's foot, nail fungus, wounds, and infections. TTO is an active component of many topical formulations used to treat cutaneous infections and is largely responsible for its antibacterial capabilities. It is promoted as a treatment for numerous illnesses at low doses and is generally accessible in nations like Australia, Europe, and North America. However, there are numerous negative effects at high concentrations, such as dermatitis, allergic rashes, and skin irritation (MARKHAM, n.d.).

1.1.1.Composition and Chemistry

Terpene hydrocarbons, primarily monoterpenes, sesquiterpenes, and the alcohols that go with them, make up TTO. The chemical makeup of tea tree oil has been defined by International Standard ISO 4730(2004) and the Comparable Australian Standard 2782-2009 ("Oil of Melaleuca, Terpinen-4-ol type"). Of more than 100 components in pure Australian TTO, it defines the level of 15 of them(DAVIS, n.d.).

1.1.2.Antibacterial action.

TTO possesses antibacterial activity *in vitro* (Papadopoulou et al., 2007). The mechanism of action of TTO against bacteria involves loss of intracellular material, inability to maintain homeostasis, and inhibition of respiration after treatment with TTO. Some reports show that few strains of *S. aureus* are slightly resistant to TTO. It has been also found that *E. coli* strains harboring mutations in the multiple antibiotic resistance (*mar*) operon, so-called Mar mutants may exhibit decreased susceptibility to TTO(Pegard, 2015b).

1.1.3.Antifungal activity

Many fungi show susceptibility to TTO like yeast, dermatophytes, and other filamentous fungi. *Candida albicans* was a commonly chosen model test organism because it easily grows in lab conditions and has a short life span. For fungi, application of TTO has been generally found to have MICs that range between 0.03 and 0.5%, and the minimal fungicidal concentration (MFC) generally ranges from 0.12 to 2% with an exception for *Aspergillus niger* of 8% (Kabir Mumu & Mahboob Hossain, 2018)

Studies show that the mechanism of antifungal action of TTO is similar to bacteria. In *C. albicans* TTO alters the permeability of the cells. Hammer et al. (2002) have reported that treatment of *C. albicans* with 0.25% TTO resulted in the uptake of propidium iodide after 30 min, and after 6 h significant staining with methylene blue showed loss of 260-nm-light-absorbing materials. Further research has demonstrated that the membrane fluidity of *C. albicans* cells treated with 0.25% TTO is significantly increased, which confirms that the oil substantially alters the membrane properties of *C. albicans*. TTO also inhibits respiration in *C. albicans* in a dose-dependent manner (Hammer, 2002). Respiration was inhibited by approximately 95% after treatment with 1.0% TTO and by approximately 40% after treatment with 0.25% TTO. Studies have also shown that germ tube formation was completely inhibited in the presence of 0.25 and 0.125% TTO in *C. albicans*.

1.2. Peppermint oil

Peppermint oil is an essential oil obtained from the leaves of the perennial herb, *Mentha piperita* L. and *M. arvensis* var. *piperascens*, a member of the labiatae family. It is a native of the Mediterranean. Peppermint oil is a colourless, pale yellow or pale greenish-yellow liquid having a characteristic odour and taste followed by a sensation of cold and is freely soluble in ethanol (70%). The oil is found on the undersides of the leaves and is extracted by steam distillation followed by rectification and fractionation before use. Mint oil and its constituents and derivatives are used in the food, pharmaceutical, perfumery, and flavoring industries. Peppermint oil shows antimicrobial properties in test tube studies, inhibiting a wide variety of pathogens. Peppermint oil shows potent antibacterial and weaker antifungal effects *in vitro*. It is active against dermatophytic fungi, which causes athlete's foot and ringworm, in test tube studies. Peppermint also has antiviral effects *in vitro*, inhibiting the growth of influenza and herpes viruses.

1.2.1.Composition and Chemistry

The chemical components of peppermint oil are menthol, menthone, 1,8-cineole, methyl acetate, methofuran, isomenthone, limonene, b-pinene, a-pinene, germacrene-d, trans-sabinene hydrate (Liang et al., 2012).

1.2.2.Antibacterial activity

Peppermint oil possesses moderate antibacterial activity against both Gram-positive (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative (*Escherchia coli* and *Klebsiella pneumonia*) bacteria. There are many reports describing the MICs and MBCs range of peppermint oil. The MIC known for the bacterial species ranges from 0.4% to 0.7%, and the lowest MIC values have been found for the *K. pneumoniae* strain (Krin et al., 2022).

Peppermint is known for its antibacterial activity. Mechanisms of antibacterial action of peppermint oil involve the loss of intracellular material, inability to maintain homeostasis, and cell lysis due to the breakdown of peptidoglycan. Agar well diffusion is one of the methods which are used to determine the antibacterial activity of peppermint oil by measuring the diameter of the growth inhibition zone at different concentrations. Figure 4 and figure 5 show the inhibitory zone of peppermint oil (1µl) on Gram-positive and Gram-negative bacterial strains, respectively. Both Gram-positive bacterial species (*S. aureus* and *S. pyogenes*) tested were sensitive to peppermint essential oil with the inhibition zone 17.2 and 13.1 mm, respectively. The inhibition zone for Gram-negative bacteria ranges from 5.1 to 12.4 mm. However, *S. aureus* was found to be the most sensitive to essential oil followed by *S. pyogenes*, *K. pneumonia*, and *E. coli*. Thus, peppermint oil is effective against Gram-positive and Gram-negative bacteria, but more effective against Gram-positive organisms when compared to Gram-negative. The lipopolysaccharides present in the outer membrane of Gram-negative bacteria might be responsible for their enhanced resistance to antibacterial substances (Allosso, 2020).

1.2.3.Antifungal activity.

Many fungal species show susceptibility to peppermint oil like yeast, dermatophytes, and other filamentous fungi. *Candida albicans* was a commonly chosen model test organism. for fungi peppermint oil, for which the MICs generally range between 0.125 and 1% while the minimal fungicidal concentration (MFC) generally ranges from 0.125 to 2% (Gadadavar et al., 2021).

1.3. Lemongrass oil

Lemongrass oil is an essential oil, which comes from the leaves of *Cymbopogon citrates* and other *Cymbopogon* species. Lemon grass is a native of Malaya or Sri lanka but is chiefly cultivated in Indonesia, Malaysia, India, and Brazil. It is a perennial grass with numerous stiff stems arising from the rhizomatous rootstock. The leaves contain 0.5% of oil with a lemon-like scent. The oil is extracted from freshly cut and slightly dried leaves by steam distillation. The oil contains 70-80% citral. The use of lemongrass was found in folk remedies for coughs, consumption, elephantiasis, malaria, ophthalmia, pneumonia and vascular disorder lemongrass holds antidepressants, antioxidants, antiseptic, astringent, bactericidal, fungicidal, nervine, and sedative properties.

1.3.1.Composition and Chemistry

The chemical components of lemongrass oil are citral, citronellol, geraniol and myrcene.

1.3.2.Antibacterial activity

Lemongrass oil possesses antibacterial activity in vitro, against both Gram-positive and Gram-negative organisms. It was observed by some scientists that Gram-positive organisms were more sensitive to the oil than Gram-negative organisms. Many bacterial organisms like *Staphylococcus aureus*, *Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia*, and *Pseudomonas aeruginosa* show susceptibility to lemongrass oil. Many reports describe the MICs and MBCs range of peppermint oil. The MIC for the bacterial species ranged from 0.03 to 0.50%(Perdana et al., 2021).

Mechanisms of antibacterial action of lemongrass oil involve the effects on cell membrane permeability which disrupts the metabolisms occurring on the cell membrane. Lemon grass is known for its antibacterial activity. Agar well diffusion and broth dilution are the methods that determine the antibacterial activity of lemon grass oil, by measuring the diameter of growth inhibition zones at different concentrations. The MIC and MBC are determined by the broth dilution method. Gram-positive organisms (*S. aureus, B. cereus,* and *B. subtilis*) are more susceptible to lemongrass oil than gram-negative organisms *(E. coli, K.pneumoniae, P. aeruginosa)* and *P. aeruginosa* was found to be highly resistant. The antibacterial activity was found to progressively increase with the increase in the concentration of oil. It was found that in the broth dilution method the bacterial organism was found to be inhibited by lemongrass oil at a very low concentration as compared to agar diffusion methods. *S. aureus* and *B. cereus* were found to be more sensitive and got inhibited at 0.03% concentration (MIC). *B. subtilis* and *E. coli* were found to be inhibited at a concentration of 0.06% (MIC) and 0.12% (MBC). Compared to other test organisms *K. pneumoniae* showed a higher MIC (0.25%) and MBC (0.5%) values (Bilichodmath, 2019).

1.3.3.Antifungal activity

Many fungi like yeasts, dermatophytes, and non-dermatophytic filamentous fungi show susceptibility to lemongrass oil. Lemongrass oil was found to be highly fungicidal, as it showed the lowest MIC and MFC values and the highest growth inhibition.

The mechanisms of antifungal action of lemongrass oil involve loss of pigmentation and disrupted conidiophore structure. Lemongrass oil was strongly active against spore germination. Spore germination of *A. niger* was significantly inhibited by Lemongrass oil volatile at a dose of 10 µl. And spores *of A. flavus* lost their viability when exposed to a concentration of 15 µl. The MIC and MFC of lemongrass oil against both *Candida spp* were 0.5 and 1µl/ml. lemongrass oil and its major constituents also affect the cell wall of candida spp like citral, geraniol, linalool, menthol, and thymol, which are the major components of essential oils, alter cell permeability by penetrating between the fatty acyl chains making up the membrane lipid bilayers, disrupting lipid packing and changing membrane fluidity (Perdana et al., 2021).

2. MATERIALS & METHODS

This experimental study was conducted in the Department of Botany, University of Delhi. This paper described the comparison of antimicrobial activities of different essential oils and extracts. Basically, three essential oils namely Tea Tree oil, Peppermint oil, and lemongrass oil had taken. Two types of assays to demonstrate the activities of essential oils had been used again four bacteria, namely, *E. coli* strain DH5α, *Bacillus cereus, Staphylococcus aureus,* and *Klebsiella pneumoniae*. Similarly, antifungal activities of all the three oils were analyzed using fungal cultures of *Aspergillus flavus, Aspergillus niger,* and yeast *Candida albicans*. There are two methods that were conducted to compare the antimicrobial activities of these three essential oils (Balouiri et al., 2016).

2.1. Disc diffusion assay: growth inhibition

In this assay, all the bacterial and fungal cultures were grown overnight in a Luria Bertani medium at 37°C with shaking at 150 rpm for 16 h. After shaking, 100 µl of the resulting culture was spread onto LB agar plates as a lawn. Sterile 7 mm glass fiber discs were immediately placed on the surface of the bacterial plates and essential oil (10 µl) was added to each disc. Grapeseed (*Vitis vinifera* seed) oil was used as a negative control and it was not expected to exhibit any antibacterial activity (*An in Vitro Study of the Antimicrobial Effect of Indigofera Daleiodes Plant Tinctures Using Disc Diffusion and Well Diffusion Assay*, 2020). Following growth at 37°C for 24 h, the zones of inhibition (halos) were measured with the help of a ruler to an accuracy of 0.5 mm. An absence of antibacterial activity would produce a halo of 7 mm in diameter, the size of the glass fiber discs.

2.2. Disc diffusion assay: zone of clearance

In this assay, all the bacterial and fungal cultures grown overnight (100 µl) were spread aseptically onto LB agar plates, and after that culture was grown overnight at 37°C for 16 h. Sterile 7 mm glass fiber discs containing 10 µl essential oil were added to each disc. Grapeseed oil was used as a control (*A Controlled in Vitro Study of the Effectiveness of the Plant Tinctures, Commiphora Molmol, Hydrastis Canadensis and Warburgia Salutaris against Candida Albicans Using the Disc Diffusion Assay*, 2004). The plates were grown at 37°C for a further 2 days and the diameters of the zones of clearance (*Determination of the Antimicrobial Properties of Withania Somnifera and Xysmalobium Undulatum Plant Tinctures in Terms of the Disc Diffusion Assay and the Agar Dilution Sensitivity Test*, 2007)were measured with the help of a ruler to an accuracy of 0.5 mm.

2.3. Statistical analysis

The results were interpreted with the standard deviation. The data were statistically analyzed to know the significant difference in antimicrobial susceptibility of each of the three essential oil using SPSS software (version 17).

3. RESULTS & DISCUSSION

3.1. Disc diffusion assay: growth inhibition

The result of this assay was shown that the halo diameters were produced by tea tree (*Melaleuca alternifolia*) oil, lemongrass oil, peppermint oil, and the grapeseed oil negative control are 27 +/- 1.5 mm for tea tree, 18 +/- 1 mm for lemongrass, 17.7 +/- 3.5 mm for peppermint and 7 +/- 0 mm for the grapeseed oil (Fig.1). Among these, the largest halo diameter was produced by tea tree oil, and the results were highly significantly different from the control in each case.

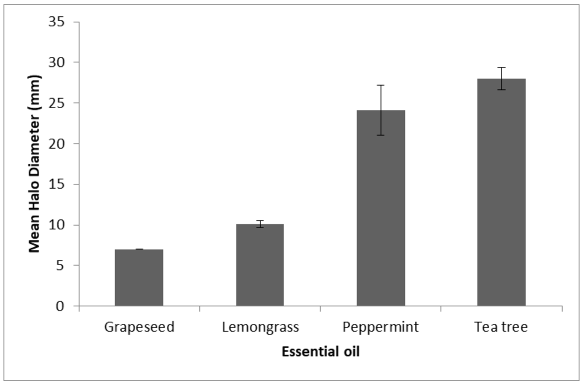

Figure 1: Antibacterial activity of essential oils in disc diffusion assays

3.1 Disc diffusion assay: zone of clearance

The result of this assay showed that peppermint and tea tree oils exhibited strong antibacterial activity in the zone of clearance assay as compared to lemongrass oil, with mean halo diameters 25.8 +/- 1.8 mm and 28.0 +/- 1.4 mm 10.1 +/- 0.4 mm respectively (Fig.2), compared to a mean halo diameter of 7 +/- 0 mm for grapeseed oil. This demonstrates that tea tree and peppermint oils have strong antibacterial activity toward *E. coli* as compare to lemongrass oil.

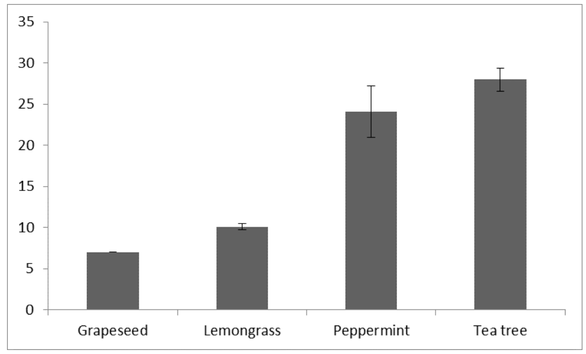

Figure 2: Antibacterial activity of essential oils in the zone of clearance assays.

3.2 Comparison of MIC, MBC, and MFC of three essential oils.

Table 1. Comparison of MIC and MBC of three essential oil three essential oil

	Tea tree oil % (µl/ml)		Peppermint oil % (µl/ml)		Lemon grass oil % (µl/ml)	
Bacterial species	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli	0.08 -2	0.25-4	0.04-0.7	0.03-0.9	0.06	0.12
Bacillus cereus	0.3± 0.5		0.06	0.012	0.03	0.06
Staphylococcus aureus	0.5-1.25	1-2	0.03-0.5	0.02-0.6	0.03	0.06
Klebsiella pneumoniae	0.25-0.3	0.25	0.02-0.4	0.03-0.8	0.25	0.5

Table 2. Comparison of MIC and MFC of three essential oil three essential oil

Variable	Tea tree oil % (µl/ml)		Peppermint oil % (µl/ml		Lemon grass oil % (µl/ml	
Fungal species	MIC	MFC	MIC	MFC	MIC	MFC
Candida albicans	0.06-80	0.12-1	0.125	0.125	0.5	1
Aspergillus flavus	0.31-0.7	2-4	1	2	1.25-10	1.25-10
Aspergillus niger	0.0016-0.4	2-8	0.5	1	6.5-15	8.5-17

After comparing the MIC, MBC, and MFC concentration of these three essential oils it has been observed that out of three essential oils peppermint oil was found to be the highest antibacterial and antifungal activity, as it showed the lowest MIC and MBC, and MFC values and the highest growth inhibition (Table 1 and 2).

4. CONCLUSION

In comparison to lemongrass and peppermint essential oils, the aforementioned data demonstrate that tea tree oil displayed considerable antibacterial activity against *E. coli* in the disc diffusion assay. The antibacterial activity of lemongrass and peppermint oils against *E. coli* is moderate. When compared to lemongrass oil, peppermint and tea tree oils have stronger antibacterial action against *E. coli* in the zone of clearance assay. From the present study, it is clear that tea tree oil, peppermint oil, and lemongrass oil possess a high antimicrobial activity against four bacteria like *E. coli* strain DH5α, *Bacillus cereus*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*. Similarly, they possess antifungal activities against fungal cultures of *Aspergillus flavus*, *Aspergillus niger*, and yeast *Candida albicans*. The result obtained from different assays and methods supports that these essential oils are highly effective against many infections and diseases causing organisms. In this study, we have also compared the MIC, MBC, and MFC values of these essential oils against a few microorganisms and after comparison, it has been found that among these three essential oils peppermint oil was found to be the highest antibacterial and antifungal activity, as it showed the lowest MIC and MBC and MFC values and the highest growth inhibition. It has been also noted that a few bacterial strains also show resistance to these essential oils like *Klebsiella pneumoniae* was found to be highly resistant to lemongrass oil. Some reports show that few strains of *Staphylococcus aureus* are slightly resistant to tree tea oil.

5. CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCE

[1] *A controlled in vitro study of the effectiveness of the plant tinctures, Commiphora molmol, Hydrastis canadensis and Warburgia salutaris against Candida albicans using the disc diffusion assay*. (2004). [Durban University of Technology]. https://doi.org/10.51415/10321/323

[2] Adaszynska-Skwirzynska, M., & Dzieciol, M. (2018). Comparison of Chemical Composition and Antimicrobial Activity of Essential Oils Obtained from Different Cultivars and Morphological Parts of *Lavandula angustifolia*. *Journal of Essential Oil Bearing Plants*, *21*(6), 1532–1541. https://doi.org/10.1080/0972060X.2018.1526123

[3] Allosso, D. (2020). Prize Medal Oil of Peppermint. In *Peppermint Kings* (pp. 77–98). Yale University Press. https://doi.org/10.12987/yale/9780300236828.003.0005

[4] *An in vitro study of the antimicrobial effect of Indigofera daleiodes plant tinctures using Disc Diffusion and Well Diffusion Assay*. (2020). [Durban University of Technology]. https://doi.org/10.51415/10321/3879

[5] Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*, *6*(2), 71–79. https://doi.org/10.1016/j.jpha.2015.11.005

[6] Bilichodmath, S. (2019). Antimicrobial Property of Lemongrass Oil against Porphyromonas Gingivalis. *Journal of Dentistry and Oral Sciences*. https://doi.org/10.37191/Mapsci-2582-3736-1(2)-013

[7] Chahal, K. (2021). Bacteria Emerging as an Opportunistic Pathogen. *International Journal of Current Science Research and Review*, *04*(05). https://doi.org/10.47191/ijcsrr/V4-i5-12

[8] DAVIS, G. R. (n.d.). TEA TREE OIL DISTILLATION. In *TEA TREE*. Taylor & Francis. https://doi.org/10.4324/9780203303603_chapter_8

[9] *Determination of the antimicrobial properties of Withania somnifera and Xysmalobium undulatum plant tinctures in terms of the disc diffusion assay and the agar dilution sensitivity test*. (2007). [Durban University of Technology]. https://doi.org/10.51415/10321/326

[10] Chahal, K. Drug Repurposing and Traditional Drug Discovery: An Overview. (2021). *International Journal of Green and Herbal Chemistry*, *10*(3). https://doi.org/10.24214/IJGHC/HC/10/3/21520

[11] Gadadavar, S., Rutvi, & Vagarali, M. (2021). Antimicrobial Efficacy of tea tree oil and peppermint oil against Periopathogen Porphyromonas gingivalis. *Indian Journal of Medical Microbiology*, *39*, S12. https://doi.org/10.1016/j.ijmmb.2021.08.041

[12] Hammer, K. A. (2002). In vitro activity of Melaleuca alternifolia (tea tree) oil against dermatophytes and other filamentous fungi. *Journal of Antimicrobial Chemotherapy*, *50*(2), 195–199. https://doi.org/10.1093/jac/dkf112

[13] Kabir Mumu, S., & Mahboob Hossain, M. (2018). Antimicrobial Activity of Tea Tree oil against Pathogenic Bacteria and Comparison of Its Effectiveness with Eucalyptus Oil, Lemongrass Oil and Conventional Antibiotics. *American Journal of Microbiological Research*, *6*(3), 73–78. https://doi.org/10.12691/ajmr-6-3-2

[14] Krin, A., Quesada Moreno, M. M. Q., Pérez, C., & Schnell, M. (2022). A Scent of Peppermint—A Microwave Spectroscopy Analysis on the Composition of Peppermint Oil. *Symmetry*, *14*(6), 1262. https://doi.org/10.3390/sym14061262

[15] Liang, R., Xu, S., Shoemaker, C. F., Li, Y., Zhong, F., & Huang, Q. (2012). Physical and Antimicrobial Properties of Peppermint Oil Nanoemulsions. *Journal of Agricultural and*

Food Chemistry, *60*(30), 7548–7555. https://doi.org/10.1021/jf301129k

[16] MARKHAM, J. L. (n.d.). BIOLOGICAL ACTIVITY OF TEA TREE OIL. In *TEA TREE*. Taylor & Francis. https://doi.org/10.4324/9780203303603_chapter_9

[17] Papadopoulou, C., Sakkas, H., Arvanitidou, M., & Leveidiotou, S. (2007). The antimicrobial activity of origanum oil, basil oil, chamomile blue oil, tea tree oil and thyme oil against Gram-positive bacterial strains. *Planta Medica*, *73*(09). https://doi.org/10.1055/s-2007-986947

[18] Pegard, A. (2015a). Antimicrobial activity of essential oil of Bulnesia sarmienti Lorenz (Gaiac Wood) and of a rectified fraction of this oil. Comparison with Melaleuca alternifolia L. (Tea Tree) essential oil activity. *Phytothérapie*, *13*(6), 384–388. https://doi.org/10.1007/s10298-015-0966-7

[19] Pegard, A. (2015b). Antimicrobial activity of essential oil of Bulnesia sarmienti Lorenz (Gaiac Wood) and of a rectified fraction of this oil. Comparison with Melaleuca alternifolia L. (Tea Tree) essential oil activity. *Phytothérapie*, *13*(6), 384–388. https://doi.org/10.1007/s10298-015-0966-7

[20] Perdana, M. I., Ruamcharoen, J., Panphon, S., & Leelakriangsak, M. (2021). Antimicrobial activity and physical properties of starch/chitosan film incorporated with lemongrass essential oil and its application. *LWT*, *141*, 110934. https://doi.org/10.1016/j.lwt.2021.110934