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Phytochemical and Antibacterial Screening of *Chromolaena odorata* Leaf Extract

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ABSTRACT

The discovery of antibacterial drugs from natural sources as a substitute for preventing bacterial resistance has become necessary due to the increase in bacterial drug resistance. *Chromolaena odorata* has been reported to have antibacterial effects. Therefore, this study investigated the phytochemical components and antibacterial properties of *C. odorata* leaves. *C. odorata* leaves were collected, prepared, and extracted using a standard procedure with 95% methanol and 95% ethanol, respectively. Both extracts were subjected to phytochemical screening and Gas Chromatography-Mass Spectrometry to detect phytochemical compounds. *Chromolaena odorata* methanolic extract at a concentration of 50 mg/mL, 100 mg/mL, 150 mg/mL and 200 mg/mL was carried out for antibacterial screening using the agar well diffusion tested on *Staphylococcus aureus* (ATCC 43330), *Bacillus subtilis* (B29), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 15442). The methanolic extract was present with tannin, terpenoid, alkaloid, and saponin while ethanolic extract was present with tannin and alkaloid. The GC-MS chromatogram identified as many as 27 and 19 compounds for ethanolic and methanolic extract, respectively. The *C. odorata* ethanolic extract consists mainly of Phenol (0.51%), Sesquiterpenoid (20.69%) and Fatty acid (39.2%). Meanwhile, *C. odorata* methanolic extract consists of Sesquiterpenoid (62.26%) and Fatty acid (17.11%). Antibacterial activity of *S. aureus* was the highest inhibition zone diameter with 7.67 mm to 10.33 mm from 50 mg/ml to 200 mg/mL concentration of *C. odorata* extract. *Escherichia coli* and *B. subtilis* inhibition zones diameter was 200 mg/mL concentration of *C. odorata extract*. No antibacterial activities were obtained for *P. aeruginosa*. This study suggests that *C. odorata* consists of a variety of phytochemicals and contains antibacterial properties. Thus, *C. odorata* represents a promising natural source for antibacterial resistance drugs.

Keywords: *Chromolaena odorata*, phytochemicals, antibacterial agents

1. INTRODUCTION

Chromolaena odorata is a perennial weed belonging to the Asteraceae (Compositae) family and is found worldwide, including in Malaysia. Locally, this plant has traditionally been known as Pokok Kapal Terbang or Pokok Malaysia (Matawali et al., 2019). *Chromolaena odorata* can be found in grasslands, marginal lands, and small forests that are able to grow throughout the year. *Chromolaena odorata* can threaten other plants because of its ability to inhibit other plant growth (Zahara, 2019). The plant are traditionally used to cure burns, soft tissue wounds, dysentery, headaches, toothaches, stop bleeding, and skin diseases (Abdullah et al., 2020). It has been demonstrated that *C. odorata* leaves has therapeutic benefits against a

variety of infections and disorders, including antibacterial, antioxidant, anti-inflammatory, anticancer, anti-diabetic, and anti-hepatotoxic properties. (Sirinthipaporn & Jiraungkoorskul, 2017). Previous studies reveal that *C. odorata* rich in various active compound such as alkaloids, tannins, flavonoids, and other phenolic compounds (Akinmoladun & Ibukun, 2007). A chemical compound produced by this plant include isosakuranetin, subcandenin, and odaretin (Putri & Fatmawati, 2019), germacrene D (Yahya & Ramlee, 2013), caryophyllene, α-murolene and β-pinene (Alara & Abdurahman, 2019). Several studies also showed that the *C. odorata* leaves extract has an effect on bacterial activity by forming inhibitory zones (Odutayo et al., 2017; Omeke et al., 2019). The leaf extract used in phytochemical screening for potential biological activities, particularly antibacterial activity, can aid in studying medicinal plants for drug discovery. Therefore, a deeper study should be carried out to suggest *C. odorata* as one of the medicinal plants for bacterial drug resistance. The active compound should be identified to evaluate its characteristics on bacterial activities. Thus, the current study aims to determine the phytochemical composition and assess the antibacterial properties of *C. odorata* leaves.

2. MATERIALS AND METHODS

2.1. Plant Material and Extract Preparation

Chromolaena odorata leaves were collected around Teluk Intan, Perak. The leaves were rinsed with running water, dried, blended, weighed, and stored in bottles at room temperature. *Chromolaena odorata* extract was prepared using the maceration method (Hanphanphoom et al., 2016). Twenty grams of dried leaves were extracted with 200 mL of 95% ethanol and 95% methanol solvents, respectively. The mixture was extracted for 48 h using a vertical shaker rotating at 150 rpm. The extract was then concentrated in a rotary evaporator at 50°C after being filtered with Whatman filter paper No.1. The filtrate was used as a sample in Gas Chromatography-Mass Spectrometry analysis and antibacterial activity.

Figure 1. *Chromolaena odorata*

2.2. Phytochemical Screening

Phytochemical screening of the *C. odorata* methanolic and ethanolic extracts was conducted using the Harbone method (Odutayo et al., 2017) for qualitative determination. The *C. odorata* extract was screened to identify the presence of alkaloid, flavonoid, tannin, saponin, terpenoids and phenols.

2.3. Gas Chromatography-Mass Spectrometry (GCMS) Analysis

The GC-MS was performed at Institute Biosains, Universiti Putra Malaysia, Selangor. The GC-MS machine used was Shimadzu GCMS-QP2010 Ultra combined with GC-2010 using HP-5 MS capillary column (30 × 0.25 × 0.25 µm). The carrier was helium gas with a flow rate

of 0.8 mL/min. The column oven temperature ranged between 50°C and 300°C every two minutes. The interface temperature was 250°C, whereas the ion source was 200°C. The mass scanning ranged from 40-700 *m/z* at 3.0 scans/s. One microliter of *C. odorata* extract was injected automatically into the GC-MS column. The GC-MS chromatogram of *C. odorata* extract was compared with the database of the National Institute of Standards and Technology (NIST), NIST14.LIB, WILEY229.LIB and FFNSC1.LIB.

2.4. Antibacterial Screening

The strain of bacteria used in this study consist of *Staphylococcus aureus* ATCC 43330, *Bacillus subtilis* B29, *Escherichia c*oli ATCC 25922 and *Pseudomonas aeruginosa* ATCC 15442 which obtain from Institute Biosains, Universiti Putra Malaysia, Selangor. All bacterial strains were cultivated in Mueller Hinton Broth (MHB) (Oxoid) and incubated overnight at 37ºC under aerobic conditions before being employed as an inoculum. The inoculum turbidity was adjusted to a standard density corresponding to 0.5 McFarland standards with approximately 10^8 cells/mL. *Chromolaena odorata* methanolic extract was performed in the antibacterial screening. *Chromolaena odorata* extract was diluted to 50 mg/mL, 100 mg/mL, 150 mg/mL and 200 mg/mL with 20% dimethyl sulfoxide (DMSO). The diluted *C. odorata* extract were kept in the refrigerator at 4 ºC until further used.

2.5. Antibacterial Activity

The methanolic extract of *C. odorata* was tested against bacterial isolates using the agar well diffusion method (Omeke et al., 2019). A sterile cotton bud was used to apply the bacterial suspension evenly across the Mueller Hinton Agar (MHA) plate. The bacterial agar was punctured using 6 mm sterile cork borers to create wells. Twenty microliters of *C. odorata* extract with 50 mg/mL, 100 mg/mL, 150 mg/mL, and 200 mg/mL concentrations were poured into the well. Chloramphenicol disc (30 µg) was utilized as a positive control, while 20% Dimethyl Sulfoxide (DMSO) was employed as a negative control. All the plates were left overnight inside the incubator with temperature control, 37°C. The diameter of the zone inhibition formed around the well was measured after the incubation period. The zones of inhibition were compared with the inhibition zones of standard chloramphenicol (Alabi et al., 2019).

3. RESULTS AND DISCUSSION

Phytochemicals are organic substances that plants use to protect themselves from environmental hazards such as pollution, stress, dehydration, UV light, and disease attacks (Nyamai et al., 2016). Phytochemicals found in herbs have various therapeutic effects. Finding novel phytochemicals to combat infections with resistance is a current area of intense investigation.

3.1. Phytochemical Analysis

The observation of phytochemical screening showed the *C. odorata* methanolic extract was found to be highly present with tannin, terpenoid and alkaloid. However, *C. odorat*a ethanolic extract was found to be highly present with tannin and alkaloid. Saponins can only be found in methanolic extract. Unexpectedly, both extracts are not detected with flavonoid and phenol compounds. Some studies also reported a little presence or absence of flavonoid compounds as studied by Igboh et al., (2009) and Jualang et al. (2013). The difference in

phytochemicals found in methanolic and ethanolic extracts could be related to the polarity of the two solvents. Methanol is more polar than ethanol will affect the solubility of target phytochemicals, which could explain the observed differences. Plant extraction effectiveness is influenced by the ratio of plant material to solvent, extraction time, and plant particle size (Manousi et al., 2019).

Table 1. Phytochemical screening of ethanolic and methanolic *C. odorata* extract

Phytochemical constituents	Ethanol extract	Methanol Extract
Flavanoid	-	-
Tannin	++	++
Terpenoid	-	++
Alkaloid	++	++
Saponin	-	+
Phenols	-	-

Notes: ++ = highly present ; + = present; - = absent

3.2. Gas Chromatography-Mass Spectrometry (GCMS) Analysis

Quantitative GC-MS analysis was performed to identify compounds present in the *C. odorata* ethanolic and methanolic extract. The GC-MS chromatogram revealed 41 compounds from ethanolic extract (Figure 2) and 24 compounds from methanolic extract (Figure 3). After comparison with database, the GC-MS identified 27 (Table 2) and 19 (Table 3) compounds for ethanolic and methanolic extract, respectively. The *C. odorata* ethanolic extract consists mainly of Sesquiterpenoid (20.69%), Fatty acid (39.2%) and Phenol (0.51%). Meanwhile, *C. odorata* methanolic extract only consists of Sesquiterpenoid (62.26%) and Fatty acid (17.11%). The hexadecenoic acid, a fatty acid with molecular formula $C_{38}H_{68}O_8$ from ethanolic extract, is the highest compound with 24.43%. Germacrene-D ($C_{15}H_{24}$) is the highest compound with 19.87% for methanolic extract. These two extracts identified the same compounds: alpha-copaene, alpha-humulene, germacrene-D, delta-cadinene, neophytadiene, hexadecanoic acid, phytol, linoleic acid, and octadecanoic acid. In addition, phytol is acyclic diterpene alcohol also found in methanolic and ethanolic extracts, containing 12.35%, and 3.67% respectively. The majority of the components in the methanolic extract *of C. odorata* were terpenoids (62.26%), compared to the ethanolic extract's fatty acids (39.2%).

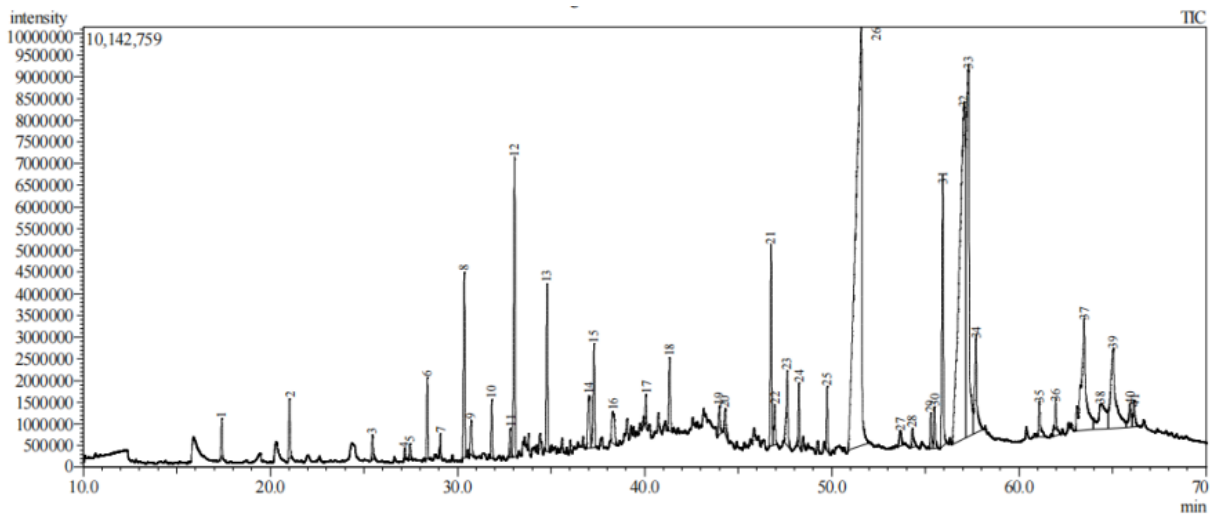

Figure 2. GC-MS chromatograph of the *Chromolaena odorata* ethanolic extract

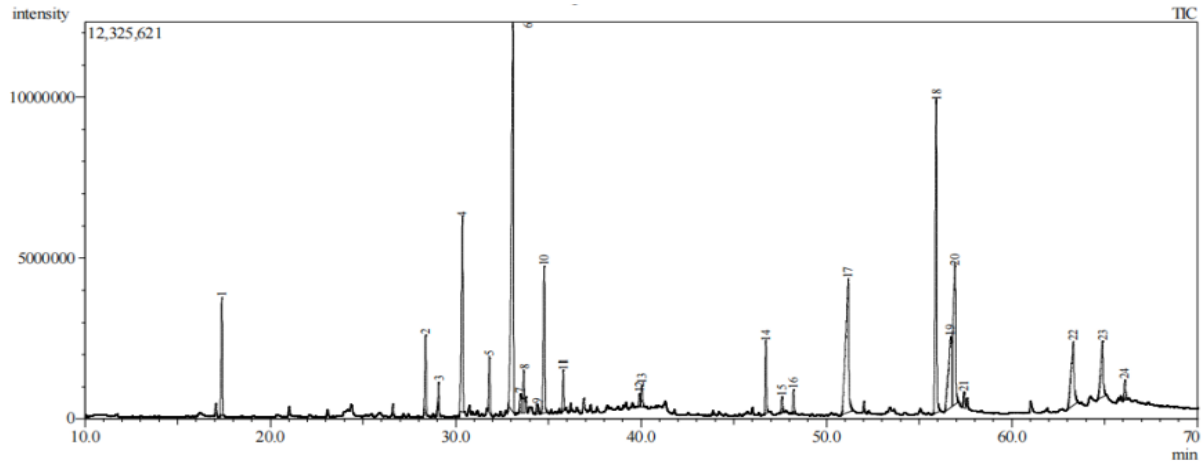

Figure 3. GC-MS chromatograph of the *Chromolaena odorata* methanolic extract

Table 2. Phytochemical compounds identified in *Chromolaena odorata* ethanolic extract

Peak No	Retention Time	Compound Name	Peak Area (%)	Molecular Formula	Molecular Weight	Nature of compound
2	21.03	2-3 Dihydrobenzafuran	0.73	C_8H_8O	120	Sesquiterpene
3	25.45	4-Vinyl Guaicol	0.32	$C_9H_{10}O2$	150	Phenol
4	27.23	α-Cubebene	0.09	$C_{15}H_{24}$	204	Sesquiterpene
5	27.45	Caryophyllic acid/ eugenol	0.19	$C_{10}H_{12}O_2$	164	Phenol
6	28.39	α-Copaene	0.91	$C_{15}H_{24}$	204	Sesquiterpene
8	30.36	Caryophyllene	2.35	$C_{15}H_{24}$	204	Sesquiterpene
10	31.83	α-Humulene	0.68	$C_{15}H_{24}$	204	Sesquiterpene
11	32.81	γ-Murolene	0.32	$C_{15}H_{24}$	204	Sesquiterpene
12	33.05	Germacrene D	3.97	$C_{15}H_{24}$	204	Sesquiterpene
13	34.78	δ-Cadinene	2.08	$C_{15}H_{24}$	204	Sesquiterpene
14	37.02	Spathulenol	1.17	$C_{15}H_{24}$	220	Sesquiterpene
15	37.29	Caryophyllene oxide	1.49	$C_{15}H_{24}O$	220	Sesquiterpene
18	41.33	5-Cyclodecen-1-ol	1.05	$C_{15}H_{24}O$	220	Fatty acid
19	43.99	Tetradecanoic acid	0.43	$C_{14}H_{28}O_2$	228	Sesquiterpene
21	46.76	Neophytadiene	2.49	$C_{20}H_{38}$	278	Sesquiterpene
22	46.95	Hexahydrofamegyl acetone	0.63	$C_{18}H36_O$	268	Fatty acid
24	48.24	Neophytadiene	0.74	$C_{20}H_{38}$	278	Fatty acid
25	49.75	Methyl palmitate	0.75	$C_{17}H_{34}O_2$	270	Fatty acid
26	51.57	Hexadecanoic acid	24.43	$C_{38}H_{68}O_8$	652	Fatty acid
28	54.30	Octadecanoic acid	0.34	$C_{18}H_{36}O_2$	284	Fatty acid
29	55.29	9,12-Octadecadienoic acid methyl ester	0.49	$C_{19}H_{34}O_2$	294	Sesquiterpene
30	55.50	9,12,15- Octa-decatrienoic acid methyl ester	0.59	$C_{19}H_{32}O_2$	292	Fatty acid
31	55.93	Phytol	3.67	$C_{20}H_{40}O$	296	Fatty acid
33	57.31	Linolenic acid	9.64	$C_{18}H_{30}O_2$	278	Fatty acid
34	57.71	Octadecanoic acid	2.10	$C_{18}H_{36}O_2$	284	Fatty acid
35	61.10	Fumaric acid	0.64	$C_{26}H_{49}NO_4$	439	Fatty acid
41	66.17	3-Cyclopentylpropionic acid	0.43	$C_{12}H_{23}NO_2$	213	Fatty acid

Total compound: 41 (100%)
Total of identified compounds: 27 (62.72%)
Total of unidentified compounds: 14 (37.8%)

Table 3. Phytochemical compounds identified in *Chromolaena odorata* methanolic extract

Peak No	Retention Time	Compound Name	Peak Area (%)	Molecular Formula	Molecular Weight	Nature of compound
1	17.39	Geijerene	3.87	$C_{12}H_{18}$	162	Sesquiterpene
2	28.37	alpha- copaene	2.39	$C_{15}H_{24}$	204	Sesquiterpene
4	30.36	trans-caryophyllene	7.44	$C_{15}H_{24}$	204	Sesquiterpene
5	31.81	alpha-humulene	1.84	$C_{15}H_{24}$	204	Sesquiterpene
6	33.09	Germacrene- D	19.87	$C_{15}H_{24}$	204	Sesquiterpene
7	33.48	Muurola-4(4),5, diene	1.14	$C_{15}H_{24}$	204	Sesquiterpene
8	33.67	Bicyclogemacrene	1.56	$C_{15}H_{24}$	204	Sesquiterpene
9	34.37	Gamma-cadinene	0.44	$C_{15}H_{24}$	204	Sesquiterpene
10	34.78	Delta-cadinene	5.43	$C_{15}H_{24}$	204	Sesquiterpene
11	35.80	Alpha-elemol	1.23	$C_{15}H_{26}O$	222	Sesquiterpene
12	39.90	Eudesm-4(14)-en-11-ol	0.44	$C_{15}H_{26}O$	222	Sesquiterpene
13	40.04	Cadin-4-en-10-ol	0.70	$C_{15}H_{26}O$	222	Sesquiterpene
14	46.73	Neophytadiene	2.31	$C_{20}H_{38}$	278	Sesquiterpene
15	47.60	Neophytadiene	0.52	$C_{20}H_{38}$	278	Sesquiterpene
16	48.22	Neophytadiene	0.73	$C_{20}H_{38}$	278	Sesquiterpene
17	51.17	Hexadecanoic acid	11.01	$C_{16}H_{32}O_2$	256	Fatty acid
18	55.93	Phytol	12.35	$C_{20}H_{40}$	296	Sesquiterpene
20	56.94	Linolenic acid	5.44	$C_{18}H_{30}O_2$	278	Fatty acid
21	57.41	Octadecanoic acid	0.66	$C_{18}H_{36}O_2$	284	Fatty acid

Total compound: 24 (100%)
Total of identified compounds: 19 (79.37%)
Total of unidentified compounds: 5 (20.63%)

According to a prior study, the methanolic extract of *C. odorata* taken from South Africa had relative levels of fatty acids esters (31.7%), sesquiterpenes (24.0%), and monoterpenes (30.8%) (Lawal et al., 2015). Discovery by Felicien et al., (2012) from Benin, the essential oil of *C. odorata* was dominated by hydrogenated monoterpenes and sesquiterpenes. Thus, this study demonstrates that local *C. odorata* has different distribution of phytochemical composition from those countries. According to Pandey & Tripathi, (2014), the quality of plant extracts is affected by factors such as the plant material's season, location, climate, and country of origin. Malaysia, which has an equatorial climate and scattered tropical, explains that the extracts obtained have different phytochemical content than those studied from other countries. In addition, some of the phytochemicals were well-known compounds, had been isolated from a variety of plant species and reported potential biological activities. The essential oil from the leaves of *Garcinia celebica*, which contains α-copaene (61.25%), germacrene D (6.72%), and β-caryophyllene (5.75%), has cytotoxic and antibacterial properties (Tan et al., 2019). Phytol, produced from the *Aster yomena* plant, exerts antibacterial effects on *Pseudomonas aeruginosa* via inducing oxidative stress-mediated DNA damage in bacteria (Lee et al., 2016). According to Yoo and Jwa (2019), β-caryophyllene may improve periodontal health by reducing inflammation produced by lipopolysaccharide and neutralizing volatile sulfur compounds (VSCs). Other substances included hexadecenoic and linolenic acid, which belong to a class of fatty acids with have antimicrobial, antibacterial, and antioxidant properties (Fadzir et al., 2018; Tyagi & Agarwal, 2017).

3.3. Antibacterial Activity

Since Hanphanphoom et al., (2016) reported that methanol was the optimum extraction solvent of *C. odorata* leaves, investigation of antibacterial activity was done using methanolic *C. odorata* extract against four different types of bacteria: *S. aureus, E. coli, B. subtilis, and P. aeruginosa*. The selected bacteria tested in this investigation against *C. odorata* extract are the most frequent pathogens (Onwuezobe et al., 2016). *C. odorata* methanolic extract has been used

to determine the antibacterial activity with a concentration of 50 mg/mL, 100 mg/mL, 50 mg/mL and 200 mg/mL. As presented in Table 4, Figure 4 to 7, the results obtained showed an increase in the inhibition zone diameter for all antibacterial activities except for *P. aeruginosa.* The phytochemical presence in methanolic *C. odorata* extract significantly contributes to the antibacterial property. These phytochemicals have been discovered to possess antibacterial activities, including the interruption of cell division, forming an extracellular soluble protein, acting as an antioxidant as well as cell membrane disruption (Hridhya & Kulandhaivel, 2017). Antibacterial activity for *S. aureus* was the highest by showing an inhibition zone from 7.67 mm to 10.33 mm with increasing in *C. odorata* extract concentration. While *E. coli* and *B. subtilis* showed low inhibition zone of 200 mg/mL concentration of C. *odorata* methanolic extract with 7.94mm and 8 mm, respectively. No significant activities were obtained for *P. aeruginosa* accordance with previous studies by Hanphanphoom et al. (2016) although the concentration had been increased from 10% to 20%. The results also implied that gram-negative bacteria are more resistant to the antibacterial effects of the *C. odorata* extract than gram-positive bacteria. According to Farrag et al. (2019), the tested substance causes rupture of the cell wall because the gram-negative bacteria's phospholipid membrane is impermeable to lipophilic solutes. However, the gram-positive bacteria's outer peptidoglycan layer has a poor permeability barrier, making it difficult for chemicals to be inhibited.

Treatment of illnesses brought on by antibiotic-resistant bacteria has become challenging due to the emergence of multidrug-resistant bacteria (Jubeh et al., 2020). Numerous studies have been done on the *C. odorata* composition, and all of the bioactive compound that have been found in it offer therapeutic promise including serving as antibacterial agents. The methanolic *C. odorata* extract contains a high phytocompound of germacrene D, have been demonstrated to have a broad range of antibacterial activity against many gram-positive and gram-negative bacteria (El-Kalamouni et al., 2017; Kelechi & Mary, 2020). Phytol (3,7,11,15-tetramethylhexadec-2-en-1-ol) is a product of the metabolism of chlorophyll in plants also known to have high antimicrobial activity (Kelechi & Mary, 2020). Other than that, Hexadecanoic acid is known to have the capacity to inhibit the growth of bacteria and fungi (Gobalakrishnan et al., 2020).

In a different earlier investigation, it has been found that the dichloromethanolic and ethanolic crude extracts of *C. odorata* are equally efficient against 22 different types of microorganisms, including various gram-positive and gram-negative bacteria and yeasts (Kanase & Shaikh, 2018). According to Vijayaraghavan et al. (2018), the ethanolic extract could suppress the growth of human infections such as *Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus subtilis, Bacillus cereus*, and *Escherichia coli*. Therefore, *C. odorata* has been shown to be effective against multidrug-resistant bacteria, and provides a promising alternative to conventional drug (Alabi et al., 2019). Thus, the *C. odorata* extract can be used as a potential antibacterial agent in future.

Table 4. Zone of inhibition (mm) of *C. odorata* methanolic extract

Bacteria	**The concentration of *C. odorata* methanolic extract (mg/mL)**				**Chloramphenicol**
	50	**100**	**150**	**200**	
*S. aureus** ATCC 43300	7.67	8.67	9.67	10.33	24
*B. subtilis** B29	NI	NI	NI	8	25
*E. coli** ATCC 25922	NI	NI	NI	7.94	28
P. aeruginosa ATCC 15442	NI	NI	NI	NI	14

Significant* ($p<0.05$). Confidence interval level at 95% NI= no inhibition

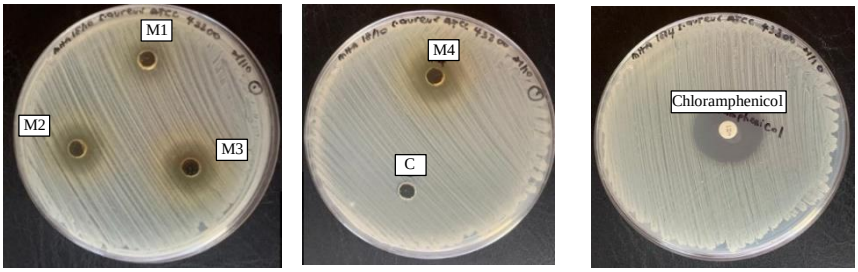

Figure 4. Zone of inhibition (mm) of *Staphyloccocus aureus* against *C. odorata* extract [M1: 50 mg/mL, M2: 100 mg/mL, M3: 150 mg/mL, M4: 200 mg/mL C: DMSO]

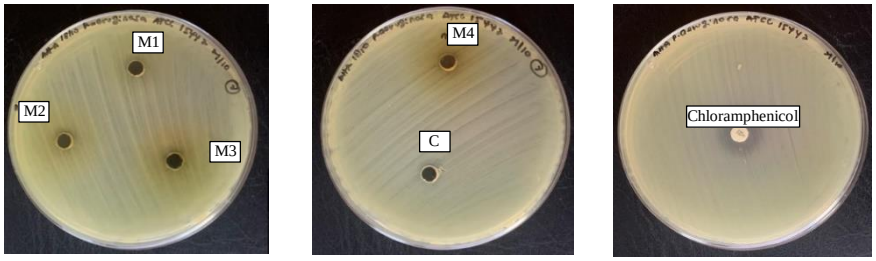

Figure 5. Zone of inhibition (mm) of *Pseudomonas aeruginosa* against *C. odorata* extract [M1: 50 mg/mL, M2: 100 mg/mL, M3: 150 mg/mL, M4: 200 mg/mL C: DMSO]

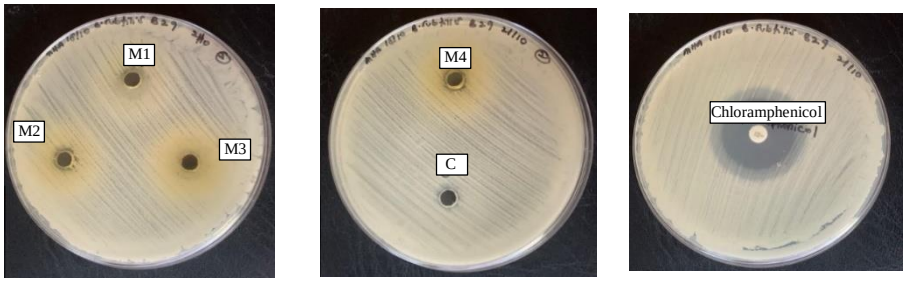

Figure 6. Zone of inhibition (mm) of *Bacillus subtilis* against *C. odorata* extract [M1: 50 mg/mL, M2: 100 mg/mL, M3: 150 mg/mL, M4: 200 mg/mL C: DMSO]

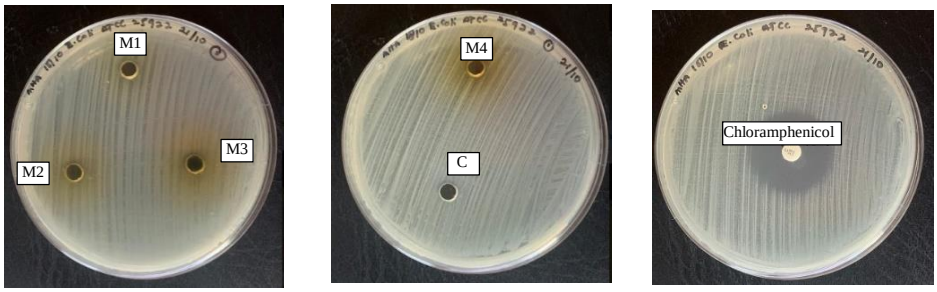

Figure 7. Zone of inhibition (mm) of *Escherichia coli* against C. *odorata* extract [M1: 50 mg/mL, M2: 100 mg/mL, M3: 150 mg/mL, M4: 200 mg/mL C: DMSO]

4. CONCLUSION

In conclusion, phytochemical screening and GC-MS analyses identified multiple bioactive compounds in the local *C. odorata* extract. Additionally, this investigation demonstrated that the *C. odorata* methanolic extract has antibacterial activity at 200 mg/mL against *S. aureus*, *B. subtilis*, and *E. coli*. As a result, *C. odorata* leaf extract can be used as an alternative in treating skin infection because it inhibits the growth of bacteria. In addition, *C. odorata* leaf extract can be a source of antibacterial agents to reduce the emergence of antibiotic-resistant strains with the development of antibiotic drugs. However, further research

is recommended to fully validate the antibacterial efficacy and biosafety assay of the *C. odorata* extracts.

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