J. Chem. Soc. Nigeria, Vol. 49, No. 6, pp 969 – 980 [2024] https://doi.org/10.46602/jcsn.v49i6.1025

ANTIMICROBIAL ASSAY OF ESSENTIAL OIL FROM *Eucalyptus globulus* LEAF

M. S. Isyaka [1,3], M. J. Sudi[1], V. J. Anyam [1,3], H. S. Labaran [2,3] and A. A. Muhammad[3,5]

[1]Department of Chemical Sciences, North-Eastern University, Gombe.
[2]Department of Biological Sciences, North-Eastern University, Gombe.
[3]Centre for African Medicinal Plants Research (CAMPRE), North-Eastern University, Gombe.
[4]Department of Chemistry, Federal University of Kashere, Gombe State, Nigeria.
[5]Department of Chemistry, Directorate of Science, Remedial and General Studies, Federal University of Health Sciences Azare, Bauchi State, Nigeria.

Correspondence authors email: abdullahi.muhammadjika@fuhsa.edu.ng

ABSTRACT

Investigating the antibacterial test of essential oil derived from *Eucalyptus globulus* leaves against clinically significant microbial strains is the goal of this investigation. *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Escherichia coli* are the isolates of Gram-negative bacteria, whereas *Staphylococcus aureus* is an isolate of Gram-positive bacteria. In the current study, essential oil was extracted using microwave-assisted hydrodistillation, and the oil's antibacterial properties were assessed. The physical method was used to determine the oil's color, odor, and solubility. The essential oil of fresh *Eucalyptus globulus* leaves exhibits zone of inhibition against three of the studied species *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhi* at all concentrations of oil per disc but no action against *Salmonella typhi* at 1.43 mL/disc. At all concentrations, the essential oil did not, however, exhibit any anti-*Pseudomonas aeruginosa* activity. With a zone of inhibition of 17.7 mm and a concentration of 5.72 mL/disc, the oil was most effective against *Staphylococcus aureus*. As concentration drops, the zone of inhibition shrinks as well; at 2.86 mL/disc and 1.43 mL/disc, it is 14.7 mm and 9.7 mm, respectively. The essential oil of dried *Eucalyptus globulus* leaves exhibits no action against *Salmonella typhi* at 1.43 mL/disc, but it does exhibit a zone of inhibition against three of the examined organisms: *Salmonella typhi*, *Staphylococcus aureus*, and *Escherichia coli*, at all oil concentrations per disc. At all concentrations, the essential oil did not, however, exhibit any anti-*Pseudomonas aeruginosa* activity. With a zone of inhibition of 13.3 mm and a concentration of 5.72 mL/disc, the oil was most effective against *Staphylococcus aureus*. As concentration drops, the zone of inhibition shrinks as well; at 2.86 mL/disc and 1.43 mL/disc, it is 11.1 mm and 8.3 mm, respectively. It is advised that the essential oil's effectiveness be evaluated against fungus in order to investigate the best combinations and uses to maximize its medicinal potential. For direct use, in vivo assay is advised.

Key word: *Eucalyptus globulus*, microwave, extraction, antimicrobial, organisms, essential oil.

INTRODUCTION

Eucalyptus globulus, also known as river red gum, is a specie of *Eucalyptus* tree that belongs to the *Myrtaceae* family appreciated for its varied medicinal benefits and industrial applications. Indigenous to Australia, it has transcended geographical limits and is now found in numerous places worldwide. The therapeutic potential of *Eucalyptus globulus* lies primarily in its bioactive compounds, which are abundant in its leaves. Because of their antibacterial qualities, these substances have attracted a lot of attention and are useful in both manufacturing and

ISSN 0795 - 22066

medicinal settings [1],[2]. Leaf extracts from *Eucalyptus globulus* have several uses in the industrial industry in addition to their therapeutic benefits. Its leaves yield essential oils that are used as main constituents in personal care, cosmetic, and medicinal goods. They are especially sought after for creating topical antiseptics, disinfectants, and oral care products because of their antibacterial qualities [3]. Additionally, eucalyptus essential oil is widely used in the fragrance business as a natural ingredient in detergents, soaps, and perfumes [4]. Because of their historical use in traditional medicine and their relevance in modern healthcare, *Eucalyptus camaldulensis* leaf extracts are being investigated for their antimicrobial activity. The bioactive compounds found in *Eucalyptus globulus* leaf as shown in Fig. 1 present a promising avenue for the development of alternative antimicrobial agents, which is urgently needed given the growing threat of antimicrobial resistance [5]. Additionally, *Eucalyptus globulus* extracts' industrial uses emphasize their economic significance and the value of researching their biological activities for a range of business objectives [6]. The medical usage of *Eucalyptus* by indigenous Australian populations stretches back generations, with traditional treatments derived from its leaves, bark, and essential oils [7]. Scientific interest in examining the medicinal potential of *eucalyptus* and its bioactive components has increased as a result of these traditional practices. Numerous bioactive chemicals, including essential oils rich in monoterpenes like 1,8-cineole (eucalyptol), α-pinene, and limonene, have been found in eucalyptus leaves through phytochemical research [1]. These substances have shown strong antibacterial action against a variety of pathogens, such as viruses, fungi, and bacteria [3]. *Eucalyptus* contains a wide variety of bioactive chemicals that offer a wealth of antibacterial agents with potential uses in the pharmaceutical and agricultural industries. However, thorough research clarifying *Eucalyptus globulus's* antibacterial efficacy against clinically important microbial strains is still required, despite the plant's widespread traditional use and scientific interest. In order to better understand *Eucalyptus globulus's* potential therapeutic and industrial uses, the purpose of this study is to examine the antibacterial activity of essential oil derived from the plant's leaves against clinically relevant microbial strains. Many bioactive substances, including as phenolic compounds, flavonoids, terpenoids, and essential oils, are known to be present in *E. camaldulensis* leaves [8]. The pharmacological characteristics of these substances, such as their antibacterial action, have been thoroughly investigated. The antibacterial properties of *Eucalyptus* species have been shown in numerous investigations. For example, a leaf extract from *Eucalyptus globulus* demonstrated strong antibacterial activity against Escherichia coli and Staphylococcus aureus [9]. Likewise, the essential oil of *Eucalyptus citriodora* demonstrated significant antifungal

ISSN 0795 - 22066

action against *Candida albicans* [10]. *Eucalyptus camaldulensis* has also been investigated for its antimicrobial properties in a study conducted by [11]. Strong antibacterial activity was shown by *E. camaldulensis* leaf extract against both Gram-positive (like Bacillus subtilis) and Gram-negative (like *Pseudomonas aeruginosa*) microorganisms. The leaf extract's bioactive components, including terpenoids, flavonoids, and tannins, were thought to be responsible for the antibacterial activity. Additionally, the capacity of *Eucalyptus camaldulensis* leaf extracts to break down bacterial cell membranes and block important microbial enzymes has been connected to their antibacterial action [12].

Figure 1. Eucalyptus *globulus* Leaf

Natural antimicrobial agents often exhibit broad-spectrum activity against a wide range of pathogens, including bacteria, fungi, and viruses [13]. Natural substances are more adaptable and useful in the fight against infectious diseases because of their wide range of activities. Plant-derived chemicals are typically regarded as safe for human usage and have lower toxicity than synthetic antimicrobials, in addition to their antibacterial activity [14]. This safety profile reduces the possibility of harmful impacts on human health, which is especially beneficial for therapeutic applications. Additionally, because natural antibacterial chemicals are biodegradable and present little chance of pollution or accumulation, they are advantageous for the environment [15]. Solvent extraction is a frequently used extraction technique in which the bioactive components of *E. camaldulensis* leaves are dissolved by macerating or Soxhlet-extracting them with an appropriate solvent. Ethanol, methanol, and water are common solvents that have varying extraction efficiencies according to their polarity [16]. Bioactive chemicals from *Eucalyptus camaldulensis* leaves can also be extracted using the supercritical fluid extraction (SFE) method. Carbon dioxide (CO_2) is frequently utilized as the solvent in SFE at high temperatures and pressures, which effectively extracts non-polar substances like essential oils

ISSN 0795 - 22066

[17]. Additionally, volatile chemicals, especially essential oils, are commonly extracted from *E. camaldulensis* leaves by steam distillation. This method involves passing steam through the plant material, which carries volatile compounds. The distillate is then collected after the constituents condense [11]. As an alternative, certain classes of bioactive chemicals from leaf extracts of Eucalyptus camaldulensis can be purified and concentrated using solid-phase extraction (SPE) procedures. Target chemicals are adsorbed onto a solid-phase sorbent in SPE, and the desired analytes are then eluted and recovered [18]. The extraction and antibacterial properties of essential oil from *Eucalyptus globulus* using the microwave-assisted hydro-distillation method of extraction have not received much attention, despite extensive study on the subject. Furthermore, there aren't many reports on the antibacterial properties of *Eucalyptus globulus* essential oil using the disc diffusion method.

MATERIALS AND METHODS

Materials and Reagents

Weighing balance, beakers, funnels, measuring cylinder, microwave extraction set-up, Mueller Hinton Agar (MHA), petri dish, autoclave, standard antibiotic discs, incubator, hot air oven, etc.

Collection, Identification, and Preparation of Plants Materials

North-Eastern University in Gombe, Gombe state, Nigeria, is where the plant material was gathered. While another sample of the same plant material was air dried at room temperature away from direct sunlight to minimize photolytic conversion, the fresh young plant was kept in a cool environment to prevent drying [28].

Extraction of Essential Oil by Microwave Assisted Hydro-Distillation

Using a home microwave oven (600 W, Daewoo, China), the essential oils were extracted using a modified version of the procedure outlined by [19]. A 500 mL flask with a flat bottom was filled with 150 g of the sample (fresh young leaves), and then distilled water was added as the solvent. To enable heating of the herb-water blend and the resulting production of vapours, the microwave oven was turned on and the desired conditions of time (10 min) and power (60%) were set. The flask containing the sample was then placed inside the microwave oven and adjusted to a condenser connected to a cold-water recirculation system. The flask could not be rotated or stirred, but the water content permitted sufficient convection, and sufficient homogeneity was achieved. When the extracted liquid entered a trap, the essential oil was recovered and its volume was calculated. Vapors started to ascend into the flask's neck until they reached the condenser, where they were cooled. The extracted oils were pooled after the procedure was carried out twice more using new samples. The yields were expressed as a percentage of the plant sample's weight (mass of extracted oil). Before being analyzed, the oil was refrigerated

and put into screw-cap amber test tubes. The process outlined above was repeated to extract essential oil from the dried sample, and the yield percentage for each sample was determined.

Preparation of Antibacterial Reagents and Apparatus

Every piece of equipment and glassware utilized in the antibacterial research was autoclave sterilized for 15 minutes at 121 degrees Celsius and then dried in a hot air oven. To maintain constant sterility until they were used, the dried glassware and equipment were kept in a laminar flow chamber under UV light [20].

Preparation of Stock Solution

A 10 mL test tube that has been cleaned and sterilized was utilized. *Eucalyptus globulus* ultimate concentrations were 143 mL/mL, 71.5 mL/mL, and 35.75 mL/mL after 0.5 mL of the plant's essential oil was measured, dissolved in 3.5 mL of trichloromethane, and serially diluted [21].

Preparation of Discs

25 sterile discs manufactured with filter paper (6 mm) were generated by immersing them in 0.5 mL of each of the following solutions: 143 mL/mL, 71.5 mL/mL, and 35.75 mL/mL. This produced 5.72 mL, 2.86 mL, and 1.43 mL per disc, respectively. All bacterial strains were tested using commercially available antibiotic diffusion discs that contained several conventional antibiotics as positive reference standards. Trichloromethane-impregnated discs were used to create negative control. The discs and solutions were made using a modified version of the [22] approach.

Source and Maintenance of Organism

Both of the microorganisms utilized were clinical isolates that were acquired and verified at the Federal Teaching Hospital's Research Laboratory of the Department of Medical Microbiology and Parasitology in Gombe, Nigeria. *Salmonella typhi, Pseudomonas aeruginosa, and Escherichia coli* are the isolates of Gram-negative bacteria, whereas *Staphylococcus aureus* is an isolate of Gram-positive bacteria. To produce pure colonies, they were kept and sub-cultured on nutrient agar (NA).

Preparation of McFarland Standard

99.5 mL of 0.18 M H_2SO_4 (corresponding to 1% v/v) was mixed with 0.5 mL of a solution containing 0.0448 M of BaCl2 (1.17% weight/volume of $BaCl_2$). This was carried out while being constantly stirred. According to [23], the resultant standard solution was separated into screw-capped bottles, firmly sealed to stop evaporation, and kept out of direct sunlight.

Preparation of Mueller-Hinton Agar (MHA)

The manufacturer's instructions were followed in the preparation of the MHA. A sterile conical flask was filled with 38 g of MHA and 1000 cm^3 of distilled water. Until the broth completely dissolved, the suspension was slowly heated while being stirred periodically. After being

ISSN 0795 - 22066

securely wrapped in aluminum foil, the dissolved medium was autoclave sterilized for 15 minutes at 121 degrees Celsius. After sterilization, the medium was allowed to cool in a laminar flow.

Preparation of the Inoculums

The test organism that had been subcultured on NA at 37 °C was taken in a loopful and suspended in a standard saline solution (0.85%, w/v) NaCl. In order to achieve the turbidity of the 0.5 M McFarland standard, which corresponds to around 1.0 x 106 cfu/mL of the bacteria, the density of the organism suspension was adjusted against a black line [24].

Antibacterial Assay by Disc-Diffusion Method

Twenty milliliters of a base layer of molten Mueller Hinton agar were used to construct 90 mm Petri dishes. Ten microliters of each bacterial suspension (106 CFU/mL) were added to each Petri dish. 6 mm diameter discs containing the essential oil were set on the medium after drying in a sterile hood. Discs impregnated with trichloromethane served as the negative control, while discs with commercially available multiple standard antibiotics served as the positive control. The plates were incubated at 37 °C for a whole day. Millimeters were used to measure the zones of inhibition's sizes. The mean of the inhibitory diameters (mm) generated was used to express the bacterial activity, and each test was run in triplicate [22].

RESULTS AND DISCUSSION

In the current study, essential oil was extracted via microwave-assisted hydro-distillation, and the oil's antibacterial properties were assessed. As shown in Table 1, the physical approach was used to determine the oil's color, odor, and solubility. The formula was also used to determine the essential oil's yield percentage.

Table 1. Physical Parameters of the Essential Oil

Parameter	**Characteristics**	
	Fresh leaves	**Dried leaves**
Colour	Pale yellow	Pale yellow
Odour	Camphor-like aroma	Camphor-like aroma
Solubility	In trichloromethane	In trichloromethane
Yield	10.7	7.9

ISSN 0795 - 22066

Antimicrobial activity of Eucalyptus Globulus Essential Oils

The antibacterial assay for the *Eucalyptus globulus* essential oil was carried out usinga disc impregnated with different concentrations of the oil, following the method used by [22] with little modification. The inhibition zone was measured after 24 hours of incubation at 37^{o}C Table 2. fresh leaves and Table 3. driedleaves. The control experiments were done using trichloromethane and standard commercially available antibiotic diffusion discs containing multiple standard antibiotics used as control Table 4.

Table 2. Antimicrobial Activity of *Eucalyptus globulus* Essential Oils Fresh Leaves

Sample	**Concentration**	**Zones of inhibition of organisms in mm**			
		Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*	Pseudomonas aeruginosa
Eucalyptus	5.72 mL/disc	9.0	17.7	9.0	Nd
globulus					
	2.86 mL/disc	7.0	14.7	7.5	Nd
	1.43 mL/disc	6.0	9.7	Nd	Nd

According to Table 2, the essential oil of fresh *Eucalyptus globulus* leaves exhibits no action against *Salmonella typhi* at 1.43 mL/disc but a zone of inhibition against three of the studied organisms including *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi* at all oil concentrations per disc. At all concentrations, the essential oil did not, however, exhibit any anti-*Pseudomonas aeruginosa* activity. With a zone of inhibition of 17.7 mm and a concentration of 5.72 mL/disc, the oil was most effective against *Staphylococcus aureus*. As concentration drops, the zone of inhibition shrinks as well; at 2.86 mL/disc and 1.43 mL/disc, it is 14.7 mm and 9.7 mm, respectively. The reported activity was comparable to [25]. It was concentration-dependent since the zone of inhibition shrank as the oil concentration per disc dropped. At doses of 5.72 mL/disc, 2.86 mL/disc, and 1.43 mL/disc, respectively, the *E. coli* zone of inhibition was 9.0, 7.0, and 6.0 mm. *Salmonella typhi* showed no activity at 5.72 mL/disc, 2.86 mL/disc, and 1.43 mL/disc, respectively, and a zone of inhibition of 9.0 and 7.5 mm. Additionally the oil from *E. globulus* shown encouraging efficacy against *Staphylococcus aureus* [27].

ISSN 0795 - 22066

Table 3. Antimicrobial Activity of *Eucalyptus globulus* Essential Oils Dried Leaves

Sample	Concentration	Zones of inhibition of organisms in mm			
		Escherichia coli	*Staphylococcus aureus*	*Salmonella typhi*	*Pseudomonas aeruginosa*
Eucalyptus	5.72 mL/disc	8.0	13.3	11.0	Nd
globulus					
	2.86 mL/disc	6.0	11.0	7.0	Nd
	1.43 mL/disc	4.0	8.3	Nd	Nd

The essential oil of dried *Eucalyptus globulus* leaves, as shown in table 3, exhibits a zone of inhibition against three of the tested organisms, namely *Salmonella typhi, Staphylococcus aureus,* and *Escherichia coli,* at all oil concentrations per disc, but no activity against *Salmonella typhi* at 1.43 mL/disc. Additionally, the essential oil did not exhibit any activity against *Pseudomonas aeruginosa* at any concentration. The oil was most effective against *Staphylococcus aureus* at a concentration of 5.72 mL/disc, with a zone of inhibition of 13.3 mm; the zone of inhibition decreases as concentration decreases, measuring 11.1 mm at 2.86 mL/disc and 8.3 mm at 1.43 mL/disc, respectively. This activity was comparable to that reported by [25]. The zone of inhibition shrank as the concentration of oil per disc dropped, indicating that activity was concentration-dependent where it happened. With doses of 5.72 mL/disc, 2.86 mL/disc, and 1.43 mL/disc, respectively, the zone of inhibition for *E. coli* was 8.0, 6.0, and 4.0 mm. *Salmonella typhi* showed no activity at 5.72 mL/disc, 2.86 mL/disc, and 1.43 mL/disc, respectively, and a zone of inhibition of 11.0 and 7.0 mm. Notably, the young, fresh leaves produced more oil than the dried leaves, and they also exhibited greater activity against the examined organisms than the dried leaves, with the exception of *Salmonella typhi*, which was more active against the dried leaves' oil at 5.72 mL/disc. Furthermore, at 2.86 mL/disc, there is no discernible difference between the two samples' activity against *Salmonella typhi*. Furthermore, at all concentrations, neither oil had any action against *Pseudomonas aeruginosa.*

ISSN 0795 - 22066

Table 4. Antimicrobial Activity of C**ommercially Available Antibiotic Diffusion Discs Containing Multiple Standard Antibiotics Used and Trichloromethane as Control**

Antibiotic disc	**Concentration per disc (µg)**	***Zones of inhibition of organisms in mm***			
		Escherichia coli	*Staphylococcus Aureus*	*Salmonella typhi*	*Pseudomonas Aeruginosa*
AZ	12	16	18	17	19
OFX	10	15	Nd	9	20
PEF	30	17	Nd	Nd	21
CN	30	19	13	10	19
AU	10	20	Nd	Nd	18
AM	30	Nd	9	Nd	17
CPX	30	17	Nd	11	19
SP	10	18	Nd	13	20
CF	10	17	Nd	13	21
LEV	20	19	15	14	19
TCM		Nd	Nd	Nd	Nd

The zone of inhibition for common antibiotics and the solvent used (TCM) is displayed in Table 4. While the solvent exhibited no activity against any of the test organisms, the activities of many conventional antibiotics varied. The conventional disc's maximum activity against *E. coli* was 20 mm by AU, which is less than what the essential oil demonstrated against the same organism. AM displayed no activity, whereas OXF recorded the lowest at 15 mm. At this concentration, the oil exhibited a 24 mm 5.72 mL/disc inhibition, suggesting more action than the reference

ISSN 0795 - 22066

medication. The oil's activity at other concentrations, however, was lower than the disc's lowest activity. The standard disc's maximum activity against *Salmonella typhi*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* was 15 mm, 17 mm, and 21 mm for LEV, AZ, PEF, and CF, respectively. In every instance, the regular disc's lowest activity was higher than the oil's lowest.

CONCLUSION

When compared to other techniques used in the distant and recent past, the yield of oil from the microwave-assisted extraction of essential oil from *Eucalyptus globulus* demonstrated high efficiency. Apart from *Pseudomonas aeruginosa*, the essential oil of *Eucalyptus globulus* has strong antibacterial action against the studied bacterial pathogens. Its mechanisms of action may be explained by the suppression of biofilm formation and the rupture of bacterial cell membranes. Its uses in medical and food preservation, as well as comparative studies to support its effectiveness and promise as a natural antibiotic substitute, highlight the need for more research.

REFERENCES

[1] Silva, J. C., Ramos, A., & Gonçalves, M. J. (2010). Chemical composition and bioactivity of different oregano (Origanum vulgare) extracts and essential oil. *Journal of the Science of Food and Agriculture*, 90(11), 1755-1762.

[2] Sadgrove, N. J., & Jones, G. L. (2014). Chemical composition of the essential oils of Eucalyptus species and the implications for biological activity. *Forests*, 5(6), 1352-1370.

[3] Carson, C. F., Hammer, K. A., & Riley, T. V. (2006). Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. *Clinical Microbiology Reviews*, 19(1), 50-62.

[4] Švarc-Gajić, J. V., Gajić, G., Đurović, S., & Zlatković, B. (2018). Essential oils in cosmetics: challenges and perspectives. *Cosmetics*, 5(1), 11.

[5] Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. P & T:A *Peer-Reviewed Journal for Formulary Management*, 40(4), 277-283.

[6] Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – a review. *Food and Chemical Toxicology*, 46(2), 446-475.

[7] Hegarty, M. P. (2021). Eucalyptus: A survey of the eucalypts of the world. Nelson.

[8] Fatiha, B. M., Sonia, G., Zahia, K., Nadia, S., & Nawel, B. (2017). Phytochemical screening and evaluation of antibacterial activity of *Eucalyptus camaldulensis*. *Journal of Pharmacognosy and Phytochemistry*, 6(6), 817-822.

[9] Saeed, S., Tariq, P., & Zafar, A. (2012). Antibacterial activity of Eucalyptus globulus (blue gum) against bacterial strains isolated from burn wound infections. *African Journal of Microbiology Research*, 6(3), 484-490.

[10] Wannissorn, B., Jarikasem, S., Siriwangchai, T., & Thubthimthed, S. (2005). Antibacterial properties of

ISSN 0795 - 22066

essential oils from Thai medicinal plants. *Fitoterapia*,76(2), 233-236.

[11] Hashemi, S. M. B., Khodadadi, I., & Ali, A. M. (2018). Chemical composition and antimicrobial activity of the essential oil of *Eucalyptus camaldulensis* Dehnh. against pathogenic bacteria. *Asian Pacific Journal of Tropical Biomedicine*,8(1), 50-53.

[12] Bibi, Y., Nisa, S., Chaudhary, F. M., Zia, M., (2016). Antibacterial activity of some selected medicinal plants of Pakistan. *BMC Complementary and Alternative Medicine*, 11(1), 52.

[13] Ghasemzadeh, A., Jaafar, H. Z., & Rahmat, A. (2010). Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). *Molecules*, 15(6), 4324-4333.

[14]Nascimento, G. G. F., Locatelli, J., Freitas, P. C., & Silva, G. L. (2000). Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazilian Journal of Microbiology*, 31(4), 247-256.

[15] Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564-582.

[16]Mao, Y., Huang, X., Ma, X., & Ai, W. (2019). Ultrasonic-assisted extraction and antioxidant activities of natural antioxidants from the *Eucalyptus camaldulensis* leaves. Heliyon, 5(5), e01531.

[17] Benelli, G., Pavela, R., Maggi, F., Petrelli, R., & Cappellacci, L. (2018). Eucalyptus essentials oils: From extraction to health benefits. *Frontiers in Pharmacology*, 9, 1-5.

[18] Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/ partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues inproduce. *Journal of AOAC International*. 86(2):412–431, https://doi.org/10.1093/jaoac/86.2.412

[19] Cardoso-Ugarte, G. A., Juárez-Becerra, G. P. Sosa-Morales, M. E. & López-Malo, A. (2013). Microwave-assisted Extraction of Essential Oils from Herbs. J*ournal of Microwave Power and Electromagnetic Energy, 47 (1): 63-72*

[20] Webber, D. M., Wallace, M. A., Burnham, C. D., (2022). Stop waiting for tomorrow: Disk diffusion performed on early growth is an accurate method for antimicrobial susceptibility testing with reduced turnaround time. *Journal of Clinical Microbiology*. **60**(5): 115-120.

[21] Licien, B. V., Douglas, M. H., Scott, P. M., John, K. H., and Michael, J. M., (2023). Antimicrobial susceptibility testing to evaluate minimum inhibitory concentration values of clinically relevant antibiotics. *Star Protocols*. **4**(3): 102512.

[22] Dibala, C. I., Konate, K., Diao, M., Maurice, O. M. and Dicko. (2014). PhytoconstituentsAnalysis, Antioxidant capacity and Antimicrobial properties of extracts from *Chrozophora senegalensis*. *Journal of Pharmacy and Pharmaceutical Sciences* **6**(7):172-178.

[23] Andrews J. M. (2001). Determination of minimum inhibitory concentrations. *The Journal of antimicrobial chemotherapy, 48 (1)*, 5 –16. https://doi.org/10.1093/jac/48.suppl_1.5

[24] Yohanna, C., Kwaji, A., & Atiko, R. (2021). Antibacterial Activity, Antioxidant Potential and Stigmasterol

ISSN 0795 - 22066

Isolation from Laggera aurita Linn (Asteraceae). *International Journal of Biochemistry Research and Review*, 24–39.

[25] Ghalem, B. R. & Mohamed, B. (2008). Paper Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and Pharmacology 2(10)*. 211-215

[26] DeSiqueira M, V., Turrini, R. N. T., & De Brito Poveda, V. (2015). Antimicrobial activity of Eucalyptus globulus oil, xylitol and papain: a pilot study. *Revista Da Escola De Enfermagem Da USP*, *49*(2), 0216–0220. https://doi.org/10.1590/s0080-623420150000200005

[27] Elangovan, S., & Mudgil, P. (2023). Antibacterial Properties of Eucalyptus globulus Essential Oil against MRSA: A Systematic Review. *Antibiotics*, *12*(3), 474. https://doi.org/10.3390/antibiotics12030474

[28] Abdullahi, M. A., Isyaka, M. S., and Ahmad, N. S., (2024). Biological investigation and chemical constituents of *Croton nigritanus scott* Elliot. *Journal of Pharmacognosy and Phytochemistry* **13**(1):32-37.

ISSN 0795 - 22066