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# Inhibitory effect of 14 essential oils against resistant pathogenic bacteria and fungi and chemical compositions of selected essential oils

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#### **ABSTRACT**

Current antimicrobial agents have become useless against multidrug-resistant pathogens. There is a necessity to discover novel antimicrobial compounds to combat these pathogens. The global trend promotes a greener and more sustainable alternative, such as essential oils (EOs). EOs are complex volatile bioactive compounds, which assist plants in the defense against pests and pathogens. A total of 14 EOs were tested for antibacterial and antifungal activity against resistant pathogens. The thyme EO completely inhibits the plant pathogenic fungi at all concentrations (100% inhibition), followed by cinnamon which completely inhibits all plant pathogens at 500 and 1,000 µl/l concentration (100% inhibition). While lemongrass was the most active EO against all bacteria except for *Enterococcus caecium*, it had the highest zone of inhibition (22 mm) against *Mannheimia haemolytica*. Tea tree was the second active EO. Thyme was the most sensitive EO against all pathogenic bacteria except for *Listeria monocytogenes* (ATCC 19115) and *Salmonella enterica*. In conclusion, there is strong evidence that EOs provide a suitable sustainable alternative to conventional therapeutic agents, which could decrease the minimum effective dose of the drugs, thus reducing their possible adverse effects and the costs of treatment.

#### INTRODUCTION

Essential oils (EOs) are natural, volatile complex bio-compounds that have distinctive strong fragrances and are produced by aromatic plants [1–4]. Previously used for aromatherapy, flavoring agents, or preservatives in food, EOs are now predominantly used to treat a variety of infections as they possess antibacterial and antifungal activities [5–7]. The antimicrobial activity of EOs involves among others, the disruption of the cell membrane of microorganisms, interfering with their metabolic processes or inducing oxidative stress. This ability to exert an antimicrobial property is due to the presence of a wide range of bioactive compounds such as terpenoids, phenolics, and aldehydes [8]. Indeed, there are

\*Corresponding Author Madira Manganyi, Department of Biology and Environmental Sciences, Sefako Makgatho Health Sciences University, Pretoria, South Africa. E-mail: mmanganyi @ wsu.ac.za numerous *in vivo* and *in vitro* studies conducted that have shown different responses of microbes on treatment with EOs [9,10]. The biosynthesis of the bioactive compounds in the EOs is a complex and dynamic process that involves the coordinated action of multiple enzymes and pathways. The specific composition and chemical properties of EOs are determined by a combination of genetic, environmental, and developmental factors. Furthermore, there are variations in exhibiting this activity as different amounts of specific bioactive compounds vary and are widely dependent on the plant species, the part of the plant from which the oil is extracted, and the extraction method used.

However, there are few scientific studies validating the bacterial inhibition potential of these commercially available EOs. A body of evidence has shown that EOs consist of bioactive compounds which play an important role in exhibiting beneficial activities such as antiviral, antifungal, as well as antibacterial against resistant pathogens [11–14]. Current antimicrobial agents, which are regarded as the foundation

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of contemporary clinical medicine, are seriously threatened by the emergence of antimicrobial resistance in a number of bacteria [14]. The agricultural sector is no exception, to prevent food losses brought on by fungi, farmers today rely heavily on synthetic fungicides. The fungicide-resistant pathogens have also emerged, and concerns have been raised about their longterm effects on the environment and public health [15]. Drawing on this, the global trend is toward greener and more sustainable approaches to tackling these issues.

Similar studies were conducted previously [16,17] that reported on the antimicrobial potential of EOs. This study, therefore, aims to investigate the antimicrobial potential of commercially available 14 plant EOs—clove bud (Syzygium aromaticum), lemongrass (Cymbopogon citratus), Rosemary (Rosmarinus officinalis), Ylang-ylang (Cananga odorata), Cinnamon (Cinnamomum zeylanicum), Geranium (Pelargonium graveolens), Tea tree (Melaleuca alternifolia), Myrrh (Commiphora myrrha), Eucalyptus (Eucalyptus globulus), Peppermimt (Mentha piperita), Sweet basil (Ocimum basilicum), Thyme (Thymus vulgaris), Sage (Salvia officinalis), and Camphor (Cinnamomum camphora) on inhibition of Botrytis cinerea, a necrotrophic fungus, Fusarium oxysporum, a Gramnegative bacteria, Fusarium graminearum, a phytopathogenic fungus, and Collectotrichum gleosporoides, a fungus pathogen. Figure 1 represents a visual summary of the main findings of this research paper.

#### MATERIAL AND METHODS

# Selection and purchasing of EOs

A total of 14 EOs were purchased from a reliable supplier in Johannesburg, South Africa. The selection of EOs was based on the literature on the antimicrobial effect of EOs against pathogenic microorganisms.

## Selection of plant pathogenic fungi

A total of four plant pathogenic fungi were obtained from the South African National Collection of Fungi, Mycology Unit, Biosystematics Division, Plant Collection Institute (PPRI), Agricultural Research Council located at KwaMhlanga road, Pretoria, South Africa. Table 1 shows plant pathogenic fungi used in this study, their host, and geographic location.

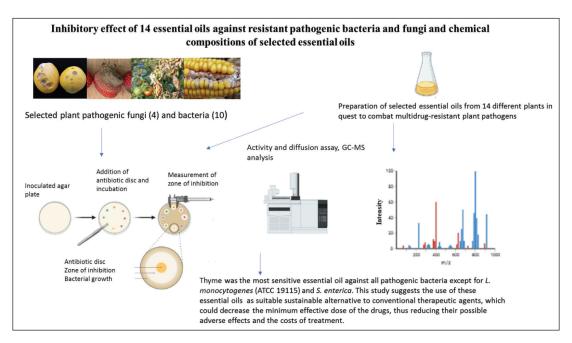
## Selection of pathogenic bacteria

A total of 10 different bacteria, 6 Gram-positive and 4 Gram-negative genera, were used in this study. The Gram-positive strains were *Bacillus cereus* (ATCC 19115), *Enterococcus faecalis* (ATCC 29212), *Enterococcus faecium* (ATCC 700221), *Listeria monocytogenes* (ATCC 19115), and *Enterococcus gallinarum* (ATCC 700425). Gram-negative bacteria included *Salmonella enterica* (MG663463), *Mannheimia haemolytica*, and *Escherichia coli* 0177 (ATCC 0177) as listed in Table 2. They were all collected from a reliable source. They were all stored in a refrigerator. These bacteria were selected based on their problematic effect on human health and the fact that they cause outbreaks and spread in hospitals.

# Antifungal activity (Toxic medium assay)

Potato dextrose agar (PDA) was prepared aseptically and supplemented with Tween-20 (200  $\mu$ l) as a surfactant. The EOs were evaluated at concentrations of 300, 500, and 1,000 ppm. The agar was poured into 90 mm Petri dishes to solidify. A 5 mm agar plug of fungal mycelia was inoculated in the center of each PDA plate. After 10 days, the mycelial growth was observed and measured (mm) with a ruler. Each test isolate and control were prepared in triplicates. The percentage inhibition of mycelial growth was determined according to the formula [18].

% inhibition =  $(C-T/C) \times 100 (1)$ 



Figur e 1 Visual summary of the main findings of this research paper.

**Table 1.** The pathogenic fungi used in this study and their host.

PPRI no.	Fungal name	Host/substrate	Locality
13071	Botrytis cinerea	Chrysanthemum flower	Gauteng, Tarlton
2929	Fusarium oxysporum	Wheat	Free states
10139	Fusarium graminearum	Maize	North West
12517	Collectotrichum gleosporoides	Papaya	Mpumalanga, Nelspruit

**Table 2.** Shows pathogenic bacteria used in this study and their sources.

Bacteria		Source
Listeria monocytogenes	Control	ATCC 19115
Listeria monocytogenes	Environmental	Water
Bacillus cereus	Control	ATCC 10876
Mannheimia haemolytica	Environmental	
Enterococcus faecium	Control	ATCC 700221
Salmonella enterica	Control	MG663463
Salmonella enterica	Environmental	
Enterococcus faecalis	Control	ATCC 29212
Enterococcus gallinarum	Control	ATCC 700425
Escherichia coli 0177	Environmental	ATCC 0177

where C = growth in control plates and T = growth in test plates.

#### Antibacterial activity using disc diffusion assay

The antibacterial effect of tested EOs was determined by disc diffusion assay [19]. Bacterial species were spread in agar plates using sterile earbuds. Filter paper discs were soaked with each EO. The bacterial cultures with discs soaked with EOs were incubated at 37°C for 24 hours, depending on the indicator microorganism. After incubation, the inhibition zones were measured. All experiments were performed in triplicate, and the results are presented as an average of three replications.

# Gas chromatography mass spectrometry (GC-MS) analysis

An Agilent 6890N gas chromatography (GC) system was used to analyze the EO composition while being connected directly to a 5973 MS (Agilent Technologies, Santa Clara, CA). Using a split ratio of 200:1, 1 µl of the sample was injected at a pressure of 24.79 psi and a temperature of 250°C. An HP-Innowax polyethylene glycol column measuring 60 m by 250 m and having a film thickness of 0.25 m was installed in the GC system (Agilent Technologies, Santa Clara, CA). Initially set to 60°C for 10 minutes, the oven temperature program increased to 220°C at a rate of 4°C/minute and held for 10 minutes before increasing to 240°C at a rate of 1°C/minute. As a carrier gas, helium was used at a constant flow rate of 1.2 ml/minute. Electron impact at 70 eV was used to acquire spectra while scanning from 35 to 550 m/z. The MarkerLynxTM application manager software was used to import the exported GC-MS chromatograms and perform peak alignment and selection [20].

#### Statistical analysis

The results were expressed as the mean of the data obtained in each replicate after each analysis was carried out in triplicate.

#### RESULTS AND DISCUSSION

## **Antifungal activity**

It can be clearly seen that the antifungal effect of EOs increased with their increasing concentration until it reached the maximum diameter of the inhibition zone. Table 3 outlines the antifungal activities of EOs on plant pathogenic fungi. Thyme EO inhibited all the pathogenic fungi at different concentrations (100% inhibition), followed by cinnamon at 500 and 1,000 μl/l concentration (100% inhibition). Clove bud EO also showed an inhibitory effect against understudied pathogens fungi at 1,000 µl/l (100% inhibition). The findings also showed that the sage EO has a low inhibitory effect at all concentrations, with an inhibitory effect that is less than 40% at 300 μl/l. These results are supported by Hu *et al.* [21], who also found that cinnamon EO has the strongest antifungal activity against plant pathogenic fungi. Numerous studies demonstrate that thyme EO has antifungal effects [22-25]. The antimicrobial activity of EOs depends on their chemical constituents. The antimicrobial activity of thyme EO is related to the presence of phenolic compounds such as thymol and terpene hydrocarbons (γ-terpinene) because they are lead compounds. Due to their lipophilic nature and low molecular weight, these compounds can cause structural and functional damage in the cells of organisms by disrupting the membrane permeability and osmotic balance of the cell, inhibiting the activity of certain enzymes, and interfering with ergosterol biosynthesis [26].

Results displayed that *F. graminearum* is more resistant to most of the EOs (39%), followed *F. oxysporum* (26%). *Collectotrichum gleosporoides* is the most sensitive or least resistant plant pathogenic fungi (14%), followed by *Botrytis cinearea* (21%). These findings are against the results of Perczak *et al.* [19] where they found that *F. graminearum* is more susceptible to oregano and cinnamon EOs. Inhibitors of deoxynivalenol production by *F. graminearum* are useful for protecting crops from deoxynivalenol contamination [27]. These results were similar to Hong *et al.* [28] findings where the *C. gleosporoides* was more sensitive to cinnamon EO. Krzyśko-Łupicka *et al.* [26] identified horizontal gene transfer (HGT) process within Fusarium. HGT is an important mechanism of eukaryotic genome evolution, particularly in unicellular organisms.

Table 4 shows the results on the antibacterial activity of EOs against pathogenic bacteria. Lemongrass was the most active EO against all pathogenic bacteria except for *E. faecium*. It had the highest zone of inhibition (22 mm) against *M. haemolytica*. Tea tree was the second active EO against all pathogenic bacteria except for *E. faecium*. It had the second-highest zone of inhibition (21 mm) against *L. monocytogenes*. Thyme was the most sensitive EO against all pathogenic bacteria except for *L. monocytogenes* (ATCC 19115) and *S. enterica*, as shown in Table 1. These findings

**Table 3.** Inhibitory effect (in %) of all selected EOs on plant pathogenic fungi.

FO1/I*	Plant pathogenic fungi			
EOs μl/l*	13071	2929	12517	10139
		Inhibitory effect	(%)	
		Peppermint		
300	$39.47 \pm 1.70$	$31.11 \pm 1.10$	$36.90 \pm 0.25$	$24.44 \pm 0.50$
500	$47.37 \pm 0.20$	$23.33 \pm 0.40$	$48.80 \pm 1.00$	$44.44 \pm 0.20$
1,000	$52.63 \pm 0.50$	$53.33 \pm 1.00$	$59.52 \pm 1.20$	$66.67 \pm 0.50$
	,	Geranium		
300		$23.33 \pm 0.33$	$41.67 \pm 0.10$	-
500	$21.05 \pm 0.25$	$44.44 \pm 0.10$	$47.62 \pm 0.46$	$0.00 \pm 0.55$
1,000	$65.79 \pm 0.80$	$55.56 \pm 0.50$	$99.17 \pm 0.10$	$0.00 \pm 0.25$
		Sage		
300	1.31 ± 1.15		$38.10 \pm 0.35$	
500	$14.47 \pm 0.04$	$8.89 \pm 0.25$	$52.38 \pm 0.15$	_
1,000	$21.05 \pm 0.75$	$16.67 \pm 0.33$	$60.71 \pm 0.25$	_
	21.03 = 0.73	Sweet basil	00.71 = 0.23	
300	$48.68 \pm 0.05$	$16.67 \pm 0.11$	$38.10 \pm 0.20$	
500	$52.63 \pm 0.03$	$22.22 \pm 1.00$	$47.62 \pm 0.50$	_
1,000	$52.03 \pm 0.01$ $55.26 \pm 0.25$	$38.89 \pm 0.05$	$58.33 \pm 0.02$	-
1,000	33.20 ± 0.23	Rosemary	38.33 ± 0.02	
200		$11.11 \pm 0.34$	$53.57 \pm 0.5$	
300	11.04 + 0.60			-
500	$11.84 \pm 0.60$	$12.22 \pm 0.08$	$54.76 \pm 0.5$	-
1,000	$14.47 \pm 0.11$	$12.22 \pm 0.25$	$64.29 \pm 0.15$	
200	57.00 . 0.05	Eucalyptus	45.04 + 0.05	
300	$57.89 \pm 0.05$	$34.44 \pm 0.10$	$45.24 \pm 0.25$	-
500	$100.00 \pm 0.15$	$41.11 \pm 0.25$	$52.38 \pm 0.05$	$23.33 \pm 0.22$
1,000	$100.00 \pm 0.33$	52.22 ± 0.22	$55.95 \pm 0.25$	$33.33 \pm 0.30$
		Ylang-ylang		
300	$45.52 \pm 0.00$	$36.67 \pm 0.10$	$45.24 \pm 0.33$	$40.00 \pm 0.11$
500	$60.52 \pm 0.05$	$55.56 \pm 0.25$	$52.38 \pm 0.66$	$44.44 \pm 0.25$
1,000	$60.52 \pm 0.11$	$67.78 \pm 0.75$	$53.57 \pm 0.15$	$47.77 \pm 0.15$
		Camphor		
300	$14.47 \pm 0.5$	-	-	-
500	$26.32 \pm 0.5$	20.00	59.52	-
1,000	$44.74 \pm 0.5$	21.11	59.52	
		Thyme		
300	$100.00 \pm 0.01$	$100.00 \pm 0.18$	$100.00 \pm 0.33$	$100.00 \pm 0.00$
500	$100.00 \pm 0.00$	$100.00 \pm 0.50$	$100.00 \pm 0.11$	$100.00 \pm 0.15$
1,000	$100.00 \pm 0.15$	$100.00 \pm 0.68$	$100.00 \pm 0.00$	$100.00 \pm 0.66$
Lemongrass				
300	$100.00 \pm 0.35$	$100.00 \pm 0.25$	$100.00 \pm 0.02$	-
500	$100.00 \pm 0.05$	$100.00 \pm 0.02$	$76.19 \pm 0.20$	$48.89 \pm 0.25$
1,000	$100.00 \pm 1.01$	$100.00 \pm 0.33$	$100.00 \pm 0.15$	$50.00 \pm 0.12$
		Tea tree		
300	$60.52 \pm 2.10$	$22.22\pm0.11$	$44.05\pm0.15$	-
500	$71.05\pm0.50$	$55.56 \pm 0.25$	$66.67 \pm 0.68$	$17.78\pm0.15$
1,000	$78.95 \pm 0.02$	$100.00 \pm 0.33$	$70.23 \pm 0.88$	$27.78 \pm 1.05$
1,000	$42.11 \pm 0.33$	$47.78 \pm 0.5$	$61.90 \pm 0.35$	$20.00 \pm 0.02$

EO1/I*	Plant pathogenic fungi				
EOs μl/l*	13071	2929	12517	10139	
		Inhibitory effect	(%)		
		Clove bud			
300	$53.95 \pm 0.05$	$72.22 \pm 0.25$	$46.43 \pm 0.30$	$61.11 \pm 0.05$	
500	$100.00 \pm 0.11$	$100.00 \pm 0.02$	$99.40 \pm 0.15$	$100.00 \pm 0.25$	
1,000	$100.00 \pm 1.01$	$100.00 \pm 0.60$	$100.00 \pm 0.85$	$100.00 \pm 0.11$	
		Cinnamon			
300	$60.52 \pm 0.33$	$74.44 \pm 1.02$	$71.42 \pm 0.02$	$77.78 \pm 0.38$	
500	$100.00 \pm 0.25$	$100.00 \pm 0.34$	$100.00 \pm 1.10$	$100.00 \pm 0.25$	
1,000	$100.00 \pm 0.05$	$100.00 \pm 0.04$	$100.00 \pm 0.67$	$100.00 \pm 0.12$	
Myrrh					
300	$38.16 \pm 0.01$	$31.11 \pm 0.25$	$37.78 \pm 0.66$	-	
500	$40.00\pm0.5$	$37.78 \pm 0.15$	$58.33 \pm 0.05$	$17.78\pm1.05$	
1,000	$42.11 \pm 0.33$	$47.78 \pm 0.5$	$61.90 \pm 0.35$	$20.00\pm0.02$	

2929 = Fusarium oxysporum; 13071 = Borytis cinerea; 10139 = Fusarium graminearum; 12517 = Collectortrichum gleosporioides; \* Values are means ± Standard Deviation (SD) of triplicate.

are supported by Naik et al. [29] who also observed that lemongrass was the most active EO against all test organisms except for Pseudomonas aeruginosa. The inhibition effect of lemongrass may be due to its components such as phenols and flavonoids. Phenolic compounds have been found to inhibit pathogenic microorganisms. Active compounds in lemongrass are myrcene, limonene, citral, geraniol, citronellol, geranyl acetate, neral, and nerol. Citral and geraniol serve as an antibacterial agent [30]. EOs are known to produce secondary metabolites, which are chemically bioactive compounds. Most of these bioactive compounds exhibit antimicrobial activity as initial sources of chemical defense in stressful conditions. The results of this study showed that M. haemolytica and E. faecalis (ATCC 29212) were the most resistant pathogens having the greatest numbers of no activity from different EOs. However, L. monocytogenes (ATCC 19115) was the most sensitive pathogen. In a study by Amat et al. [31], they found that the EOs ajowan, thyme, and fennel inhibited M. haemolytica which is contradictory to our findings. Mannheimia haemolytica has been reported to be a major cause of bovine pneumonia, and antibiotics are used in large amounts to control the pneumonia [32].

In addition, another study by Benbelaïd *et al.* [33] observed that EOs showed good antimicrobial activity and high ability in *E. faecalis* biofilm eradication. These findings are supported by Mazzarrino *et al.* [34] who also observed that EOs had a good activity on *L. monocytogenes*. A study conducted by Dobre *et al.* [35] found that *B. cereus* was sensitive against selected EOs and that was also observed in this current study. Most EOs tested showed some inhibition, but only three (lemongrass, tea tree, and cinnamon) showed large inhibition zones against multiple pathogenic strains (Table 4). Lemongrass, thyme, cinnamon, and tea tree were very effective against both Gram-positive and Gram-negative bacteria, while clove bud only inhibited Gram-positive organisms. Previous studies have also shown that cinnamon, clove, and rosemary were inhibitors of bacteria [36]. The least

Table 4. Antibacterial activity of EOs against pathogenic bacterial species.

Bacterial species	Camphor	Rosemary	Lemongrass	Sage	Thyme	Geranium	Eucalyptus	Ylang	Myrrh	Sweet	Clove	Cinnamon	Teatree	Peppermint
Listeria monocytogenes ATCC 19115	++ (11.6) ± 0.01	++ (8.7) ± 0.15	+++ (20) ± 0.35	++ (10) ± 0.65	+++ (29) ± 0.58	++ (8) ± 0.25	++ (9.7) ± 0.01	+ (6.7) ± 0.02	++ (10) ± 0.5	++ (12) ± 0.03	+++ (15) ± 0.66	+++ (16) ± 0.00	+++ (16) ± 0.11	++ (8) ± 1.00
Listeria monocytogenes Water	+ (7) ± 0.25	++ (8.3) ± 0.85	+++ (16) ± 0.11	++ (10) ± 0.12	1	++ (10) ± 0.33	++ (12) ± 0.11	+ (6.7) ± 0.35	++ (8.7) ± 0.5	+++ (16) ± 0.18	++ (10) ± 0.24	+++ (23) ± 0.19	++ (13) ± 0.25	++ (12) ± 0.00
Bacillus cereus ATCC 10876	ı	++ (8) ± 0.09	+++ (17.3) ± 0.25		1	++ (11) ± 0.86	++ (9.7) ± 0.24	+ (6.7) ± 0.16	++ (14) ± 0.5	++ (11) ± 1.01	++ (10) ± 1.06	++ (13) ±0.05	(21)	
Mannheimia haemolytica	ı	++ (11) ± 1.09	+++ (22) ± 0.25	++ (11) ± 0.35	1	++ (11.6) ± 0.25	++ (12.3) ± 0.15	1	++ (8) ± 0.5				ı	
Enterococcus faecium ATCC 700221	ı				1	++ (14) ± 0.16	+ (6.7) ± 0.25		++ (11) ± 0.5	++ (10) ± 0.25	+++ (15) ± 0.17	+++ (17) ± 0.88	+++ (17) ± 0.11	+++ (17) ± 0.05
Salmonella enterica MG663463	++ (13.6) ± 0.00	++ (10) ± 0.00	+++ (21.7) ± 0.02	++ (9) ± 0.05			++ (12) ± 0.15		++ (9) ± 0.5		++ (10.7) ± 0.25	++ (12) ± 0.13	++ (12) ± 0.45	++ (13)
Salmonella enterica	++ (1.3) ± 0.08	++ (8) ± 0.11	+++ (16.7) ± 0.65	++ (9.7) ± 0.85	+++ (15.3) ± 0.31	+ (7) ± 0.15	+++ (17.7) ± 0.15		++ (11.7) ± 0.5				++ (10)	++ (13) ± 0.25
Enterococcus faecalis ATCC 29212			+++ (18.3) ± 0.15	+(7) ± 0.15		++ (10) ± 0.03	++ (11.7) ± 0.12		++ (9.7) ± 0.5	++ (12.7) ± 0.14		1	++ (12) ± 0.55	•
EnterococcusGallinari ATCC 700425	++ (7) ± 1.05	+ (7) ± 0.05	+++ (19.3) ± 0.25	+ (6.7) ± 0.05		++ (9) ± 0.45	++ (9.7) ± 0.14		++ (10.7) ± 0.5	++ (10.7) ± 0.15			++ (11) ± 0.11	•
Escherichia coli 0177 ATCC 0177	+ (7.3) ± 0.33		+++ (17.3) ± 0.86		,	++ (11) ± 0.25	++ (11) ± 0.11	+ (7) ± 0.26	++ (8) ± 0.5	++ (9) ± 0.74	++ (13) ± 0.11	ı	+++ (15) ± 0.12	+ (7) ± 0.02

The scale was represented as follows, colony growth of over 14 mm (+++) shows strong inhibition activity, from 8 to 14 mm (++) is moderate effect, 1 to 7 mm (+) represents weak inhibition, and 0 mm (-) growth shows no activity. Values are means ± Standard Deviation (SD) of triplicate.

**Table 5.** Percentage area of EO analysis of lemongrass and thyme oils.

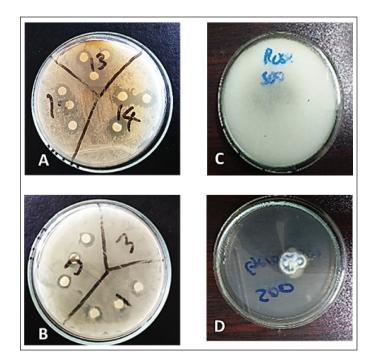
Compounds -	% Area calcula	ated from the FID
Compounds	Thyme	Lemongrass
α-Pinene	0.9	0.7
Camphene	tr	0.2
β-Pinene	0.1	0.1
Myrcene	0.4	1.6
Limonene	0.3	0.2
1,8-Cineole	0.5	0.4
<i>p</i> -Cymene	19.1	14.4
Camphor	0.4	0.3
Linalool	6.4	4.9
Terpinen-1-ol	1.2	1.1
Fenchol	0.3	-
β-Terpineol	0.6	0.5
β-Caryophyllene	0.9	1.2
Isoborneol	0.5	0.5
Neral	0.2	8.5
α-Terpineol	12.6	8.5
λ-Terpineol	2.0	1.5
Borneol	1.2	0.9
Geranial	0.3	15.0
Geraniol	0.1	1.2
Thymol	49.0	36.6
Total	92.5	98.3

active EOs were thyme and ylang ylang showing the greatest numbers of no-inhibition activity against most pathogenic bacteria that were used. Figure 2 displays the inhibitory activity against bacteria (Fig. 1A and B) and against fungi (Fig. 1C and D).

Gas chromatography data reveals that the active EOs comprise one or more high-concentration compounds. The thyme EO was richer in thymol (49%), p-Cymene (19.1%), and  $\alpha$ - terpineol (12.6%), and this was in agreement with studies conducted by Santurio *et al.* [37] and Ahmed *et al.* [38]. In addition, thymol (36.6%), geraniol (15%), and p-Cymene (14.4%) were the main biocompounds of lemongrass EO. Table 5 shows the breakdown of all the constituents in thyme and lemongrass EOs. Drawing from the results, thymol and *p*-Cymene are in both EOs at high concentrations, hence we might attribute the activities to these EOs. As anticipated, the EO of lemongrass was found to comprise high levels of thymol and possess some antimicrobial as well as preservation action [39] and insecticidal potential [40].

# **CONCLUSION**

In this study, we established that EOs possess excellent antimicrobial properties against pathogenic-resistant microorganisms. Aromatic plants that produce EOs are rich



**Figure 2.** Zone of inhibition of (A) thyme oil and (B) lemongrass oil against resistant bacteria; inhibitory effects (C) control without oil (D) treated with thyme oil against *C. gleosporoides*.

sources of natural bio compounds exhibiting antibacterial, antifungal, antiviral, insecticidal, and antioxidant activities. Drawing from this, EOs may act as an excellent candidate to decrease the resistance upward trend. Moreover, the utilization of EOs will decrease the minimum effective dose of the drugs, thus reducing their possible adverse effects and the costs of treatment. Subsequently, isolating beneficial compounds and exploring the synergistic effects may lead to toxicity investigation for product development. In conclusion, these EOs have the potential to be used as natural antimicrobial agents in the medical, pharmaceutical, and agricultural industries. This suggests that the EOs tested in this study could be considered as potential alternatives for synthetic antimicrobial agents with modification as their structures could lead to the development of new classes of antibacterial and antifungal compounds.

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## **AUTHOR CONTRIBUTIONS**

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJEs) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

## ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

#### DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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