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Antimicrobial Activity of Commercial Essential Oils Available in the Market of Kathmandu

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ABSTRACT

Given the increasing global apprehension surrounding antibiotic resistance, there has been growing interest in exploring natural compounds, specifically essential oils, as potential substitutes for combating microbial pathogens. This research work aimed to evaluate the antimicrobial activity exhibited by commercial essential oils and by diluting them with the carrier oils at equal concentration. The screening of antimicrobial activity was conducted using the agar well diffusion method with eight essential oils. Using the broth dilution method, the antibacterial activity of eight commonly used essential oils: lavender, tea tree, eucalyptus, peppermint, thyme, citrus, rosemary, and cinnamon were assessed. Both Gram-positive and Gram-negative species were selected for antimicrobial activity. The minimum inhibitory concentration (MIC) was determined by the broth dilution method. Following MIC determination, a sub-culture was performed on nutrient agar plates to ascertain the minimum bactericidal concentration (MBC). All the antibiotics used in the study demonstrated sensitivity when subjected to the antibiotic susceptibility test. Thyme, cinnamon and peppermint strongly inhibited the growth of *S. epidermidis* 46 mm, 30mm, and 34 mm respectively. Findings revealed distinct variations in the MIC values among the different essential oils and bacteria tested. Thyme and tea tree oils exhibited the broadest antibacterial spectrum, inhibiting both Gram-positive and Gram-negative bacteria at relatively low concentrations. Thyme (15.625 µl/ml) and tea tree (31.25 µl/ml) oil demonstrated promising activity against Gram-positive pathogen, while tea tree oil (15.625 µl/ml) displayed better efficacy against Gram-negative bacteria. A significant outcome was observed when the essential oils were diluted with carrier oils, indicating promising results in terms of antibacterial activity. These findings highlight the potential of essential oils, particularly thyme and tea tree oils, as effective natural antimicrobial agents.

Keywords: Essential oils, antimicrobial activity, minimum inhibitory concentration, minimum bactericidal concentration, carrier oils

Introduction

Essential oils (EOs) are secondary metabolites produced by aromatic plants and are volatile, natural, complex molecules with a strong odor (Bakkali et al., 2008). Essential oils are liquids that are lipid soluble, rarely colored, and often have a lower density than water. All plant organs, including buds, flowers, leaves, stems, twigs, seeds, fruits, roots, wood, or bark, are capable of synthesizing essential oils, which are then stored in secretory cells, cavities, canals, epidermal cells, or glandular trichome. Terpenes, aldehydes, alcohols, esters, polyphenols, ethers, and ketones are only a few of the numerous chemical compounds that give essential oils derived from medicinal and aromatic plants their distinctive scents and biological effects (Kalemba & Kunicka, 2005; Swamy & Sinniah, 2015).

Essential oils are often produced through hydro- or steam-distillation as well as expression (for citrus peel oils) (Seow et al., 2014). The most popular way for obtaining essential oils commercially is using a process known as steam or hydro-distillation, which was first invented by Arabs in the medieval times (Chouhan et al., 2017). Despite the fact that these methods have been used for EO extraction for a long time, their use has revealed a number of drawbacks, including the loss of some volatile compounds, low extraction efficiency, the degradation of unsaturated or ester compounds through thermal or hydrolytic effects, and the possibility of toxic solvent residues in extracts or EOs (Reyes-Jurado et al., 2015). Supercritical fluid extraction (SFE), pressurized

liquid extraction, pressurized hot water extraction, membrane-assisted solvent extraction, solid-phase micro-extraction, microwave-assisted and ultrasound-assisted extraction, among other new techniques, are currently available for the extraction of EOs. These alternatives to traditional extraction techniques may improve production efficiency and aid in environmental protection (Jurado et al., 2016).

EOs are used as analgesic, sedative, anti-inflammatory, and spasmolytic products. Additionally, EOs are widely known for having a broad range of antibacterial, antifungal, and even antiviral actions. They are also able to stop the development of drug-resistant microbial strains, which are challenging to treat with conventional antibiotics (Lunz & Stappen, 2021). The antibacterial characteristics of EOs have also attracted the attention of food, pharmaceutical, and cosmetic industries, since the use of natural additives has gained prominence as a trend in the replacement of synthetic preservatives (Murbach Teles Andrade et al., 2014). Since the nineteenth century, root canal therapy, temporary fillings, and periodontal care have all benefited from the use of essential oils, which are complex mixes of organic molecules that represent the odoriferous principles of plants. Essential oils have also been used for thousands of years to decrease mouth odor and pain (Shapiro et al., 1994). Today, a variety of medical ailments, such as cancer, pain, stress, and infectious diseases, are treated with essential oils (Abers et al., 2021).

EOs have been the central focus of the scientific community due to the increased consumer demand for the development of natural, safe, and effective health products (Ni et al., 2021). According to Grand View Research 2020, the market for essential oils is predicted to expand at a compound annual growth rate of 7.5% between 2020 and 2027. Increased customer demand for natural ingredients in foods, as a result of worries about the negative health consequences of synthetic preservatives, is the main reason for this rise. The rising appeal of unusual flavors and their expanded use in sectors like perfumery, cosmetics, hygiene, and aromatherapy are further significant contributors. Growing demand for processed meals and beverages is another (Fuentes et al., 2021).

Hence, plant essential oils and the main chemical components within them are being explored for its usage in various fields. This study aims to evaluate the potential antimicrobial activities of eight commercial essential oils available in the market of Kathmandu using agar well diffusion. The study also focuses on determining the minimum inhibitory concentration of the essential oil against *Escherichia coli* and *Staphylococcus aureus* by broth dilution method and minimum bactericidal concentration by plating out on nutrient agar plates. Likewise, the antibacterial activity of the essential oils was evaluated by diluting them with different carrier oils (Mustard, Olive and Castor oils) at equal concentration.

Materials and methods

A total of eight different essential oils were used to evaluate antimicrobial properties. The essential oils used were Lavender (*Lavender angustifolia*), Eucalyptus (*Eucalyptus globulus*), Rosemary (*Salvia rosmarinus*), Peppermint (*Mentha piperita* L.), Tea tree (*Melaleuca alternifolia*), Cinnamon (*Cinnamomum verum*), Thyme (*Thymus vulgaris*), and Citrus oil, and the carrier oils used were Castor (Ricinus communis), Olive (*Olea europaea* L.), and Mustard (*Brassica juncea*). All the research work was carried out at the Microbiology Laboratory of St. Xavier's College, Maitighar, Kathmandu.

Different bacteria were tested for their susceptibility to the selected essential oils. The bacteria were *Staphylococcus aureus (*ATCC 6538P*), Staphylococcus epidermidis (*ATCC 12228*), Escherichia coli (*ATCC 8739), *Bacillus subtilis* (isolated from soil sample), *Pseudomonas aeruginosa* (ATCC 9027), *Listeria monocytogenes* (ATCC 19115), *Salmonella* Typhi (ATCC 14028), *Salmonella* spp. (isolated from fecal matter of dog), and *E. coli* (isolated from fecal matter of dog). All the ATCC cultures were provided by the Department of Plant Resources, Thapathali, Kathmandu, and the test organisms were available at the laboratory of St. Xavier's College.

Inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of bacteria in 5ml of nutrient broth following incubation at 37 °C for 3 hours. The turbidity developed was matched with 0.5 McFarland standards.

Screening of antibacterial activity

The essential oils were stored at 4 °C before processing. Agar well diffusion method was used for the evaluation of antimicrobial activity of the essential oils (Balouiri et al., 2016). Sterile MHA plates were prepared, and with the help of sterile cotton swabs, the inoculum was carpet cultured on the surface of the plates. On the plates, wells of diameter 4mm were made with a cork borer to which 10 µl of the essential oils was filled. The plates were then incubated at 37 °C for 24 hours. The plates were observed, and the zone of inhibition was measured (Sharma et al., 2014).

Antibiotic susceptibility test

Antimicrobial susceptibility testing was done by the Kirby-Bauer disc diffusion method as recommended by CLSI guideline 2022. An inoculum of 0.5 McFarland standard turbidity was prepared in a nutrient broth from an isolated colony of test organisms. The sample was inoculated on Mueller-Hinton Agar (MHA) plate by the lawn culture method. The antibiotic discs (Hi Media Labs, India): Amikacin (30 mcg), Amoxyclav (30/10 mcg), Ampicillin (10mcg), Azithromycin (15 mcg), Cefalexin (30 mcg), Ceftriaxone (30 mcg), Chloramphenicol (30mcg), Ciprofloxacin (5mcg), Clindamycin (2 mcg), Erythromycin (15 mcg), Gentamicin (10mcg), Tetracycline (30mcg), Penicillin G (1 unit), and Vancomycin (30mcg) were placed on the agar plate and incubated at 37 °C for 18 hours. The zone of inhibition was measured, and the results were interpreted as resistant (R), intermediate (I) and sensitive (S) to the respective antibiotics according to the zone interpretation chart of CLSI 2022.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of various essential oils was determined using the broth dilution method. Each essential oil was subjected to a 2-fold serial dilution, starting from a stock solution of 1000 µl in 1 ml Muller Hinton broth to 15.625 µl/ml concentration. 1ml inoculum of 5×10^5 CFU/ml was added in the dilution series. Following 16 hours of incubation at 37 °C, the MIC was determined as the lowest concentration of the essential oil that inhibited visible bacterial growth. After obtaining the MIC of oil against organisms, they were subcultured in essential oil free media to determine the MBC (CLSI, 2018).

Antimicrobial susceptibility test of EOs using carrier oil

AST of EO using carrier oil was performed by the agar well diffusion method. Sterile MHA plates were prepared, and with the help of sterile cotton swabs, the inoculum was lawn cultured on the surface of the plates. On the plates, wells of 4mm diameter were made to which the essential oils (5 µl) and carrier oil (5 µl) were filled, i.e., 10 µl. The plates were then incubated at 37 °C for 24 hours. Zone of inhibition was measured and noted. The carrier oils used were mustard, olive and castor oils.

Quality control: The essential oils, without mixing with any of the carrier oils, were used as control.

Data management and analysis: The data were analyzed by comparing with the standard interpretative chart provided along with the antibiotics.

Results

Antimicrobial activity of essential oils

The following scale was used for measurement (including the diameter of the well): strong inhibition having zone of inhibition ≥ 28mm, moderate inhibition having zone of inhibition ≥ 16mm and ＜28mm, mild inhibition having zone of inhibition ≥ 12mm and ＜16mm, and no inhibition having zone of inhibition ＜12mm (Semeniuc et al., 2017).

Out of all the essential oils tested, thyme oil was found to be most effective against five strains of bacteria used. *Staphylococcus epidermidis* was the most susceptible bacterial stain showing the largest zone of inhibition of 46mm followed by *Salmonella* spp. (fecal isolate from dog), *Bacillus subtilis* (soil isolate), *E. coli* (fecal isolate from dog), and *Pseudomonas aeruginosa* with 42, 35, 30, and 28 mm, respectively.

Citrus essential oil showed moderate inhibition against *E. coli* (fecal isolate from dog), *S. aureus*, *S. epidermidis,* and *S.* Typhi. However, its effect was milder against *E. coli* (ATCC). Conversely,

Citrus showed lesser to negligible inhibition against *Bacillus*, *Listeria* (ATCC), *Salmonella* (dog fecal specimen isolate), and *P. aeruginosa* (ATCC). The zone of inhibition against *E.coli* (ATCC) and *S. aureus* was 13 mm and 19 mm, respectively.

Rosemary essential oil exhibited strong inhibition for *E. coli* (fecal isolate from dog), moderate inhibition for *S. epidermidis*, *Salmonella* spp. (fecal isolate from dog), *S.* Typhi, and *P. aeruginosa*, mild inhibition for *Bacillus,* and no inhibition for *E. coli* (ATCC), *S. aureus,* and *Listeria*.

Peppermint showed strongest antimicrobial activity against *S. epidermidis* (34 mm), moderate antimicrobial activity against *E. coli* (fecal isolate from dog), i.e., 18mm, and mild antimicrobial activity against *Bacillus* (14mm) and *Listeria* (15mm). The oil could not inhibit the growth of *E. coli* (ATCC), *S. aureus*, *Salmonella* spp, *S.* Typhi, and *P. aeruginosa*.

S. aureus and *S.* Typhi were strongly inhibited by tea tree oil with zone of inhibition 33mm and 28mm, respectively. *E. coli* (ATCC), *E. coli*, *S. epidermidis*, *Salmonella* spp., and *P. aeruginosa* were moderately inhibited by the essential oil with zone of inhibition 20mm, 18mm, 17mm, 23mm, and 16mm, respectively, followed by no inhibition for *Bacillus* and *Listeria* with inhibition of 9mm and 10mm.

S. epidermidis was strongly inhibited by cinnamon oil with a zone of inhibition 30mm. *E. coli* (ATCC), *E. coli* (fecal specimen isolate from dog), *S. aureus*, *Bacillus*, *Listeria*, *Salmonella*, *S.* Typhi, and *P. aeruginosa* were moderately inhibited by cinnamon oil with zones of inhibition of 26mm, 25mm, 18mm, 20mm, 20mm, 25mm, 26mm, and 27mm, respectively.

Thyme essential oil emerged as a potent agent, exerting strong inhibitory effects on a wide spectrum of bacteria. *E. coli* (fecal isolate from a dog), *S. epidermidis*, *Bacillus*, *Salmonella*, and *P. aeruginosa* were notably affected, showcasing zones of inhibition measuring 30mm, 46mm, 35mm, 42mm, and 28mm, respectively.

Comparatively, among the range of bacteria tested, thyme essential oil demonstrated the most robust inhibitory activity. However, it displayed a somewhat less potent yet still noteworthy effect against *E. coli* (ATCC), *S. aureus*, *Listeria*, and S. Typhi, registering zones of inhibition at 26mm, 27mm, 18mm, and 28mm, indicating a moderate level of inhibition.

Table 1. Antimicrobial activity of essential oils using agar well diffusion method. The diameter of the zone of inhibition includes the well (4 mm)

S.N	Name of Organism	Zone of Inhibition(mm)							
		Cit	R	Pp	Tt	Cin	Thy	L	Eu
1	*E. coli* (ATCC 8739)	13	11	10	20	26	26	0	30
2	*E. coli* (isolated from feces of dog)	26	29	18	18	25	30	6	28
3	*S. aureus* (ATCC 6538P)	18	9	6	33	18	27	7	12
4	*S. epidermidis* (ATCC 12228)	17	16	34	17	30	46	7	28
5	*Bacillus subtilis* (isolated from soil)	10	15	14	9	20	35	11	11
6	*L. monocytogenes* (ATCC 19115)	2	8	15	10	20	18	0	8
7	*Salmonella* spp. (isolated from feces of dog)	9	22	7	23	25	42	0	15
8	*S.* Typhi (ATCC 14028)	24	26	0	28	26	24	0	16
9	*P. aeruginosa* (ATCC 9027)	10	22	8	16	27	28	0	20
Cit= citrus, R= rosemary, Pp= peppermint, Tt= tea tree, Cin= cinnamon, Thy= thyme, L= lavender, Eu= eucalyptus, 0= no zone of inhibition									

Antibiotic susceptibility test

All of the bacteria tested were found resistant to lavender essential oil as little to no zone of inhibition was observed. The recorded zone of inhibition was 6mm against *E. coli,* 7mm against both *S. aureus* and *S. epidermidis,* and 11mm against *B. subtilis*.

Eucalyptus had strong inhibition against three bacteria, *E.coli* (ATCC), *E.coli* (dog isolate), and *S. epidermidis* having zone of inhibition 30mm, 28mm, and 28mm, respectively, moderate inhibition against *S.* Typhi, and mild inhibition against *S. aureus* and *Salmonella* spp. The oil failed to inhibit the growth of *Bacillus* and *Listeria* spp.

Each of the antibiotics used in the study was sensitive against all the bacteria used. 30 mcg of tetracycline used for *E. coli* (ATCC) and *E. coli* (fecal specimen isolated from dogs) exhibited a zone of inhibition of 28mm and 22mm, respectively. Similarly, Amoxyclav (AMC30) used against *S. aureus* possessed a zone diameter of 31mm. Likewise, Cefalexin (CN30) acquired a zone diameter of 35mm against *S. epidermidis*. Erythromycin (EI5) acquired a zone diameter of 25mm against *B. subtilis* (soil isolate). For *L. monocytogenes*, Ceftriaxone (CTR30) had a zone of inhibition of 25mm. Ciprofloxacin (CIP5) demonstrated a zone of inhibition of 31mm against *Salmonella* spp. (fecal isolate from dog). *S.* Typhi and *P. aeruginosa* exhibited a zone of inhibition of 40mm and 38mm, respectively.

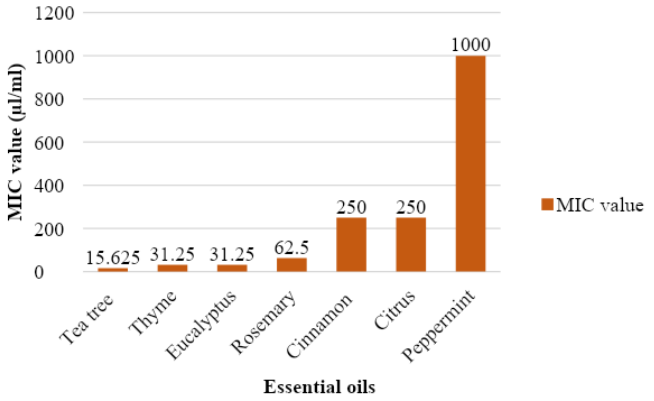


Figure 3. Graph representing MIC value of different EOs against *E.coli*

Table 2. Antibiotic susceptibility test for different bacteria

S.N	Name of Organism	Antibiotics	Interpretative criteria (mm)			Zone of Inhibition (mm)
			S	I	R	
1	*E.coli*	Tetracycline	≥15	12-14	≤11	28
2	*S. aureus*	Amoxyclav	≥18	14-17	≤13	31
3	*S. epidermidis*	Cefalexin	≥14	-	-	35
4	*B. subtilis* (soil isolate)	Erythromycin	≥23	14-22	≤13	34
5	*L. monocytogenes*	Ceftriaxone	≥23	20-22	≤19	25
6	*Salmonella (*fecal isolate from dog)	Ciprofloxacin	≥25	19-24	≤18	31
7	*S. Typhi*	Ciprofloxacin	≥25	19-24	≤18	40
8	*P. aeruginosa*	Ciprofloxacin	≥25	19-24	≤18	38
9	*E.coli* (fecal isolate from dog)	Tetracycline	≥15	12-14	≤11	22

S-sensitive, I-intermediate, and R-resistant

The MIC values of essential oils obtained by broth macrodilution followed by streaking on NA plates are presented in Figure 1. Evidently, the antimicrobial efficacy against *E. coli* displayed notable divergence among the various oils, ranging from negligible inhibitory effects observed with lavender to complete inhibition achieved by tea-tree essential oil. Based on their antimicrobial effectiveness against *E.coli*, the essential oils can be ranked as follows: Tea-tree > Eucalyptus = Thyme > Rosemary > Cinnamon = Citrus > Peppermint. Tea tree essential oil demonstrated the most potent inhibition against *E. coli*, achieving a MIC of 15.625µl/ml, surpassing all other oils tested. The MIC values generally aligned with the screening test's inhibitory trends, except for eucalyptus, cinnamon, and tea tree essential oils. Eucalyptus EO had the highest zone of inhibition than other EOs during agar diffusion assay, whereas MIC value of tea tree EO was lowest, indicating a higher antimicrobial activity than eucalyptus EO during the broth dilution.

Antimicrobial effects of essential oils on S. aureus

Thyme EO had the lowest MIC value of 15.625µl/ml, indicating it to be the most effective essential oil in inhibiting the growth of *S. aureus.* Tea tree oil had a MIC value of 31.25µl/ml, suggesting it to be effective but slightly less potent than thyme. Peppermint oil had a MIC value of 62.5µl/ml, which is higher than thyme and tea tree oil but still shows effectiveness.

Similarly, citrus oil has a higher MIC value of 125, indicating it to be less effective in inhibiting the growth of *S. aureus* compared to the previous oils. Rosemary and cinnamon oils both have a MIC value of 250, implying they have a similar level of effectiveness against *S. aureus*. Eucalyptus oil had the highest MIC value of 1000, indicating it is the least effective in inhibiting the growth of *Staphylococcus aureus* among the oils listed.

Antibacterial activity of EOs in combination with carrier oils

The antimicrobial properties of the essential oil compounds, alone and in combination with carrier oils, were determined using the agar well diffusion assay. In this study, the antimicrobial activity of different essential oils showing higher antagonistic effects in combination with three carrier oils against nine pathogens were determined, and the results are shown in Table 3. Among the nine bacterial strains, seven bacterial strains were able to show moderate inhibition to each combination except *E. coli* from Dog and *S.* Typhi when tested by the disc diffusion method. The diameter of the zone of inhibition varied depending on the EOs and bacterial strains used. Cinnamon oil in combination with mustard oil, castor oil, and olive oil showed a noteworthy synergistic effect against *S. epidermidis* (ATCC 12228). Other bacterial strains displayed moderate to low and no zone of inhibition as shown in Table 3.

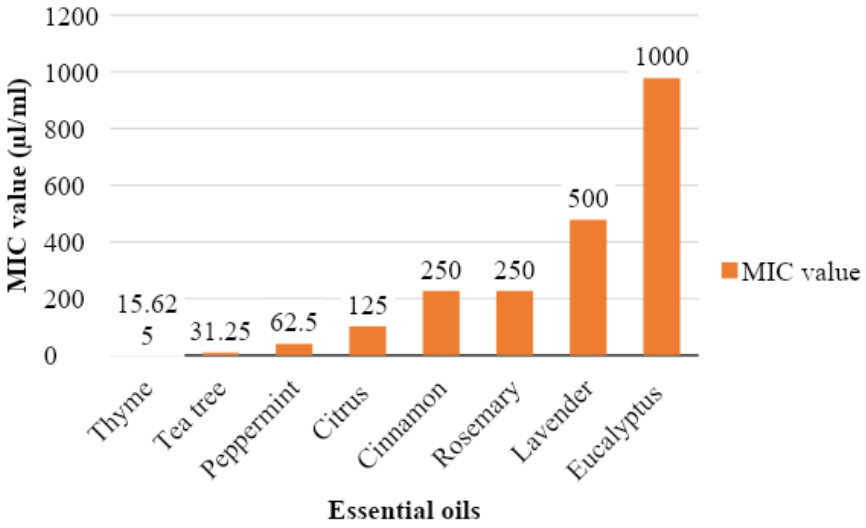


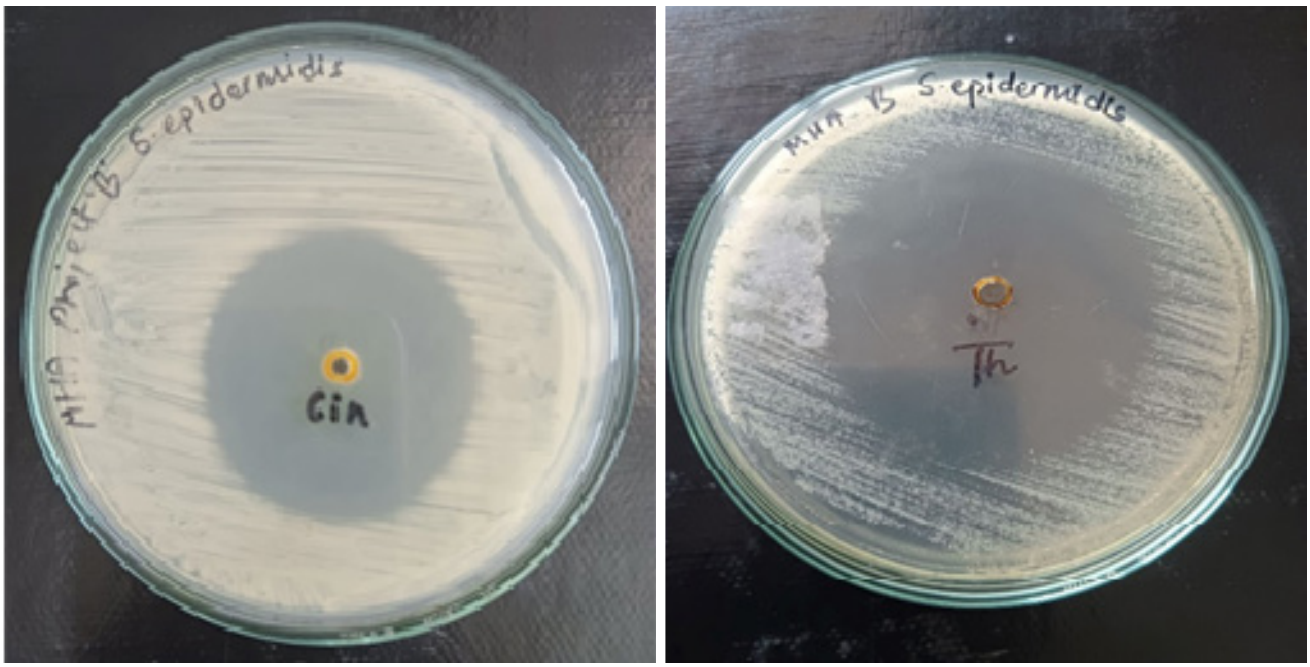
Figure 4. Graph representing MIC value of different EOs against *S. aureus*

Table 3. Antimicrobial activity of EOs in combination with carrier oils

S.N	Organisms	EOs	Zone of inhibition in combination with carrier oils (mm)		
			Mustard	Olive	Castor
1.	*E. coli*	Eucalyptus	10	9	0
2.	*E. coli* (fecal isolate from dog)	Rosemary	0	0	0
3.	*S. aureus*	Tea tree	14	11	0
4.	*S. epidermidis*	Cinnamon	25	16	19
5.	*B. subtilis* (soil isolate)	Peppermint	0	0	6
6.	*L. monocytogenes*	Cinnamon	0	18	7
7.	*Salmonella* spp. (fecal isolate from dog)	Thyme	7	6	0
8.	*S.* Typhi	Tea tree	0	0	0
9.	*P. aeruginosa*	Cinnamon	14	0	19

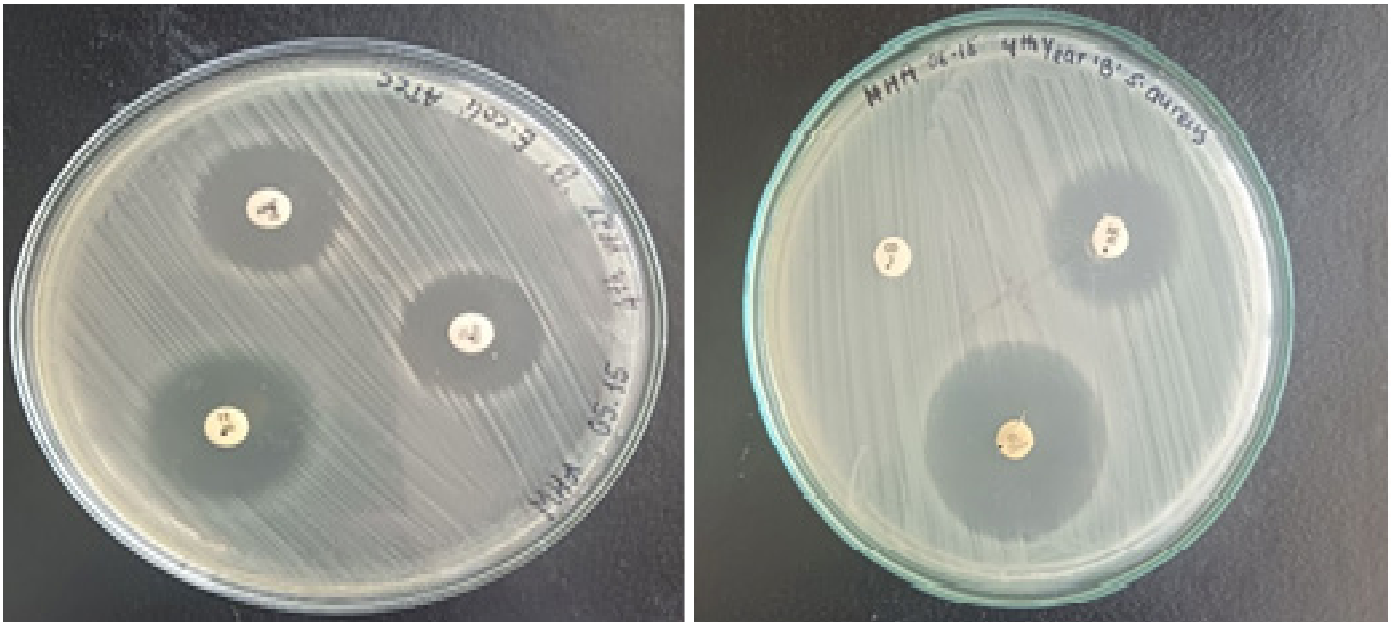
Photograph 1. Eight different commercial essential oils used in the study (lavender, rosemary, peppermint, cinnamon, thyme, eucalyptus, citrus and tea tree respectively)

Photograph 2. Zone of inhibition shown by each essential oil against *E. coli*
1= cinnamon oil, 2= citrus oil, 3= rosemary oil, 4= tea tree oil, 5= lavender oil, 6= thyme oil, 7= eucalyptus oil, and 8= peppermint oil

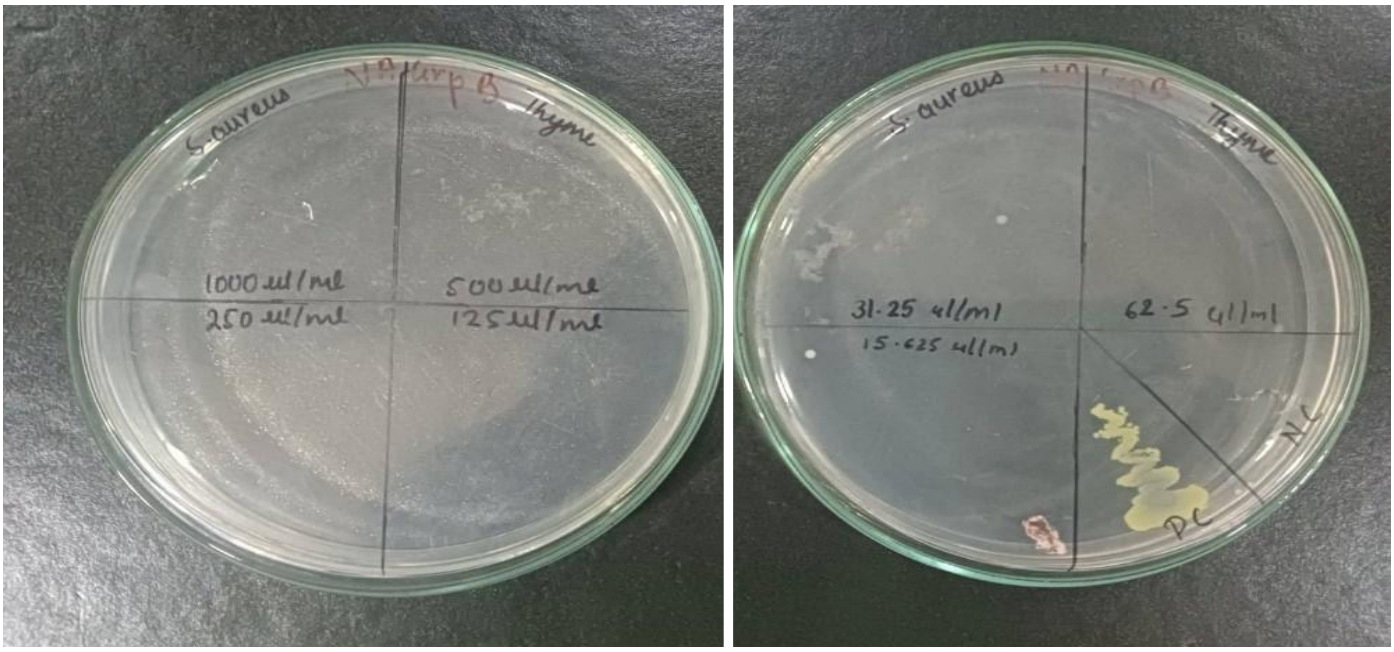


Photograph 3. Zone of inhibition shown by cinnamon and thyme oil against *S. epidermidis*

Photograph 4. Antimicrobial activity of cinnamon oil and tea tree oil in combination with mustard oil, castor oil, and olive oil against *S. epidermidis* and *S. aureus* respectively
1= mustard oil, 2= castor oil, and 3= olive oil



Photograph 5. Antibiotic susceptibility test of *E. coli* and *S. aureus* using different class of antibiotic disc



Photograph 6. Minimum bactericidal concentration of *S. aureus* against various concentrations of thyme oil

Discussion

This study reveals diverse antimicrobial activities among the tested essential oils. Thyme oil showed the most potent inhibition against various bacteria, while lavender oil exhibited limited antimicrobial activity. Thyme oil demonstrated the maximum antimicrobial activity against *S. epidermidis* (ATCC 12228) with a remarkable 46mm zone of inhibition. It exhibited strong inhibition against the highest number of bacteria among the oils tested. Additionally, it displayed moderate inhibition against several other bacteria. Thyme oil's antibacterial compounds, thymol and carvacrol, could have contributed to its effectiveness, as supported by Moreira et al. (2007). Our findings align with Boskovic et al. (2015), who reported strong antibacterial activity of thyme oil against *Staphylococcus aureus*. Lavender oil showed limited to no antimicrobial activity against the tested bacterial strains, which contrasts with the remarkable antimicrobial activities reported in studies by Yang et al. (2021) and Inouye et al. (2001).

Eucalyptus oil exhibited strong inhibition against *E. coli* (ATCC), *E. coli* (dog isolate), and *S. epidermidis* with moderate inhibition against *S.* Typhi and mild inhibition against *S. aureus* and *Salmonella* spp. However, it did not inhibit *Bacillus* and *Listeria*. Our results align with Rustagi et al. (2014), who found Eucalyptus oil to be effective against *E. coli* but less effective against *S. aureus* and *B. subtilis*.

The findings derived from evaluating the antimicrobial efficacy of essential oils bear a semblance to the outcomes of susceptibility tests conducted for antibiotics. As evidenced in Table 1, the application of thyme essential oil against *S. epidermidis* resulted in a substantial zone of inhibition measuring 46mm, while the utilization of Cefalexin, an antibiotic, against the same bacterial strain yielded a slightly smaller zone of inhibition, measuring 35mm. This juxtaposition highlights a noteworthy difference in the inhibitory effects of thyme oil as compared to Cefalexin in this specific scenario.

Similarly, when assessing the response to *Salmonella* spp., the administration of thyme oil produced a notable 42mm zone of inhibition. In contrast, when Ciprofloxacin, a commonly employed antibiotic, was utilized against the same strain of bacteria, it elicited a zone of inhibition measuring 31mm. This discrepancy underscores the superior antimicrobial potency of thyme oil when juxtaposed with Ciprofloxacin in combating infections caused by *Salmonella* spp.

The antimicrobial activity for *E.coli* ranged from non-inhibition with lavender essential oil to complete inhibition with tea tree essential

oil. The essential oils can be ranked in the order: Tea-tree> Eucalyptus=Thyme> Rosemary> Cinnamon=Citrus> Peppermint on the basis of antimicrobial activity shown. Tea tree essential oil was able to completely inhibit the growth of *E. coli* with the minimum inhibitory concentration of 15.625µl/ml among the other essential oils tested. The MIC values confirmed the overall inhibitory pattern from the screening test except eucalyptus, cinnamon, and tea tree EOs. Eucalyptus EO had the highest zone of inhibition compared to other EOs during agar diffusion assay, whereas MIC value of tea tree EO was lowest, indicating a higher antibacterial activity than eucalyptus EO during broth dilution.

Thyme has the lowest MIC value of 15.625µl/ml, indicating it to be the most effective essential oil in inhibiting the growth of *S. aureus.* The significance of the low MIC value for thyme oil is that only a small amount is needed to effectively inhibit the growth of *Staphylococcus aureus*. This indicates that thyme oil possesses potent antimicrobial properties specifically against this bacterium. The distinctive chemical composition of thyme oil, including active compounds like thymol and carvacrol, likely plays a significant role in its potent ability to inhibit *S. aureus*. The findings were similar to Sienkiewicz, et al. (2011) who performed research on Antibacterial Activity of Thyme and Lavender Essential Oils, where the value of MIC for *Staphylococcus aureus* was 15.625µl/ml, which demonstrated a good efficacy.

Conclusion

This study showed that essential oils have the ability to inhibit the growth of pathogenic microorganisms like *E. coli, S.* Typhi*, P. aeruginosa, L.monocytogenes, S. aureus,* and *S. epidermidis* and spoilage causing bacteria like *Bacillus subtilis*. This study demonstrated that the dilution of EOs with carrier oils exhibits effective inhibition against opportunistic skin microorganisms, indicating the blend to be a good source of antimicrobial agent for topical uses. Even a small concentration of tea tree essential oil of about 15.625µl/ml is sufficient to completely inhibit the growth of *E. coli,* and the same concentration of thyme essential oil inhibits the growth of *S. aureus*. New antibacterial agents are very valuable in multidrug-resistant bacteria, and this study provides additional support to the already available data to use essential oils against various bacteria. The significant antibacterial activity of the essential oils suggests that these EOs can serve as a source for compounds with therapeutic potential for topical use.

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