International Journal of Medical Science in Clinical Research and Review

Online ISSN: 2581-8945

Available Online at http://www.ijmscrr.in Volume 04 | Issue 03 (May - June) | 2021 |

ANTIMICROBIAL POTENTIAL OF EUCALYPTUS AND LEMONGRASS OIL ALONEAND IN COMBINATION AGAINST CLINICAL BACTERIA IN PLANKTONIC AND BIOFILM MODE

[1]Iram Liaqat, [2]Uzma Hanif,

[2]Andleeb Anwar Sardar,

[1]Amna Asgar

[1]Microbiology Lab, Department of Zoology, GC University, Lahore
[2]Department of Botany, GC University, Lahore

Corresponding Author: Dr. Iram Liaqat, Associate Professor, Department of Zoology, GC University, Lahore-54000, Pakistan. Email: iramliaq@hotmail.com

How To Cite:
Anwar Sardar, 1Amna Asgar1. L. 2. H. 2. (2021). ANTIMICROBIAL POTENTIAL OF EUCALYPTUS AND LEMONGRASS OIL ALONE AND IN COMBINATION AGAINST CLINICAL BACTERIA IN PLANKTONIC AND BIOFILM MODE. *International Journal of Medical Science in Clinical Research and Review, 4*(03). Page: 01-12. Retrieved from http://ijmscrr.in/index.php/ijmscrr/article/view/147

Article Received: **01 April 2021,**
Accepted: **01 May 2021,**
Publication: **05 May 2021**

Abstract

The use of natural substances has been trending from past few years. In recent years, the synthetic products obtained from plants have gained immense importance. Essential oils and volatile products obtained from plants are the source of food flavoring, aroma products and fragrance industries. Their use is also common to cure different ailments such as cancer, skin problems and nosocomial infections. Vast research has shown the antimicrobial properties of essential oils obtained from plants. Essential oils of lemongrass (*Cymbopogon citratus*, Stapf, Family: Family: Poaceae) and eucalyptus(*Eucalyptus globulus* Labill. Family: Myrtaceae) have antiviral, antibacterial, antioxidant and insecticidal characteristics. Lemongrass and eucalyptus oils have proven antibacterial activity towards some Gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis* and some Gram negative bacteria such as *Escherishia coli* and *Klebsiellapneumonia*. Combination of these oils havealso been suggested to possess high antimicrobial activity against different strains of bacteria. This review highlights the use of essential oils of lemongrass and eucalyptus for their *in vitro* antimicrobial properties against different bacteria in planktonic and biofilm mode. Mode of action by which the oil shows its inhibitory activity will be discussed. Susceptibility shown by different bacteria towards these essential oils and their components will be described.

Keywords: eucalyptus oil, lemongrass oil, planktonic mode, biofilm mode.

1. Introduction

Medicinal plants are one of the most important sources of the traditional medicines which are being used by the whole world. Now a days, the medicinal research have shown the importance of the herbal remedies since these are the active means of bioactive compounds [1].Volatile oils also called essential oils are derived from plant husk, buds, barks, wood, seeds, leaves, flowers, twigs, herbs, roots and fruit and aromatic oily liquids. There is estimation of about 2500-3000 volatile oils; in fragrance market which are commercially important [2].Essential oils have antiviral, antibacterial, antioxidant and insecticidal characteristics[3]. Additionally, use of these oils in cancer treatment is also common. Lemongrass and eucalyptus are very well known fragrant herbal plants. Lemongrass belongs to the Poaceae family and is cultivated in tropical and subtropical regions of the world. It is widely popular in Asian cuisine and medicinal practices for its aroma, active ingredients and health benefits [4].

Eucalyptus plant belongs to the family Myrtaceae. It was originally used in Australia but now commonly found throughout the world. It is large genus and has about 900 types and subtypes. Dried leaves and hot water extracts of eucalyptus (lemon scented eucalyptus) are used in analgesic, anti-inflammatory and antipyretic treatments for the indication of respiratory inflammation, like sinus congestion, cold and flu and cure for many other diseases [5].

Lemon grass and eucalyptus plants have essential oils that are evaporative, intensive and deliquescent liquids with nice and strong odors [6].Theseoils are used in food preservatives, fragrance industry and aroma therapy [7]. Lemongrass oil is one of the most widely used ingredients for its health benefits[8].Bioactive compounds present in this oil are utilized mainly for therapeutic motives and treatment of different ailments [8].Eucalyptus oil also acts as an antioxidant and anticancer agent. *Eucalyptus globulus* and *E. radiata* essential oils are in great demand, as they are utilized for expectorant, astringent, febrifuge, fumigant, antiseptic, anesthetic, deodorant, inhalant, insect repellant and abscess disinfectant. Furthermore, its use for folk remedy, wounds, arthritis, burns, boils, asthma, bronchitis, flu, inflammation, worms and rhinitis is also common [9].

Bacterial resistance has become major problem in the field of medicine and has developed due to the continuous use of antibiotics. Development of new chemicals and drugs against bacteria is very necessary [10]. Due to resistance against antibiotics, medicines and drugs derived from plants are being tested to check their effectiveness against different bacteria especially multi-drug resistant pathogens [11]. The phytochemical compounds in essential oils can act synergistically along with other antimicrobial compounds to eliminate the resistance problem [12-14].

Biofilm formed by bacteria is another major concern in medical sector. Biofilm is the association of bacteria that attach themselves to a surface and form an extracellular matrix to attach with each other. This forms a multicellular association which is highly resistant against antibiotics [15]. Biofilm is a major cause of development of diseases for example in patients suffering with diabetes and endocarditis [16]. There are many evidences regarding the effect of plant essential oils on bacteria but much less is known about their effects on their biofilm forming ability [17].

This review will summarize information on the antimicrobial effect of eucalyptus oil and lemongrass oil and their combined effect from previous researches and studies. The effect on some Gram positive and Gram negative bacteria in planktonic and biofilm forms is being discussed in order to understand their antimicrobial potential to improve and promote their use as a medicinal compound in pharmaceutical industries and cosmetics.

1.1.Effect of eucalyptus oil on Gram positive and Gram negative bacteria

Essential oil of Eucalyptus (*Eucalyptus globulus*Labill.) is widely used as antibacterial agent. Its antimicrobial action mainly depends on their chemical constituents. Major component of eucalyptusoil is terpene 1, 8-cineole also called eucalyptol. Compounds have functional groups such as 1,8-cineole, but terpineol is a major subsidizer for activity since its proved to have 8 times greater affectivity compared to 1,8-cineole against *S. aureus*[18].

Figure 1: Some characteristics of essential oil

E. globulus oil has main constituents i.e., 1, 8-cineole, which is eucalyptol (63.81%),as compared to*E. radiata* oil, in which the major constituent is limonene (68.51%). Amount of components fluctuates from 44% to 84% and these components are mainly responsible for the antimicrobial activity[19].Essential oils are extracted from many species of eucalyptus plant for example, *E. camaldulensis*, *E. radiata* and *E. citriodora* are used to obtain oil but commercially available essential oil is extracted from *E. globulus*[5]. The essential oils of *E. globulus* and *E. radiata* are two important species; demands of their essential oils are very high in the market, and they possess antimicrobial, antioxidant and anti-quorum detecting characteristics [20].*E. citriodora* oils are used for pharmaceutical and medicinal purposes.

E. globulus oil possess antimicrobial, anti-inflammatory, anti-quroum and anti oxidant properties (Fig. 1). The effect of eucalyptus essential oil against bacteria had been widely observed by agar well diffusion assay in which wells are made in agar plates which are filled by volatile oil and showed the inhibitory affect in the form of zones of inhibition ([21-22]. Eucalyptus oil showed antimicrobial potential against different pathogenic bacterial strains such as *P. aeruginosa* and *Staphylococcusaureus*[23].By using high concentration of oil, greater inhibition zone were observed and by using low concentration of oil little or less inhibition zone were observed [24]. It was found that inhibitory effect of the eucalyptus oil is more towards Gram positive bacteria and is less towards Gram negative bacterial strains [25].

Using agar well diffusion assay, *E.globulus*oil showed antimicrobial potential against different pathogenic bacterial strains like *P.aeruginosa* and *S.aureus*. It was observed that different concentrations showed different inhibition zones, the larger zone with 100% concentration of oil was observed. Also experimentation showed that *E.globulus* oil has higher inhibitory effect against *S. aureus*which is a Gram positive bacterium compared to *P. aeruginosa* which is Gram negative bacteria[22].Mota*et al.*[26] showed inhibitory effect of eucalyptus oil against *S. aureus* and *Escherichia coli*. However, this study showed that the oil had not inhibited Gram negative bacteria such as *Pseudomonas* and *Salmonella.*

Kruthi*et al.* [21]showed that essential oil of *E. globules* was effective against other tested bacterial strains such as *S. aureus*, *Bacillus subtilis*, *E. coli* and *Klebsiella pneumonia*. It was found that Gram negative bacteria were less sensitive compared to Gram positive ones which showed more inhibitory effect of the oil. Gram negative bacterial strains were moderately affected by the eucalyptus oil.

Another study showed that oil from two species of eucalyptus that is *E. globulus* and *E. camaldulensis* inhibited bacterial growth at higher concentration of 5 to 20 micro liters when tested against *E. coli* and *S. aureus* strains. But it was not effective when used in low concentrations of 1 and 2 micro liters. Higher zones of inhibition corresponding to high concentrations were observed [27].

Essential oil of *E. camaldulensis* has shown antimicrobial activity by agar well diffusion method against a vast range of bacteria like *E. coli, S. aureus, S. typhi, P. mirabilis and K. pneumonia*. Aqueous extract of eucalyptus have shown zones of inhibition towards *E. coli* (7mm), *K. pneumonia* (9mm), *S. typhi*(12mm), *S. aureus* (12mm) and *P. mirabilis* (13mm). Likewise, acetone extract showed inhibition zones against *E. coli* (12mm) *K. pneumonia* (13mm), *S. typhi*(14mm) *P. mirabilis* (15mm)and *S. aureus*(14mm)(Table-1) [28].

Table-1: Antibacterial activity of of Eucalyptus aqueous and acetone extract against various pathogens

Bacteria	***Eucalyptus camaldulensis(Zones of inhibition in***	**Reference**

	mm)		
Clinical isolates	***Aqueous extract***	***Acetone extract***	
E. coli	7	12	(Abubakar, 2010).
K. pneumonia	9	13	
S. typhi	12	14	
S. aureus	12	14	
P. mirabilis	13	15	

1.1.1.Effect of eucalyptus oil on planktonic bacteria

Planktonic bacteria are free living bacteria which float in liquid media. Effect on planktonic mode of bacterial growth has been measured by determining minimum inhibitory concentration (MIC). MIC is the lowest amount or concentration of oil that inhibits microbial growth in case of essential oil used as an antimicrobial agent [29].It is determined by broth dilution assay in most of the studies and the result is measured in the form of turbidity or optical density [29].

Table-2: Different concentrations of oil affecting different bacterial strains

Bacterial strains	**MIC**	**References**
P. vulgaris	25%	[30]
S. pyogenes	50%	
S. epidermidis	50%	
E. coli	100%	
S. aureus	100%	

*E. globulus*oil showed effect on different bacterial strains at different concentrations in the form of MIC. Some bacterial isolates like *Proteus vulgaris* was inhibited when 25%concentration of oil was used. *S. pyogenes* and *S. epidermidis* were inhibited at 50% concentration. *S. aureus* and *E.coli* were inhibited by the highest concentration of oil i.e. 100% [30] (Table-2).

Table-3: MIC values of eucalyptus oil against different bacteria

Bacterial strains	**MIC values as %**	**References**
Gram positive bacteria		
S. aureus	0.07 - 0.5	[30-36]

B. subtilis	0.17 - 0.34	
M. luteus	0.2 - 0.4	
S. pyogenes	0.4 - 1.1	
Gram negative bacteria		
E. coli	0.15 - 3.2	[32; 34]
K. pneumoniae	0.05 - 0.32	
A. baumannii	0.05 - 0.1	[33]
V. parachaemoliticus	0.01	[34]

Gram positive bacteria had been widely used for testing the efficacy of essential oil. Eucalyptus oil obtained from *E. camaldulensis* showed moderate effect against *S. aureus* and its MIC ranged from 0.07 to 0.5%. MIC for other bacterial strains like *B. subtilis* was 0.17 to 0.34%; 0.2 to 0.4%against *M. luteus* and0.4 to 1.1% against *S. Pyogenes*[30-36].

Similarly, the MIC values against Gram negative bacterial strains were also observed. Among these the most commonly used bacteria was *E. coli* for which MIC value ranged from 0.15 to 3.2%. 0.05 to 0.32% for *K. pneumonia* (Khubeiz*et al.,* 2016;OstadAsiaei*et al.,* 2018).A multi drug resistant bacteria, *A. baumannii* exhibited MIC value of 0.05 to 0.1% [34] and the lowest value of 0.01% was obtained for the bacterium *V. parachaemoliticus*[34] (Table-3).

Another study revealed the antimicrobial potential of essential oil obtained from *E. globulus*. The oil proved to be effective against all the strains tested with lowest activity of 3.13 mg/ml for *P. aeruginosa* and *S. infantis*. The highest activity of oil was observed against *S. aureus, S. pyogenes*and *E. coli* with MIC of 0.09 mg/ml [37].

1.1.2. Anti-biofilm activity of eucalyptus essential oil

Recently, it was observed that among biofilm forming bacteria, some bacteria form strong biofilms like *E. coli* while other form moderate biofilms like *S. aureus*. The essential oil of *E. camaldulensis* had showed inhibition against these biofilms at different concentrations. Results of the study showing effect of eucalyptus volatile oil against biofilms showed that *S. epidermidis* was inhibited by all concentrations of the oil (72%, 74% and 76%). Similarly, the bacteria, *K. pneumoniae*, *E. coli* and *P. vulgaris* also showed inhibition at all concentrations but the in case of *E. coli*, the highest inhibition was observed at final concentration (97%) of the oil [30].In the above discussed experiment, the oil was applied before the formation of biofilm and had shown positive effect in inhibition of biofilm formation.

Another study done on *P. aeruginosa* confirmed that the oil from *E. camaldulensis* has antibacterial effect against its biofilm. In this experiment, the oil was used alone and in combination with ciprofloxacin against the bacterial biofilm. The results showed that oil had inhibited both planktonic and biofilm when used alone and also have synergistic effect when used in combination with ciprofloxacin. These experimental observations were obtained by determining MIC, BIC (biofilm inhibitory concentration) and checkerboard assays of oil and antibiotic against bacteria. Checkerboard assays were done by preparing serial dilution of oils and antibiotics whereas BIC was determined by using serially diluted volatile oil in 48 well plates [38].

The experimentation on oral *Streptococci* showed that eucalyptus oil has inhibitory effect on *Streptococci* biofilm. Strong inhibitory effect on biofilm was observed against seven samples out of total 10 bacterial samples. Reduction in biofilm of most oral *Streptococci* was also observed in concentration dependent way of oil. However, few

Streptococci strains were observed to stimulate biofilm formation [39].

Research on anti-biofilm activity of eucalyptus oil from *E. globulus* against *P. aeruginosa* and *S. aureus*showed that essential oil from this plant had strong inhibitory effect against these bacteria and may also affect other Gram positive and negative bacteria in similar manner. It was observed that both biofilm producers and non-biofilm producers were sensitive to eucalyptus oil. 65.43% sensitivity by *P. aeruginosa* biofilm producers and 80.32% sensitivity by non-biofilm producer strains of *P. aeruginosa* were observed. In case of *S. aureus*, the sensitivity by biofilm producers was 54.16% and that form on biofilm producers was 68.75%. Biofilm of the latter had shown high degree of sensitivity [40].The anti-biofilm activity of essential oil from eucalyptus species against *P.mirabilis*, which is a urinary tract pathogen also showed 90% inhibition of the biofilm formation by this bacteria [41].

1.2. Effect of lemongrass oil against bacteria

Lemongrass *Cymbopogoncitratus*(Family:Poaceae) is widely used as antibacterial and antifungal agent[7]. It has proved to be effective against a diverse range of bacteria including *Streptococcus* and *Pseudomonas* species; these bacteria are responsible for food poisoning, lungs and skin diseases [42]. It has also been found to be effective against fungi and is used in a large number of antifungal skin products as an active ingredient. The antimicrobial properties of lemongrass oil mainly depend on three components which are geranial, neral, and myrcene. Geranial and neral are effective against a vast range of Gram positive and Gram negative bacteria when used individually or in combination [43].On the other hand, myrcene is a weak antimicrobial agent showing moderate inhibitory activity [44].

Antimicrobial activity of essential oil of three lemongrass species i.e., Pragati, Praman and Suvarna were tested against Gram positive bacteria (*S.aureus* and *B.subtilis*) and Gram negative bacteria (*E.coli*and*P.aeruginosa*). The powerful antimicrobial effects were showed by essential oil obtained from Suvarna.These three distinct essential oils showed effective antimicrobial action towards all microbes except for *B. subtilis*. Likewise, *S. aureus*was found to be more inhibited by use of these essential oils [45].

Much of the research has shown the effect of active ingredients of lemongrass oil alone and in combination with other essential oils to be more effective against Gram positive bacteria than Gram negative bacteria[46]. Lemongrass essential oil was tested against *S. aureus, B. cereus, B. subtilis, E. coli, K. pneumonia and P. aeruginosa*[47]. All of the bacteria were susceptible to various concentration of lemongrass essential oil except for*P. aeruginosa* which did not respond to any of the tested concentration seven the pure one. This indicates the effectiveness of essential oils against a large bacterial population that is responsible for a wide variety of diseases[48]. Lemongrass essential oil was also found to be effective against bacteria related to food poisonings such as *Listeria monocytogenes* and *Salmonella typhimurium*as reported by[49] This proves its potential candidate for food preservation while not compromising the quality and nutrition of the food.Various studies on 1400 bacterial and fungal isolates were tested for their susceptibility to lemongrass oil and around 40% of the species were effectively eliminated using lemongrass oil at different concentrations[50].

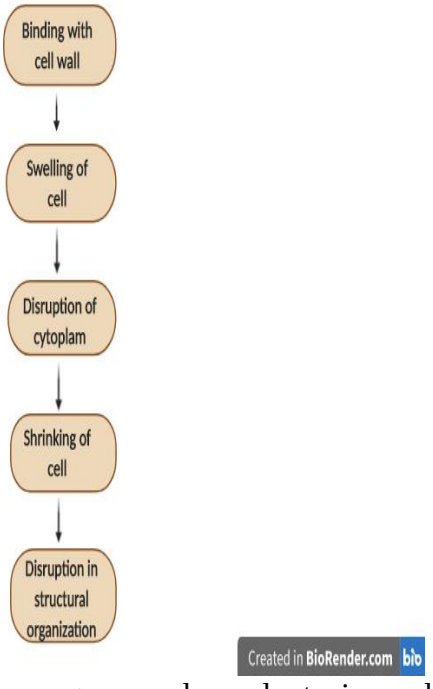
1.2.1. Antibiofilm activity of lemongrass oil

Diseases that were previously very easy to treat with antibiotics are no longer responding to even high doses giving rise to multidrug-resistant bacteria (MDR) bacteria. Those bacteria have acquired antibiotic-resistant genes that make antibiotics completely ineffective. Some bacteria however, in addition to acquiring antibiotic resistance have developed other strategies such as aggregation of bacteria in a protective coating or biofilms[51]. These biofilms protect thebacteria from a range of biotic and abiotic factors rendering most of the treatments ineffective against them. Among the many advantages of biofilms is their ability to make the colony of the bacteria more resistant to typical bactericidal agents such as antibiotics, chlorine, and detergents. That is because of the exopolysaccharide, which bacteria secrete in biofilm mode[51].

Most chronic infectious diseases in humans such as pneumonia and other life-threatening diseases are caused by *Streptococci.P. aeruginosa* forms biofilms and causes lung infections in patients suffering from cystic fibrosis. Essential oils of lemongrass have components that can break this biofilm protective covering and interferes with the molecular mechanisms of bacteria thus reducing their ability to reproduce and disrupting their cytoplasm [52]. Essential oil from lemongrass has proved to be an effective treatment for such biofilms forming bacteria.

1.3. Combined effect of eucalyptus and lemongrass oils

Lemongrass and eucalyptus in combination possess remarkable strength against different bacteria and the diseases.Ghalem and Mohamed, [53] observed great inhibitory activity by both oils against Gram positive bacteria *B. subtilis*and*S. aureus* and moderate effect on Gram negative bacteria *E. coli* and *K. pneumonia*. Combination of eucalyptus and lemongrass in 1:1 express high inhibitory effects towards *B. subtilis* and *S. aureus* and less inhibitory effect towards *E. coli* and *K. pneumonia* [54].Essential oils extracted from lemongrass and eucalyptusis used traditionally for cure of typhoid fever and malaria. In treatment of disease like stomach ache and typhoid fever, mixture of leaves and grass of both plants were found to be effective [55].There has been so much research on the antimicrobial potential of lemongrass and eucalyptus against different bacterial and fungal diseases in the eastern medicine system [56]. Some authors also suggested use of these oils as an anti-inflammatory agent because of their ability to modify the immune response in localized areas [57-58].Thus these oils have a special place in traditional herbal medicine. More research is needed to effectively manipulate the true potential of these oils as bactericidal agents without inducing resistance in bacteria and fungi [59].



1.3.1. Mode of action of eucalyptus and lemongrass oils

Lemongrass and eucalyptus oils are composed of many components and their antibacterial activity depend on site of attack on bacteria. Both plant oils attack on different sites depending on lipophilicity of constituents of oil. Thus lipophilicity performs important functions in antibacterial activity by penetrating lipid layer of cell of bacteria and due to this penetration mitochondria cause's cell to lose its structural organization [60]. Oil components first bind with surface of bacterial cell wall causing it to swell and then disrupts membrane of cytoplasm and cell shrink in response to oil components. The disruption in structural organization of cytoplasm occurs more than cell wall. Thus major component of bacterial cell which is affected by oil is cytoplasm (Fig. 2) [61].

Figure 2: Steps of mode of action of essential oil

2. Conclusions

Essential oils of lemongrass and eucalyptus are reported to have promising effects against Gram positive bacteria compared to Gram negative bacteria. Combination of both oils has proven inhibitory effect against a vast range of microbial strains either Gram positive or Gram negative bacteria in planktonic as well as biofilm mode. Antimicrobial action of the oils mainly depend on their chemical constituents. Essential oil components show antimicrobial activity by disrupting the structural organization of bacterial cells mainly by destroying cytoplasm. Therefore, they can be used to overcome the problem of

bacterial resistance which is due to the repetitive use of antibiotics. Thus, these oils can be a good and natural replacement to different drugs and medicines. Further study is needed to combine oils with other herbal plants extracts and /oils, antimicrobial drugs and products to determine their synergistic effect with those compounds. Such studies will be beneficial in developing new drugs and medicines that will flourish the area of medicine and pharmaceutics. Research efforts must be directed toward detailed mechanism understanding. Detail studies will reduce the problems related to antibiotics, reducing the burden on the healthcare system, and aversion of the antibiotic apocalypse. Furthermore, studies on herbal compounds and oils can cause pharmaceutical industries to develop different novel drugs from natural products.

3. References

1. Moosavy, M.H., and Shavisi, N. 2013. "Determination of antimicrobial effects of nisin and *Menthaspicata* essential oil against *Escherichia coli* O157: H7 under various conditions (pH, temperature and NaCl concentration)". *Pharm. Sci.*19(2): 61-67.
2. Boyanova, L., Gergova, G., Nikolov, R., Derejian, S., Lazarova, E., Katsarov, N., Mitov, I., and Krastev, Z., 2005. Activity of Bulgarian propolis against 94 Helicobacter pylori strains in vitro by agar-well diffusion, agar dilution and disc diffusion methods. *J. Med. Microbiol. 54*(5): 481-483.
3. Azzazi, M. F; Shahin, S.I, Nofal, A. M and Abd El – Rahman, A. F. 2015. "A Preliminary Study on Antimicrobial Activities of Essential Oils and Egyptian Pollen Extract Against some phytopathogenic Fungi and Bacteria". *Egypt. j. Bot.* 2: 363-374.
4. Santin, M. R., dos Santos, A.O., Nakamura, C.V., Dias Filho, B.P., Ferreira, I.C.P., and Ueda-Nakamura, T. 2009. "*In vitro* activity of the essential oil of *Cymbopogoncitratus* and its major component (citral) on *Leishmaniaamazonensis*". *J. Parasitol. Res.*105(6): 1489.
5. Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I.L., and Matos, F.J.A. 2003. "Analgesic and anti-inflammatory effects of essential oils of Eucalyptus*". J. Ethnopharmacol.*89(2-3): 277-283.
6. Babayi, H., Kolo, I., Okogun, J.I., and Ijah, U.J.J. 2004. "The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and Terminaliacatappa against some pathogenic microorganisms". 2004.
7. Rauber, C.D.S., Guterres, S. S., and Schapoval, E.E. 2005. "LC determination of citral in Cymbopogoncitratus volatile oil. *J. Pharm. Biomed. Anal*. 37(3): 597-601.
8. Oloyede, O. I. 2009. "Chemical profile and antimicrobial activity of Cymbopogoncitratus leaves". *Indian J. Nat. Prod. Resour.*2: 98-103.
9. Bachir, R. G., and Benali, M. 2012. "Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against *Escherichia coli* and *Staphylococcus aureus*". *Asian Pac. J. Trop. Biomed*. 2(9): 739.
10. Lokhande, P.D., Gawai, K.R., Kodam, K.M., Kuchekar, B.S., Chabukswar, A.R., andJagdale, S.C. 2007. "Antibacterial activity of isolated constituents and extract of roots of Inularacemosa". *Res. J. Med. Plant.* 1(1): 7-12.
11. Mulyaningsih, S., Sporer, F., Reichling, J., and Wink, M. 2011. "Antibacterial activity of essential oils from Eucalyptus and of selected components against multidrug-resistant bacterial pathogens". *Pharm Biol*. 49(9): 893-899.
12. Chaves, T.P., Fernandes, F.H.A., Santana, C.P., Santos, J.S., Medeiros, F.D., Felismino, D.C., Santos, V.L., Catão, R.M.R., Coutinho, H.D.M. and Medeiros, A.C.D. 2016. Evaluation of the interaction

between the Poincianellapyramidalis (Tul.) LP Queiroz extract and antimicrobials using biological and analytical models. *PLoS One*. 11(5): e0155532.

13. Cristo, J.S., Matias, E.F., Figueredo, F.G., Santos, J.F., Pereira, N.L., Junior, J.G., Aquino, P.E., Nogueira, M.N., Ribeiro-Filho, J., Cunha, F.A. and Costa, M.S. 2016. "HPLC profile and antibiotic-modifying activity of *Azadirachtaindica* A. Juss, (Meliaceae)". *Ind. Crops Prod*. 94: 903-908.
14. Morais-Braga, M.F.B., Sales, D.L., dos Santos Silva, F., Chaves, T.P., Bitu, V.D.C.N., Avilez, W.M.T., Ribeiro-Filho, J. and Coutinho, H.D.M. 2016. "*Psidiumguajava* L. and Psidiumbrownianum Mart ex DC. potentiate the effect of antibiotics against Gram-positive and Gram-negative bacteria". *Eur. J. Integr. Med*. 8(5): 683-687.
15. Singh, S., Singh, S.K., Chowdhury, I. and Singh, R. 2017. "Understanding the mechanism of bacterial biofilms resistance to antimicrobial agents". *Open Microbiol*. J.11: 53.
16. Thwaites, G.E., Edgeworth, J.D., Gkrania-Klotsas, E., Kirby, A., Tilley, R., Török, M.E., Walker, S., Wertheim, H.F., Wilson, P., Llewelyn, M.J. and UK Clinical Infection Research Group. 2011. Clinical management of *Staphylococcus aureus* bacteraemia. *Lancet* Infect. Dis. 11(3): 208-222.
17. Kavanaugh, N. L., and Ribbeck, K. 2012. Selected antimicrobial essential oils eradicate *Pseudomonas* spp. and *Staphylococcus aureus* biofilms. *Appl. Environ. Microbiol.* 78(11): 4057-4061.
18. Hendry, E.R., Worthington, T., Conway, B.R., and Lambert, P.A. 2009. "Antimicrobial efficacy of eucalyptus oil and 1, 8-cineole alone and in combination with chlorhexidinedigluconate against microorganisms grown in planktonic and biofilm cultures". *J. Antimicrob. Chemother*. 64(6): 1219-1225.
19. Goldbeck, J.C., do Nascimento, J.E., Jacob, R.G., Fiorentini, Â.M., and da Silva, W.P. 2014. "Bioactivity of essential oils from *Eucalyptus globulus* and *Eucalyptus urograndis* against planktonic cells and biofilms of *Streptococcus mutans*". *Ind. Crops Prod*. 60: 304-309.
20. Preethi, R., Devanathan, V.V., and Loganathan, M. 2010. "Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens". *Adv. Biol. Res*. 4(2): 122-125.
21. Kruthi, B.S., Srikari, K., SaiPriya, P., and ChJyothi, G.S. 2014. "*In vitro* testing of antimicrobial properties of lemongrass, eucalyptus and their synergistic effect". *Int. J. Sci. Res*. 4(2): 1-8.
22. Adnan, M. 2019. "Bioactive potential of essential oil extracted from the leaves of *Eucalyptus globulus* (Myrtaceae)". *J. Pharmacogn. Phytochem.* 8(1): 213-216.
23. Ishnava, K. B., Chauhan, J. B., and Barad, M. B. 2013. "Anticariogenic and phytochemical evaluation of *Eucalyptus globules*Labill". *Saudi J. Biol. Sci*. 20(1): 69-74.
24. Tyagi, A. K., and Malik, A. 2011. "Antimicrobial potential and chemical composition of *Eucalyptus globulus* oil in liquid and vapour phase against food spoilage microorganisms*". Food Chem*. 126(1): 228-235.
25. Oussalah, M., Caillet, S., and Lacroix, M. 2006. "Mechanism of action of Spanish oregano, Chinese cinnamon, and savory essential oils against cell membranes and walls of *Escherichia coli* O157: H7 and *Listeria monocytogenes". J. Food Prot*. 69(5): 1046-1055.
26. Mota, V.D.S., Turrini, R.N.T., and Poveda, V.D.B. 2015. "Antimicrobial activity of Eucalyptus globulus oil, xylitol

and papain: a pilot study". *Rev. Esc. Enferm.* US. 49(2): 0216-0220.

27. Ghalem, B. R., and Mohamed, B. 2008. "Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis"*. *Afr.* J. *Pharm. Pharmacol*. ·2(10): 211-215.
28. Abubakar, E.M.M. 2010. "Antibacterial potential of crude leaf extracts of Eucalyptus camaldulensis against some pathogenic bacteria". *Afr. J. Plant Sci*. 4(6): 202-209.
29. Rodriguez-Tudela, J.L., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., Denning, D., Donnelly, J.P., Dupont, B., Fegeler, W., Moore, C. and Richardson, M. 2003. "Method for the determination of minimum inhibitory concentration (MIC) by broth dilution of fermentative yeasts". *Clin. Microbiol. Infect.* 9(8): i-viii.
30. Khalaf, Z. Z., and Zahra, L. A. 2020. "Evaluation of the Activity of Essential Oil and Hydrosol from *Eucalyptus Camaldulensis* against Some Bacterial Species". *Iraqi J. Sci.*1282-1288.
31. Akin, M., Aktumsek, A., and Nostro, A. 2010. "Antibacterial activity and composition of the essential oils of *Eucalyptus camaldulensis*Dehn. andMyrtuscommunis L. growing in Northern Cyprus". *Afr. J. Biotechnol*. 9(4).
32. Asiaei, E. O., Moghimipour, E., andFakoor, M. H. 2018. "Evaluation of antimicrobial activity of Eucalyptus camaldulensis essential oil against the growth of drug-resistant bacteria". *Jundishapur J. Nat. Pharm. Prod.* 13(4).
33. Knezevic, P., Aleksic, V., Simin, N., Svircev, E., Petrovic, A., and Mimica-Dukic, N. 2016. "Antimicrobial activity of Eucalyptus camaldulensis essential oils and their interactions with conventional antimicrobial agents against multi-drug resistant Acinetobacterbaumannii". *J. Ethnopharmacol.*178: 125-136.
34. Khubeiz, M. J., Mansour, G., and Zahraa, B. 2016. "Chemical compositions and antimicrobial activity of leaves Eucalyptus camaldulensis essential oils from four syriansamples". *Int. J. Curr. Pharm. Res.* 7(5): 251-257.
35. Rasooli, I., Shayegh, S., and Astaneh, S.D.A. 2009. "The effect of Menthaspicata and Eucalyptus camaldulensis essential oils on dental biofilm". *Int. J. Dent. Hyg*. 7(3): 196-203.
36. Reda, F.M., El-Zawahry, Y.A., and Omar, A.R. 2017. "Synergistic effect of combined antibiotic and methanol extract of *Eucalyptus camaldulensis* leaf against *Staphylococcus aureus* and *Pseudomonas aeruginosa*". *Int. J. Appl. Sci .Biotechnol*. 5(4): 486-497.
37. Damjanović-Vratnica, B., Đakov, T., Šuković, D., and Damjanović, J. 2011. "Antimicrobial effect of essential oil isolated from *Eucalyptus globulus*Labill. from Montenegro". *Czech J. Food Sci.* 29(3): 277-284.
38. Al-Qaysi, A. M. K., Al-Ouqaili, M. T., and Al-Meani, S. A. 2020. "Ciprofloxacin-and gentamicin-mediated inhibition of *Pseudomonas aeruginosa* biofilms is enhanced when combined the volatile oil from *Eucalyptus camaldulensis*". *Sys. Rev. Pharm*. 11(7): 98-105.
39. Bahjat, S. A. 2019. "Evaluation of Antibacterial and Antibiofilm Activity of Cinnamon, Clove, Eucalyptus, and Tea Tree Oils Against Oral Streptococci". *RJS*. 28(3E): 1-14.
40. Sambyal, S. S., Sharma, P., and Shrivastava, D. 2017. "Anti-biofilm activity of selected plant essential oils against *Pseudomonas aeruginosa* and *Staphylococcus aureus*". *Int. J. Curr. Microbiol. Appl. Sci*. 6(3): 444-450.
41. Mathur, S., and Gutte, M. 2013. "Study the effect of essential oils on microbial biofilm

formation by *Klebsiella pneumonia". Scholars Acad. J. Biosci*. 1(3): 76-79.

42. Cimanga, K., Kambu, K., Tona, L., Apers, S., De Bruyne, T., Hermans, N., Totté, J., Pieters, L. and Vlietinck, A.J. 2002. "Correlation between chemical composition and antibacterial activity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo". J. *Ethnopharmacol*. 79(2): 213-220.
43. Akhila, A. (Ed.). 2009. *Essential oil-bearing grasses: the genus Cymbopogon*. CRC press.
44. Vadlapudi, V., and Naidu, K. C. 2009." In vitro bioefficiency of marine mangrove plant activity of Rhizophoraconjugata". *Int. J. Pharma. Tech. Res*. 1: 1598-1600.
45. Kumar, G. A., Muhury, R., and Ganjewala, D. 2016. "A study on antimicrobial activities of essential oils of different cultivars of lemongrass (*Cymbopogonflexuosus*)". 2016: 164-169.
46. Mith, H., Dure, R., Delcenserie, V., Zhiri, A., Daube, G., and Clinquart, A. 2014. "Antimicrobial activities of commercial essential oils and their components against food-borne pathogens and food spoilage bacteria". *Food Sci. Nutr*.2(4): 403-416.
47. Naik, M. I., Fomda, B. A., Jaykumar, E., and Bhat, J. A. 2010. "Antibacterial activity of lemongrass (Cymbopogon citratus) oil against some selected pathogenic bacterias". *Asian Pac. J. Trop. Med*. 3(7): 535-538.
48. Premathilake, U., Wathugala, D., and Dharmadasa, R. 2018. "Evaluation of chemical composition and assessment of antimicrobial activities of essential oil of lemongrass (Cymbopogoncitratus (dc.) stapf)". *Int. J. Minor Fruits, Med. Aromatic Plants*, 4: 13-19.
49. Reis-Teixeira, F.B., Sousa, I.P., Alves, V.F., Furtado, N.A.J.C., and De Martinis, E.C.P. 2019. "Evaluation of lemongrass and ginger essential oils to inhibit *Listeria monocytogenes* in biofilms". *J. Food Saf*.39(4): 12627.
50. Singh, B.R., Singh, V., Singh, R.K., and Ebibeni, N. 2011. "Antimicrobial activity of lemongrass (*Cymbopogoncitratus*) oil against microbes of environmental, clinical and food origin". *Int. Res. J. Pharm. Pharmacol.* 1(9): 228-236.
51. Watnick, P., and Kolter, R. 2000. Biofilm, city of microbes. *J. Bacteriol*.182(10): 2675-2679.
52. Brooker, I. and Kleinig, D.A. 2004. "*Field Guide to Eucalypts: Northern Australia": Volume Three*. Bloomings Books.
53. Ghalem, B. R., and Mohamed, B. 2008. "Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*". *Afr. J. Pharm. Pharmacol*. 2(10): 211-215.
54. Alma, M. H., Nitz, S., Kollmannsberger, H., Digrak, M., Efe, F. T., and Yilmaz, N. 2004. "Chemical composition and antimicrobial activity of the essential oils from the gum of Turkish pistachio (Pistaciavera L.)". *Agric. Food Chem*. 52(12): 3911-3914.
55. Kakarla, S., and Ganjewala, D. 2009. "Antimicrobial activity of essential oils of four lemongrass (*Cymbopogonflexuosus*Steud) varieties". *Med. Aromat. Plant Sci. Biotechnol*, 3(1): 107-09.
56. Gamal, A.M., and Sabrin, R.M.I., 2007. "Eucalyptone G, a new phloroglucinol derivative and other constituents from *Eucalyptus globulus*Labill". *ARKIVOC Inter. J. Org. Chem*. 281-291.
57. Akin-Osanaiye, B. C., Agbaji, A. S., and Dakare, M. A. 2007. "Antimicrobial activity of oils and extracts of *Cymbopogoncitratus* (Lemon Grass), *Eucalyptus citriodora* and *Eucalyptus camaldulensis*". *J. Med. Sci.* 7(4).

58. Dabur, R., Gupta, A., Mandal, T.K., Singh, D. D., Bajpai, V., Gurav, A.M., andLavekar, G.S. 2007. "Antimicrobial activity of some Indian medicinal plants". Afr. J. Tradit. Complement. *Altern. Med.* 4(3), 313-318.
59. Jafari, B., Ebadi, A., Aghdam, B. M., and Hassanzade, Z. 2012. "Antibacterial activities of lemon grass methanol extract and essence on pathogenic bacteria". *American-Eurasian J. Agric. Environ. Sci*. 12(8): 1042-6.
60. Burt, S. (2004). "Essential oils: their antibacterial properties and potential applications in foods—a review". *Int. J. Food Microbiol*. 94(3): 223-253.
61. Aiemsaard, J., Aiumlamai, S., Aromdee, C., Taweechaisupapong, S., &Khunkitti, W. 2011. "The effect of lemongrass oil and its major components on clinical isolate mastitis pathogens and their mechanisms of action on *Staphylococcus aureus* DMST 4745". *Res. Vet. Sci*. 91(3): e31-e37.