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SHORT COMMUNICATION

Comparative antimicrobial evaluation of synthetic antibiotics and essential oils against human pathogenic bacteria and fungi

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Abstract

In pathological conditions, surgeries, or immunodeficiency, opportunistic pathogens that normally coexist harmlessly within the human body can escalate the human system, leading to infections ranging from mild to life-threatening. Combatting such infections requires reliance on either natural or synthetic antimicrobial molecules. Plant secondary metabolites, particularly essential oils, present a potential natural remedy for addressing these infections. The study aimed to qualitatively and quantitatively assess the efficacy of eight synthetic drugs and three essential oils against various pathogenic bacteria and fungi, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, *Enterococcus faecalis*, *Malassezia furfur*, and *Candida albicans* (K4-1). Our results showed that penicillin was highly effective against *S. aureus* and *E. faecalis*, while gentamicin was effective against *S. epidermidis*. Vancomycin exhibited antimicrobial activity against all bacteria except *S. epidermidis*. Notably, clotrimazole and amphotericin-B demonstrated potent inhibition of fungal pathogens. Essential oils, particularly lemongrass displayed prominent zones of inhibition against all the examined pathogens including the resistant strains. Palmarosa oil showed substantial inhibition at a concentration of 3% v/v.

Keywords

Antibiotics, Essential oils, Fungus, Infection, Human pathogenic bacteria

Introduction

The human body harbours a vast majority of microflora that live in a mutualistic association. In an immunocompetent patient, these normal microflorae can subvert the immune system and threaten the internal organs, hence, referred to as "opportunistic pathogens" (Abers et al. 2021). A wide spectrum of bacteria, fungi, etc. fall under this terminology. Harmful microbes can be described as either strict/exclusive or opportunistic pathogens. Opportunistic pathogens are defined as pathogens that live harmoniously as normal commensals of our human body. They are considered facultative parasites that can overtake our immune system when the normal homeostasis of the body is disturbed. For instance, a diseased condition in the body, surgery, injury, some medications, malnutrition, immunodeficiency, age factors, environmental changes, etc. (Baumgartner and Xia 2003; Brown et al. 2012). They can cause relatively minor infections to even life-threatening bloodstream infections which is a major cause of concern. Some of the bacterial and fungal pathogens included are - *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acne*, *Streptococcus pyogenes*, *Escherichia coli*, *Enterococcus faecalis*, *Corynebacterium*,

Mycobacterium smegmatis, *Aspergillus fumigatus*, *Candida albicans*, etc. (Calderone and Clancy 2011).

In its role as an opportunistic pathogen, *S. aureus* has the ability to circumvent the immune system, leading to the development of soft tissue infections such as carbuncles, folliculitis, cellulitis, furuncles, along with abscesses and bacteremia (Chidambar et al. 2019). Similarly, *S. epidermidis* majorly dwells on the human skin and is prominently responsible for nosocomial infections. It is also related to infections of the implants (Cox et al. 1994; Chouhan et al. 2017). A gastrointestinal tract commensal *E. faecalis* (Domingo and Fontanet 2004; Deb et al. 2022) can cause infections of the oral cavity (Ergan et al. 2004) like those of occluded root canal (Gemeda et al. 2018) periodontitis (Ingham and Cunningham 1993) UTIs, nosocomial infections (Kalemba and Kunicka 2005), etc. A species of *Mycobacterium* called *M. smegmatis* which is not linked with causing tuberculosis in humans (Kayaoglu and Orstavik 2004) has the potential to cause soft tissue infections (Khaliq et al. 2023) pulmonary infections (in case of lipoid pneumonia) (Kibbler et al. 2003). Two of the dimorphic and polymorphic fungal pathogens taken into account are *Malassezia furfur* (Kim and Sudbery 2011) and *C. albicans* (McBride et al. 2007). *C. albicans*, an exclusively opportunistic pathogen, is well known for causing a range of infections such as oral, cutaneous, vulvovaginal candidiasis (Meliani et al. 2013; Naik et al. 2010), candidaemia (Owens et al. 1997), nosocomial infections (Poirel et al. 2011; Puvaca et al. 2021; Rabbani et al. 2024). To combat these infectious diseases, one has to depend on commercially available generalized narrow or broad-spectrum antibiotics. Various antimicrobial drugs administered nowadays belong to classes of β-lactams, aminoglycosides, sulfonamides, glycopeptides, quinolones, ansamycins, pyrimidine analogs, azoles, polyenes, etc. Nevertheless, the use of antibiotics carries risks that may result in harmful effects on essential organs (Rocas et al. 2004). Also, the indiscriminate use of conventional antimicrobials has led to the emerging phenomenon of antibiotic resistance and other complications in the human body. In an attempt to subside this concern, alternative agents can be employed to keep such unforeseen side effects at bay. For this purpose, secondary metabolites of plants like essential oils can serve as potential substitutes for synthetic antimicrobials.

Essential oils have long been used traditionally in perfumery, cosmetics, food preservation, aromatherapy, alleviating colds, etc. Also, they are commonly recognized for their diverse and advantageous pharmacological impacts including antibacterial, antifungal, antiviral, anti-inflammatory, antioxidant, anticancer, and insecticidal properties (Sharma et al. 2019; Sharma et al. 2021). As a matter of fact, essential oils have shown antimicrobial activity against a wide range of food borne and human pathogens (Shaukat et al. 2023; Silva et al. 2011; Singh et al. 2011). Their mode of action mainly relies on cell membrane disruption, and interference in enzymatic activity, cellular respiration or DNA/RNA synthesis. Such natural plant derived therapeutics pose minimal risk to the development of resistance. Therefore, essential oils, due to their low toxicity, economic viability, and biodegradability, may be better suited as medications in contrast to conventional antibiotics (Soleimani et al. 2022).

In the present evaluation of the antimicrobial efficacy of eight selected antibiotics/antifungals and three essential oils has been carried out against *S. aureus*, *S. epidermidis*, *M. smegmatis*, *E. faecalis*, *M. furfur*, and *C. albicans* and initial results have been presented.

Materials and methods

All the bacterial pathogens were procured from HiMedia laboratories (Lucknow, India). The fungal pathogenic strain *M. furfur* was isolated from human scalp while *C. albicans* K4-1 was a clinically isolated strain. The generalized antimicrobial drugs such as penicillin, gentamicin, vancomycin, rifampicin, kanamycin, ampicillin, clotrimazole, and amphotericin-B that were obtained from Cipla Pharmaceuticals, India. The three essential oils viz. Lemongrass (*Cymbopogon flexuosus*), Palmarosa (*Cymbopogon martinii*) and Peppermint (*Mentha piperita*) were procured from CSIR-Central Institute of Medicinal and Aromatic Plants (Lucknow, India).

Disk diffusion assay

On a fresh nutrient or Sabouraud agar plate, 100 µL of the prepared microbial suspension was evenly spread with the help of a glass spreader. A few 5 mm filter paper (Whatmann no. 1) disks were placed on agar plates which were later loaded with 5 µg (5 µl) of the standard antimicrobials. Following incubation at 37 °C for 24 hours and 28 °C for 48 hours for bacterial and fungal pathogens, respectively clear zones of growth inhibition were measured in millimetres. The mean of inhibition zone were determined in triplicates. Similarly, 30 µl of essential oil per 5 mm disc was applied and zone of inhibition was determined in triplicate.

Minimum inhibitory concentration (MIC)

The MIC for all the bacterial and fungal pathogens was achieved by the broth dilution method in a 96-well microtiter plate. Broth cultures were prepared for all pathogens in their respective media which were adjusted to 0.5 McFarland standard turbidity to obtain 1×10^8 Colony Forming Units (CFU)/ml. In order to achieve a concentration of 50 µg/ml, 15 µL of the selected antimicrobial drugs from the stock solution of 1 mg/mL were used for MIC determination. The well volume was then made up to 300 µL. Following a two-fold dilution series, 10 µL of the microbial suspension was added to each well except the negative control. The plates were then incubated at 37 °C for 24 hours (bacterial pathogens) or 28 °C for

48 hours (fungal pathogens). After the incubation period, the visual representation of MIC was done by a redox indicator dye that changed its color from purple to pink in the presence of the cellular reductases. The experiment was repeated thrice in duplicates and mean values of MIC were determined.

The *in vitro* antimicrobial activity of essential oils from lemongrass (*C. flexuosus*), palmorosa (*C. martinii*), and peppermint (*M. piperita*), essential oils was assessed by evaluating the in vitro inhibition of the selected bacterial and fungal pathogens following the method described previously by other authors with some modifications [38]. All the oils were dissolved in tween 80 with 3% (v/v oil). MIC was determined by the broth microdilution assay. Broth cultures of all the pathogens were prepared in their respective media and their concentrations were adjusted to 0.5 McFarland standard turbidity for obtaining 1×10^8 Colony Forming Units (CFU)/ml. The plates were then incubated at their respective temperatures and time as described earlier. Each experiment was carried out in duplicates.

Minimum bactericidal/fungicidal concentration (MBC/MFC)

The MBC/MFC for all the bacterial and fungal pathogens was deduced via spot inoculation onto a fresh agar plate. The last three wells of each row of antibiotic/antifungal/essential oil from the MIC plate were chosen for this process. The plates were divided into quadrants and each section corresponded with the well coordinates. Following incubation for the respective bacterial and fungal pathogens, the MBC/MFC was allocated to the quadrant showing no bacterial/fungal growth.

Statistical analysis

All the test were performed in triplicates and the obtained data was statistically analysed by a statistical software Minitab™ version 20 (Minitab, LLC; PA, USA). The results were presented as the mean of the triplicate values along with standard deviation and 5% statistical significance ($p < 0.05$) through Fisher's test.

Results and discussion

Disk diffusion of antibiotics and essential oils

The study evaluated the antimicrobial efficacy of commercially available drugs and essential oils against the listed bacterial and fungal strains that included Gram-positive bacteria *S. aureus*, *S. epidermidis*, *M. smegmatis*, *E. faecalis*, and fungi *M. furfur* and *C. albicans* (K4-1), respectively. The selected bacteria are particularly known for causing infectious diseases like folliculitis, cellulitis, impetigo, bacteraemia, and endocarditis whereas the fungal pathogens are linked to skin conditions like dandruff, seborrheic dermatitis, pityriasis versicolor, etc. (Van Tyne et al. 2013). Our results indicated that none of the antibiotics was commonly effective against all the tested bacterial pathogens but inhibited only *S. aureus* and *E. faecalis*. Penicillin maximum zone of inhibition (ZOI) in *S. aureus* and *E. faecalis* as compared to gentamicin, rifampin, kanamycin, vancomycin, and ampicillin (Table 1). The growth of *S. aureus* was highly suppressed by penicillin (40.0 mm), rifampicin (32.0 mm) and ampicillin (32.3 mm). Similarly, the growth inhibition of *E. faecalis* was found highest towards penicillin (30 mm) (Fig. 1). The order of inhibition for *S. epidermidis* was gentamicin > kanamycin > rifampicin. However, *S. epidermidis* exhibited no activity with penicillin, vancomycin as well as ampicillin. Furthermore, penicillin and ampicillin were found inactive against *M. smegmatis*. Two sets of pathogens, *S. aureus* and *E. faecalis*, as well as *S. epidermidis* and *M. smegmatis* exhibited nearly close ZOI to each other with gentamicin (23.7 mm and 24.0 mm, respectively). A similar trend was also observed with the pathogens *S. aureus* and *S. epidermidis* (15.0 mm and 15.3 mm, respectively) as well as the pathogens *E. faecalis* and *M. smegmatis* (19.0 mm for both) with the antibiotic kanamycin. The antibiotic vancomycin showed its activity against three of the four tested pathogens but proved ineffective against *S. epidermidis*. Among the fungal pathogens, *M. furfur* and *C. albicans*, clotrimazole proved to be highly effective against both the selected pathogens giving nearby ZOI values for each (34.0 mm and 32.0 mm, respectively). Similarly, amphotericin-B exhibited a similar trend in the ZOI values against these two pathogens (17.5 mm and 18.0 mm, respectively) (Fig. 2).

Table 1. Zone of inhibition (mm) developed by different antibiotics and essential oils against human pathogenic bacteria and fungi.

	Penicillin*	Gentamicin	Vancomycin	Rifampin	Kanamycin	Ampicillin	Amphotericin-B	Clotrimazole	Lemongrass#	Palmarosa	Peppermint
S. aureus	40.0** ± 0	23.7 ± 1.15	16.0 ± 0	32.0 ± 0	15.0 ± 0	32.3 ± 0.58	-	-	12.7 ± 0.58	8 ± 0	10 ± 1
S. epidermidis	0.0 ± 0	19.3 ± 0.58	0.0 ± 0	13.0 ± 0	15.3 ± 0.58	0.0 ± 0	-	-	20.7 ± 0.58	14 ± 1	9.3 ± 0.58
E. faecalis	33.0 ± 0	24.0 ± 0	17.0 ± 1	19.7 ± 0.53	19.0 ± 0	18.7 ± 0.58	-	-	10.3 ± 0.58	10 ± 0	10 ± 0
M. smegmatis	0.0 ± 0	19.7 ± 0.58	11.0 ± 1	11.3 ± 0.58	19.0 ± 0	0.0 ± 0	-	-	30.3 ± 0.58	7.3 ± 0.58	31.7 ± 0.58
M. furfur	-	-	-	-	-	-	17.5 ± 0.707	34 ± 0	27.0 ± 0.58	16.7 ± 0.58	13.7 ± 0.58
***C. albicans* (clinical)**	-	-	-	-	-	-	18 ± 0	32 ± 2.828	28.3 ± 0.58	13.3 ± 0.58	12 ± 0

*5 µg/ml, **mean of triplicate of each value, #3% v/v.

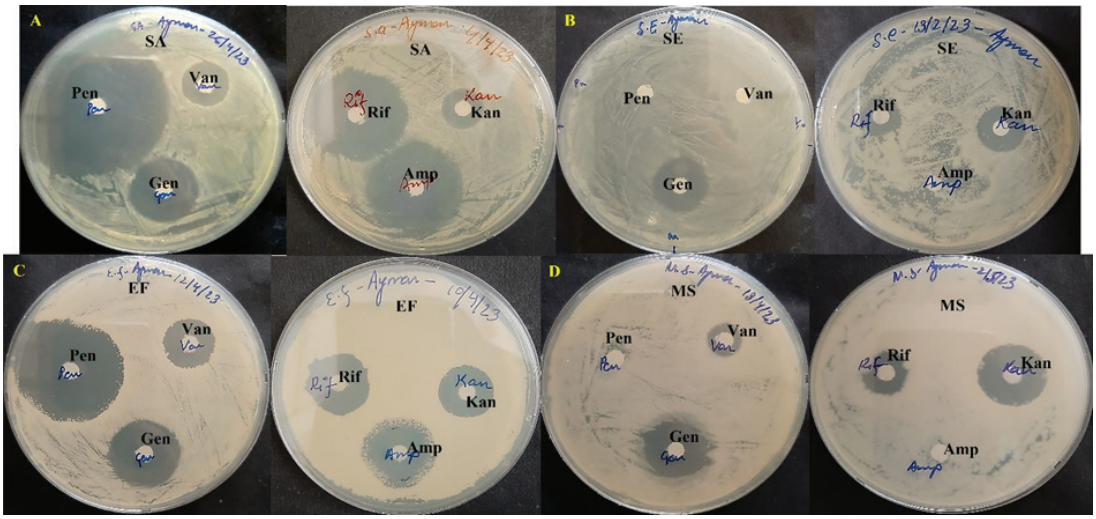

Figure 1. Zone of inhibition by antibiotics: (Pen: Penicillin, Van: Vancomycin, Gen: Gentamicin, Rif: Rifampicin, Kan: Kanamycin: Amp: Ampicillin) against pathogenic bacteria. **A.** SA (*S. aureus*); **B.** SE (*S. epidermidis*); **C.** EF (*E. faecalis*); **D.** MS (*M. smegmatis*); **E.** MS (*M. smegmatis*); **F.** CA (*C. albicans*).

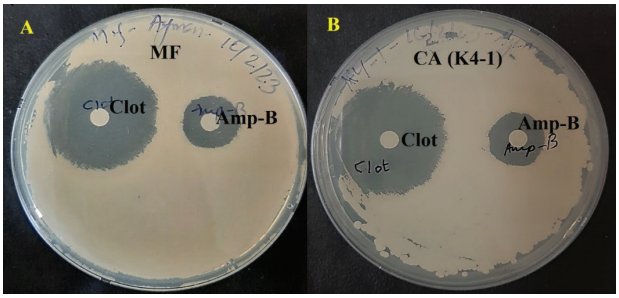

Figure 2. Zone of inhibition by antifungals: (Clot: Clotrimazole, Amp-B: Amphotericin-B) against pathogenic fungi. **A.** MF (*M. furfur*); **B.** CA (*C. albicans*).

Parallelly, the disc diffusion assay results were found to be significant in the case of *C. flexuosus* giving prominent ZOI for each of the selected bacterial and fungal pathogens (Fig. 3). Prominent zones of inhibition (27.0 mm and 28.3 mm) were noted for *M. furfur* and *C. albicans*. Similar findings have been reported by Van Vuuren and Holl (2017) who evaluated antimicrobial activity of *Cymbopogon* oil against three Gram negative and positive bacteria and two fungi. The essential oil of *C. martinii* displayed highest zone of inhibition activity against *M. furfur* (16.7 mm) as compared to all other pathogens and produced smaller zones of inhibition, measuring 8.0 mm, 10.0 mm, and 7.3 mm against *S. aureus*, *E. faecalis*, and *M. smegmatis*, respectively. The inhibitory effect of *M. piperita* essential oil was maximum against *M. smegmatis* (31.7 mm), whereas minimum inhibitory activity (10 mm) was observed against *S. aureus* and *E. faecalis*. Our findings were found similar to a study reported by Verma et al. (2011).

MIC/MBC of Antibiotics and Essential oils

Taking 50 µg/ml of each drug and following a two-fold dilution series, the most efficacious drugs required in

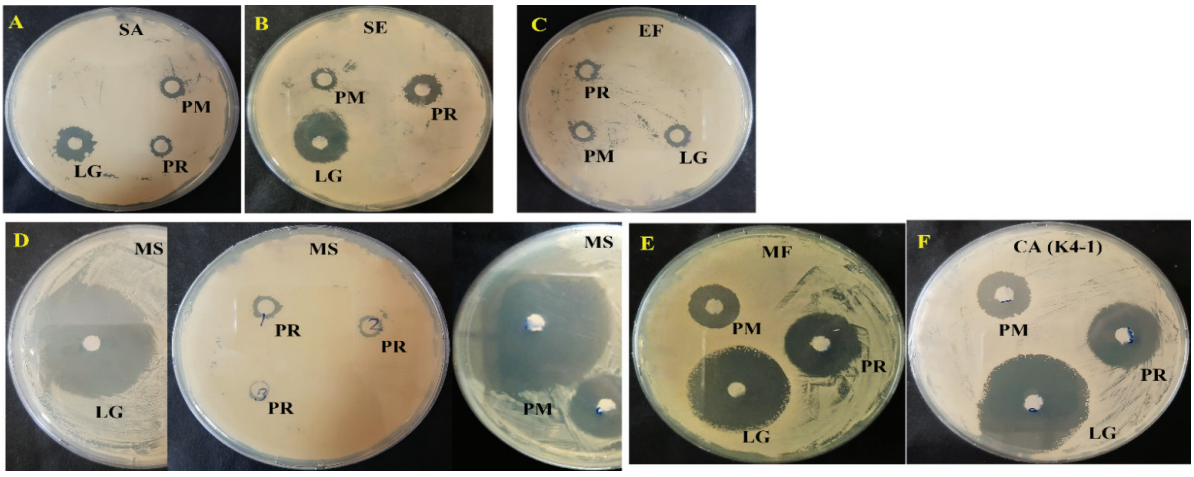

Figure 3. Zone of inhibition showing antimicrobial activity of essential oils: (LG: Lemongrass, PR: Palmarosa, PM: Peppermint) against human pathogenic bacteria and fungi. **A.** SA (*S. aureus*); **B.** SE (*S. epidermidis*); **C.** EF (*E. faecalis*); **D.** MS (*M. smegmatis*); **E.** MF (*M. furfur*); **F.** CA (*C. albicans*).

minimum quantity Our results showed that at MIC 0.10 µg/ml, penicillin and rifampicin inhibited the growth of *S. aureus* and *E. faecalis*. Similarly, gentamicin suppressed the growth of *S. epidermidis* and *M. smegmatis* at MIC 0.10 µg/ml. Kanamycin had a higher MIC (0.39 µg/ml) than penicillin (0.10 µg/ml) to restrict the growth of *S. epidermidis*. Vancomycin was effective at a slightly higher concentration for all the pathogens except *E. faecalis*. MIC values were found to be at par for rifampicin (0.10 µg/ml) in case of *S. aureus*, *S. epidermidis* and *E. faecalis*. Similarly, *S. aureus*, and *M. smegmatis* had equal MIC values for kanamycin (1.56 µg/ml) also. The favourable drug to suppress the growth of *M. smegmatis* was found to be gentamicin (MIC 0.10 µg/ml) in this study.

Likewise, clotrimazole showed antimicrobial activity at (MIC 0.10 µg/ml for *M. furfur* and *C. albicans*, while amphotericin-B was required at a higher concentration for growth inhibition and death of both the fungal pathogens (MIC 3.12 & 0.10 µg/ml respectively).

In a parallel investigation, when assessing the antimicrobial efficacy of essential oils, specifically *C. martini*, yielded the most favorable results against a variety of tested bacterial pathogens. The MIC for this oil ranged from 0.07% to 0.09% v/v. Our result was consistent with those of previous studies done by Vuong and Otto (2002). Similarly, the essential oil derived from M piperita demonstrated significant effectiveness, particularly against *S. aureus*, with an MIC value of 0.07% v/v. It also exhibited positive results against *S. epidermidis* (MIC 0.09% v/v), *E. faecalis* (MIC 0.09% v/v), and *M. smegmatis* (MIC 0.19% v/v). Almost similar efficacy was reported in a study reported by Wang et al. (2022). On the contrary the essential oil from *C. flexuosus* required slightly higher concentrations, to inhibit the growth of all the tested pathogens (MIC 0.19% v/v). Notably, the MBC values aligned with the MIC values for these bacterial pathogens. Moreover, the investigation extended to two fungal pathogens, and the essential oils from *C. martinii* and *M. piperita* demonstrated their capability to inhibit both pathogens at a concentration of 0.3% v/v of MIC/MFC, while *C. flexuosus* achieved similar inhibition at a concentration of 0.12% v/v of MIC/MFC (Table 2). These values almost coincide with the results reported by Vuong and Otto (2022).

The comparison of essential oils with antibiotics in the context of antimicrobial activity is a subject of growing interest in the field of microbiology as well as medical research. As essential oils are natural extracts from plants that contain a wide variety of bioactive compounds, including terpenes and phenolic compounds, that can have antimicrobial properties (Watts et al. 2018). In contrast, antibiotics are either synthetic or semi-synthetic compounds designed specifically to target and kill or inhibit the growth of bacteria which in due course of time can develop resistance by altering the target of the antibiotic or by developing efflux pumps to expel the drug (Wisplinghoff et al. 2004).

Conclusion

Despite the broad-spectrum activity of synthetic antibiotics, the problem of antibiotic resistance still remains a challenge to the global healthcare system in many developing nations. The rise and dissemination of multidrug-resistant pathogens have posed a serious threat to the existing antibacterial treatment. Hence, to avert this concern, natural derivatives such as essential oils have been considered as alternatives to the synthetic antimicrobials. Bioactive compounds like terpenes and terpenoids present in essential oils have bacteriostatic and bactericidal effects on various pathogens. However, the degree of antimicrobial potency of essential oils cannot surpass the standard antibiotics. On the basis of our findings, we concluded that even though differing concentrations of antibiotics and essential oils were taken into account, the antimicrobial efficacy of essential oils managed to align with synthetic drugs. Having said this, all the essential oils taken inhibited the growth of all the tested pathogens to some

Table 2. Minimum Inhibitory Concentration (MIC) and Minimum Bacterial/Fungicidal Concentration (MBC/MFC) of selected human pathogenic bacteria and fungi against different antibiotics and essential oils.

	Penicillin*		Gentamycin		Vancomycin		Rifampicin		Kanamycin	
	MIC**	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	0.09 ± 0	0.09 ± 0	0.58 ± 0.19	0.78 ± 0	1.56 ± 0	1.56 ± 0	0.09 ± 0	0.09 ± 0	1.56 ± 0	1.56 ± 0
S. epidermidis	-	-	0.09 ± 0	3.12 ± 0	-	-	0.09 ± 0	0.09 ± 0	0.39 ± 0	3.91 ± 3.31
E. faecalis	0.09 ± 0	0.39 ± 0	0.29 ± 0.14	0.39 ± 0	0.39 ± 0	1.56 ± 0	0.09 ± 0	0.78 ± 0	1.04 ± 0.45	6.25 ± 0
M. smegmatis	-	-	0.09 ± 0	1.56 ± 0	3.12 ± 0	25 ± 0	0.78 ± 0	3.12 ± 0	1.56 ± 0	12.5 ± 0
M. furfur	-	-	-	-	-	-	-	-	-	-
***C. albicans* (clinical)**	-	-	-	-	-	-	-	-	-	-

	Ampicillin		Amp-B		Clot		Lemongrass#		Palmarosa		Peppermint	
	MIC	MBC	MIC	MFC	MIC	MFC	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	0.09 ± 0	0.09 ± 0	-	-	-	-	0.19 ± 0%	0.19	0.07 ± 0.03%	0.07	0.07 ± 0.03%	0.07
S. epidermidis	-	-	-	-	-	-	0.19 ± 0%	0.19	0.09 ± 0%	0.09	0.09 ± 0%	0.09
E. faecalis	0.19 ± 0	0.39 ± 0	-	-	-	-	0.19 ± 0%	0.19	0.09 ± 0%	0.09	0.09 ± 00%	0.09
M. smegmatis	-	-	-	-	-	-	0.19 ± 0%	0.19	0.09 ± 0%	0.09	0.19 ± 0%	0.19
M. furfur	-	-	3.12 ± 0	25 ± 0	0.09 ± 0	0.19 ± 0	0.12	0.12	0.3	0.3	0.3	0.3
***C. albicans* (clinical)**	-	-	3.12 ± 0	12.5 ± 0	0.09 ± 0	0.39 ± 0	0.12	0.12	0.3	0.3	0.3	0.3

*50 µg/ml, **mean of duplicate of each value, #3% v/v.

degree, contrary to a few antibiotics that failed to suppress the proliferation of some pathogens. In fact, lemongrass essential oil depicted the most favourable results giving distinct ZOIs (12.7–30.3 mm) for the majority of the tested pathogens while also being efficient towards resistant pathogens. *M. smegmatis* was found resistant towards penicillin and ampicillin but its growth was prominently inhibited by lemongrass and peppermint essential oils. A similar pattern was seen against *S. epidermidis*, although peppermint oil showed very low inhibition. Additional research under *in vivo* conditions is needed to thoroughly assess the full therapeutic potential of these investigated essential oils that could provide valuable insights into potential treatment as substitutes against relevant diseases.

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Conflicts of Interest:

The authors declare no conflict.

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