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# A study of phytochemical screening and antibacterial effect of some methanolic plant extracts on *Pseudomonas aeruginosa* Khoulood A. Hemida<sup>1\*</sup>, Shimaa Khalid<sup>1</sup>, Abdelkarim Mohammed Abdelatif<sup>2</sup>, and Amany M. Reyad<sup>1</sup>

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# **ABSTRACT:**

Resistance of the different bacterial strains to conventional antibiotics was noticed, and the need of solutions to come over this problem turn into find natural sources. The efficacy of methanol extracts of different plants is noticed as a powerful way to solve this problem. Lantana camara, Eucalyptus sp, Menthab sp, Mangifera indicia leaves were chosen as trails. Efficacy of methanol extracts of Lantana, Eucalyptus, Mentha, Mangifera leaves were assessed against pathogenic multi-drug resistant Pseudomonas aeruginosa. The resistance of the bacterial strain was screened for all used conventional antibiotics. The extracts from the investigated leaves have been studied for their antimicrobial and antioxidant effects. The disc diffusion method was used for determining the minimum inhibitory concentration (MIC) and the antimicrobial activity. The same MIC (12.5 mg/mL) was recorded for Eucalyptus and Mentha, while MICs were 3.125, 6.25 mg/mL for Lantana and Mangifera, respectively. Chemical composition of each extract was evaluated using GC-MS giving; hexadecanol as the major compound (15.13%) for Lantana, eucalyptol was identified as the major compound (21.41%) for *Eucalyptus*, 2-methyl-5-phenyl Cyclohexen-1-one was identified as the main compound (14.31%) for Mentha, and chloroform was identified as the major compound (7.67%) for Mangifera. The total phenols, DPPH scavenging activity, and reducing power were estimated under spectrophotometric conditions and supported our GC-MS findings. In this article, different plant leaves extracts act as powerful antibacterial agents, and the MICs values were significantly low against the tested bacterial pathogen.

KEY WORDS: Plant extracts, Antimicrobial, GC-MS.

# **1. INTRODUCTION:**

The pathogenic bacterium Pseudomonas aeruginosa is an opportunistic Gramnegative bacterium. It can result in a variety of acute and chronic infections that have the potential to be fatal. Immune system-compromised patients are more likely to contract it. (Ahmed et al., 2019). According to(Alotaibi et al., 2022), it is among the most prevalent nosocomial pathogenic bacteria that infect hospitalized patients. P. aeruginosa is a common microbe that can infect patients with immunological deficiencies, burns, cystic fibrosis, and the complication of abnormal neutrophil reduction (Reynolds and Kollef, 2021). Pseudomonas aeruginosa (PA) is an opportunistic and widespread pathogen that inhabits a variety of environments, including terrestrial and aquatic ones, with temperatures ranging from 4 to 42°C and plants to mammals as hosts (Guillaume et al., 2022). With a genome that is among the largest among bacteria-nearly 6000 genes-PA has a significant advantage over other microorganisms in terms of competitiveness and high environmental adaptability patients with cystic fibrosis (CF) have been shown to have healthy PA lungs (Feigelman et al., 2017). Antibiotic treatments are no longer effective for treating PA infections once it adheres to the epithelial surface and transforms into a mucoid phenotype (Guillaume et al., 2022). This bacterium resistance to antibiotics grown, which presents several has challenges for treating it (Terreni, Taccani and Pregnolato, 2021). Clinically significant antimicrobial compounds derived from plants with diverse antibiotic mechanisms eradicate bacteria implicated in treating infections brought on by resistant microbial strains (Saeidi and Fazeli Nasab, 2019). As a potential substitute for chemically synthetic drugs, to which many infectious microorganisms have developed resistance, numerous

studies have been carried out to identify the various antimicrobial and phytochemical constituents of medicinal plants and use them for the treatment of microbial infections (both topical and systemic applications) (Obiefu et al., 2021). New active molecules are still largely sourced from medicinal plants. According to WHO estimates from 2002, 80% of Africans engaged in this behavior for personal health reasons (Dehou et al., 2021). Lantana camara L. was one of the many species that were exploited.

Lantana camara Linn, belongs to the Verbenaceae family and is valued as an ornamental garden plant as well as a notorious weed ( Barik, et al., 2020). L. camara extract has been used for centuries by traditional healers to treat conditions related to the gastrointestinal tract. inflammation, dermatology, rheumatism, fever, and headaches (Mansoori et al., 2020). According to studies conducted by(Lata, 2020), leaf extracts have been possess demonstrated to antifungal, antiproliferative, antibacterial, nematocidal, germicidal, anthelmintic, and anticancer properties.

A methanolic extract of *L. camara* leaves was found to heal stomach ulcers and inhibit the formation of duodenal ulcers in rats, Fresh leaf extracts are antibacterial and have been used for generations in Brazil as an antipyretic, carminative, and in the treatment of respiratory diseases (Barreto et al., 2010; Darwish et al., 2022). Numerous tested bacteria and fungi were dramatically inhibited in their ability to grow by the chemical composition of L. camara, which contains B-caryophyllene, geranyl acetate, terpinyl acetate, bornyl acetate, and limonene. P. aeruginosa, A. niger, F. solani, and Candida albicans were the strains that were most sensitive (Ezebo et al., 2021). Triterpenoids, steroids. flavonoids. iridoid glycosides,

oligosaccharides, phenylpropanoid glycosides, and naphthoquinones have all been identified through phytochemical investigations on *Lantana* species(Kumar, Singh and Yadav, 2020).

In Mediterranean countries, mentha is widely used to flavour food and has long been used as a remedy for conditions like upper respiratory infections, hemorrhoids, indigestion, and stomachaches (Alsaraf et al., 2021). A review has been conducted on the chemical composition and various biological properties of Mentha (Tafrihi et al., 2021). The methanolic extract of Mentha has been reported to exhibit antibacterial activities against both Gramnegative and Gram-positive organisms, such as Pseudomonas aureus, Bacillus subtilis, Escherichia coli, Pseudomonas Serratia marcescens. aeruginosa, and Streptococcus aureus (Anwar et al., 2017).

Eucalyptus is a great source of both volatile and nonvolatile compounds that have a variety of biological functions. The volatile compounds found in eucalyptus methanolic extract, on the other hand, have mostly been utilized in the pharmaceutical and food industries. Indigenous Australians have used eucalyptus as traditional bush medicine to heal wounds and treat fungal infections since time immemorial (Sharma, 2023). The chemical composition of Eucalyptus leaves has spp. been

# 2. MATERIALS AND METHODS: Bacterial strain

*Pseudomonas aeruginosa* strain DL001 that was obtained from Microbiology Lab, Botany Department, Fayoum University, and that was previously studied for its resistance by Selim et al., (2022).

# Plant collection and processing

The mature leaves of *L. camara*, *Mentha*, *Eucalyptus and Mangifera*.

were obtained from the Garden of Fayoum University in Egypt. To remove dirt and unwanted dust particles, the plant leaves

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have antioxidant. demonstrated to anticancer, insecticidal, antibacterial, antiviral, and antifungal effects (Davari and Ezazi, 2017). These chemical composition has a wide range of bioactivities, such antioxidant, as antimicrobial, insecticide, antibacterial, and fungicide characteristics (Mutlu-Ingok et al., 2020). Mangifera plant leaves have long been used in folk medicine to treat a number of inflammatory illnesses, but scientific evidence for their anti-oxidant and antiis insufficient inflammatory qualities (Awuchi et al., 2023). Traditionally, different parts of *M. indicia* have been used to treat gastrointestinal disorders (dysentery, piles, stomach distress, biliousness, persistent constipation), respiratory diseases and other ailments

(bronchitis, asthma, hiccough, throat problems), and genitourinary issues (urinary discharges, leucorrhea, and vaginal problems)(Kalita, 2014).

Therefore, our study aims to investigate the metabolic crude extract of Leaf for its phytochemical properties and antimicrobial activities .and its effect on *P. aeruginosa* in vitro study. GC-MS studies were also performed to find out functional groups and active antimicrobial compounds in the extract of *L. camara*, *Mentha*, *Eucalyptus and Mangifera*.

were rinsed with tap water. After being shaded, dried, and powdered with a mechanical blender, the leaves were stored in airtight bottles.

# **Preparing the extract**

plant material were extracted according to (*Rajagopal et al.*, 2022).

**Determination of total phenolic content total phenolics** were determined using Folin-Ciocalteu reagent method Ali, *et al.*, (2010).

Assay for reducing strength

Oyaizu's process was used to calculate the reductive potential of the extracts (**Oyaizu**, **M.** (1986)).

## **Radical scavenging action of DPPH**

Blois' method was used to evaluate the extracts' ability to scavenge and 1,1-diphenyl-2-picrylhydrazil

radical(DPPH) radicals (Blois, 1958).

# GC/MS analysis of plant extract

The GC/MS experiment was carried out on a Thermo Scientific Trace GC Ultra / ISQ Single Quadrupole MS, TG-5MS fused silica capillary column. The quantification of all observed components was investigated using a percentage relative peak area. The compounds were correctly identified by comparing the individual retention times and mass spectra of the chemicals to those of the NIST, WILLY library data from the GC/MS instrument (REYAD, 2022).

## Assay by disc diffusion

The antibacterial activity of plant extracts was determined using a disc diffusion experiment on Mueller-Hinton agar

# **3. RESULTS AND DISCUSSION:**

The antimicrobial activity of the four plant extracts under investigation against Pseudomonas aeruginosa (PA) on agar plates demonstrated that each extract inhibited the growth of Pseudomonas aeruginosa (Table 1). The filter paper discs impregnated with the investigated plant extracts develop an inhibition growth zone, or transparent halo, as a result of the inhibitory action. The strongest antibacterial effect among various plant extracts was found in Mentha plant extract, according to the results. The average diameter of the growth inhibition zones of the Mentha plant extract was found to be 1.20 cm, whereas the Lantana camara, Eucalyptus, and Mangifera plants had average diameters of 1.07 cm, 1.03 cm, and 0.77 cm, respectively. Pseudomonas aeruginosa (PA) was susceptible to the

(Himedia, India). (Ngamsurach and Praipipat, 2022).

# Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the bioactive plant extract was measured by using the micro-broth dilution process (NCCLS, 2000). The lowest extract concentration (maximum dilution) that prevented the growth of the examined microorganisms was known as the minimum inhibitory concentration (MIC) (Ari Setyati *et al.*, 2020).

## Statistical analysis

Data were statistically analyzed using a one-way analysis of variance (ANOVA test) using SPSS Statistical Package Program(Venkata et al., 2015) (SPSS, 2015) version 23. Mean of treatments were compared by Duncan multiple range test when the differences were significant (Duncan, 1955). Level of significance in all tests was P $\leq$ 0.05. The results are expressed as means  $\pm$  standard error (SE).

antimicrobial activity of several plant extracts. As shown in Table (1), The obtained results of antibacterial activity of the studied *Lantana camara, Eucalyptus, Mentha, Mangifera* against *Pseudomonas aeruginosa* on agar plates demonstrated an inhibitory microbial effect of MIC 3.125, 12.5, 12.5, 6.25 mg/ml respectively.

Some previous studies also reported that Lantana camara has antimicrobial activity against many fungi and bacteria (Hernández et al., 2005; Sonibare and Effiong, 2008) showed the MIC of Lantana camara and its constituents for Pseudomonas aeruginosa, the MIC was found 1 mg/ml and that agreed to our results. Mentha has antibacterial activity against Pseudomonas aeruginosa and showed the MIC value 5mg/ml and that agreed to our results (İşcan et al., 2002).

Additionally, *Eucalyptus* exhibits antibacterial activity against several bacteria and fungi, according to certain earlier investigations, and showed the MIC of *Eucalyptus* and its constituents for *Pseudomonas aeruginosa*, the MIC was found 30 mg/ml and that dis agreed to our results (Ait-Ouazzou et al., 2011).

This study reports that the leaf extract of *Mangifera* has antibacterial activity, and MIC value against *Pseudomonas aeruginosa is dis agreed with* Bbosa *et al.* (2007), where the value of MIC was 43.75 mg/ml.

The antimicrobial activity of *Lantana* camara, Eucalyptus, Mentha, Mangifera is due to their various components including terpenes, aldehydes, alcohols, esters, phenolics, ethers, and ketones (Arjmand and Dastan, 2020). The analysis of the

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volatile profile of *Lantana camara*, shows that it mainly contains monoterpene, 1,5dideuteriopentane, 1-Hexadecanol, Dodecachloroperylene, pregnanoic acid ethvl ester and Tetraanhydrobacterioruberin as the major compounds. The terpenes listed above have biological properties such as antitumor. nematicide, analgesic, antibacterial, anti-inflammatory, sedative, fungicide, pesticide, and insecticide. nematicide insectifuge chemopreventive hypocholesterolemic antiacne(Mariajancyrani et al., 2014). The current study results have been confirmed by the chemical compositions reported from Lantana camara, which are rich in terpenes and also supported and then supplemented previous data (El-Din et al., 2022).

 Table 1. Average of the Inhibition Zone diameters and MICs for the four tested plant extracts against *Pseudomonas aeruginosa.*

Plant extract	Inhibition Zone, cm	MIC (mg/mL)
Eucalyptus	$1.03 \pm 0.12^{ab}$	12.5
Lantana	$1.07{\pm}~0.09^{\mathrm{ab}}$	3.125
Mangifera	$0.77 \pm 0.03^{b}$	6.25
Mentha	$1.05{\pm}~0.09^{\mathrm{ab}}$	12.5

(a and b) Average in the same column having different superscripts are differ significantly ( $P \le 0.05$ ).

**Total phenolics and antioxidant activity:** The DPPH scavenging capability was used to evaluate the antioxidant behaviour of different extracts. The results in (Fig. 1) indicate that the *lantana* extract has a higher scavenging capability of DPPH and reducing power, but *Eucalyptus* has a higher content of total phenolic than the other extracts. Additionally, there was a direct correlation between DPPH scavenging capability, and the reducing power assay.

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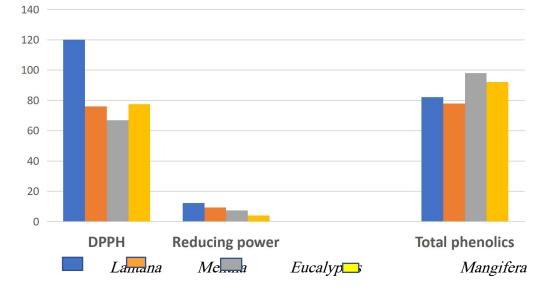


Figure 1. Physiological parameters (.DPPH scavenging capability, reducing power and total phenolic ) of *L. camara, Mentha, Eucalyptus and Mangifera.* Leaves extracts.

The antimicrobial effect of Lantana extracts is owing to their high phenolic and antioxidant content, which is consistent with the findings of Baba and Malik (2014) . They stated that Gentiana kurrooroyle methanol extracts of roots and leaves had an antibacterial effect and concluded that the root methanol extract had a strong effect against selected pathogenic bacteria due to its high phenolic content. The presence of hydroxyl groups with significant free radical scavenging activities mediates the antioxidant and antibacterial effects of phenolic compounds (Bouarab-Chibane et al., 2019). The specificity of the diverse Lantana, mentha, Eucalyptus, and Mangifera extracts against Pseudomonas aeruginosa was demonstrated by their associated behavior. which mav be attributed to the broad spectrum of

phenolic and antioxidant bioactive pharmacological compounds (Gilles et al. (2010), Husain et al., 2017). There was agreement in total phenolic content, DPPH scavenging, and reducing power activity, and these findings are supported by the results obtained by (Zhang and Wang, 2009); that demonstrated direct a relationship between antioxidant behavior and total phenolic compounds.

## **Chemical composition of plant extracts**

The GC/MS study of *Eucalyptus* methanol extract revealed 50 organic compounds, including alkaloids, terpenes, alkanes/alkenes, aldehydes/ketones, esters, ethers, and organic acids, the majority of which are Eucalyptol (21.41%), Thiophene (8.02%), dichloro methyl propyl sulfone (4.68%), and 3-Eicosene, (E) (3.02%) as the major compounds (**Table 2**).

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Chemical compound	Rt (min)	(Area % )	M.wt	M.formula	S.Structure
Eucalyptol	9.28	21.41	154	C <sub>10</sub> H <sub>18</sub> O	0
Thiophene-D1	5.22	8.02	84	C <sub>4</sub> H <sub>3</sub> DS	5 <b>S</b> 2
2,12-Dibromo- 6,7,8,9 ,16,17-hexahydro- 5h-dibenzo[f,m][1, 4,8,12]tetraazacy Clopentadecina tonickel (ii)	5.14	5.49	518	C <sub>19</sub> H <sub>18</sub> Br <sub>2</sub> N <sub>4</sub> Ni	1 Br Br
Dichloromethyl propyl Sulfone	5.35	4.68	190	$C_4H_8Cl_2O_2S$	
1-Chloro-1,1,2- Trifluoro -2-Tridecene	28.15	3.24	270	C13H22ClF3	
3-Eicosene, (E)-	23.86	3.02	280	C20H40	

Table 2. The major detectable chemical compounds in Eucalyptus using GC-MS.

RT: retention time, peak area % represents the concentration, M. wt: molecular weight, M. formula: molecular formula, C. structure: compound structure

The GC/MS study of *Lantana* methanol extract revealed 52 organic components, including 1,5-dideuteriopentane (15.13%),

1-Hexadecanol (CAS) (3.08%), and Dodecachloroperylene (2.54%) as the major compounds (**Table 3**).

Chemical compound	Rt(m in)	Area%	M.wt	M.formula	S.Structure
1,5-Dideuteriopentane	5.15	15.13	72	C <sub>5</sub> H <sub>10</sub> D <sub>2</sub>	
1-Hexadecanol (CAS)	35.61	3.08	242	C <sub>16</sub> H <sub>34</sub> O	••••
Methyl 2-methyl-4H-3,1- benzo xathiine-2- carboxylate	19.72	3.02	224	C <sub>11</sub> H <sub>12</sub> O <sub>3</sub> S	
Dodecachloroperyl ene	19.62	2.54	660	C <sub>20</sub> Cl <sub>12</sub>	
3,5-Cyclo-6- methoxy-à (iodotetradecyl) Pregnanoic acid ethyl ester	53.70	2.47	696	C38H651O	
Tetraanhydrobacte rioruberin	53.96	2.24	668	C50H68	

**RT:** retention time, peak area % represents the concentration, M. wt: molecular weight, M. formula: molecular formula, C. structure: compound structure.

The GC/MS study of *Eucalyptus* methanol extract revealed 50 organic components, including alkaloids, terpenes, alkanes/alkenes, aldehydes/ketones, esters, ethers, and organic acids (Table 4), the majority of which are Cyclohexen-1-one,

2-methyl-5-(1-methyle phenyl (14.31%), Methane oxybis [dichloro] (5.33%), methyl-6,8-diox-abicyclo[3.2.1]octane (3.87%), and 4-(Acetylacetyl)-5-hydroxy-3- (4-chloropheneth yl)-1-phenylpyrazole (2.65%) as the major compounds (**Table 4**).

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Table (4). The major detectable chemical compounds in Mentha using GC-MS.					
Chemical compound	Rt (min)	(Area%)	M.wt	M.formula	S.Structure
2-Cyclohexen-1- one, 2-methyl-5-(1- methylet henyl)-, (R)- (CAS)	15.37	14.31	150	C <sub>10</sub> H <sub>14</sub> O	
Methane, oxybis[dichloro-	5.13	5.33	596	C34H26C12N2O4	
(exo)-4,5,7,7- Tetrameth yl-6,8- dioxabicyclo[3.2 .1]octane	32.06	3.87	170	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	
5(Dibromomethyl)- 1,3 - bis(tribromomethy l) benzene	35.62	2.90	744	C9H4Br8	
4-(Acetylacetyl)- 5-hydr oxy-3-(4'- chloropheneth yl)-1-	5.21	2.65	382	C21H19CIN2O3	
<u>phenylpyrazole</u> Phenol, 2,4-bis(1,1-dimethyleth - (CAS)	22.30	2.62	206	C14H22O	

RT: retention time, peak area % represents the concentration, M. wt: molecular weight, M. formula: molecular formula, C. structure: compound structure.

The GC/MS analysis of Mangifera revealed a total of 48 organic compounds, the majority of which are Chloroform (7.67%), decachloro-2,3-benzo- fluorene (3.27%), 1-bromo-2-chloro-1,1-d ifluoro-

2-tridecene (2.89%), and Formy-2- (2hydroxy ethyl)-2-devinylorin trimethyl ester (2.34%) as the major compounds (Table 5).

Chemical compound	Rt (min)	(Area%)	M.wt	M.formula	S.Structure
Chloroform	5.11	7.67	118	CHC <sub>13</sub>	
Dodecachloro-2,3- benzo Fluorene	5.26	3.27	624	C17Cl12	
1-Bromo-2-chloro- 1,1-d ifluoro-2-tridecene	32.06	2.89	330	C <sub>13</sub> H <sub>22</sub> BrClF <sub>2</sub>	Br F CI
3,3',5'-Tri-iodo-4'- isopro poxy-6-methoxy- 2'-met hyl-2- nitrobiphenyl	16.13	2.64	679	C17H16I3NO4	
Grcchpacgknysc/3	7.55	2.62	1495	N/A	
ë-Formyl-2-(2- hydroxy ethyl)-2- devinylorin e6 trimethyl ester	6.57	2.34	684	C38H44N4O8	
Ethyl)-2-devinylorin e6 trimethyl ester	8.90	2.45	818	C44H28Br2N4Ti	

Table 5. The major detectable chemical compounds in *Mentha* using GC-MS

**RT:** retention time, peak area % represents the concentration, M. wt: molecular weight, M. formula: molecular formula, C. structure: compound structure.

The analysis of the compound profile of *Mentha*, shows that it mainly contains 2-Cyclohexen-1-one, 2-methyl-5-(1-methyletphenyl), Phenol, (1,1-di methyl Ethyl) and Tri bromo methyl benzene as the major compounds, that's disagree with Mahboubi and Kazempour, 2014 who stated that menthol as the main component of mentha. Plant extract compositions can vary greatly based on geographical region, plant types and age, and extraction procedures.

The chemical analysis of *Eucalyptus* showed that Eucalyptol, Thiophene, Eicosene and Dichloro methyl propyl Sulfone as the major compounds, that's confirmed and agreed with results of Gilles et al. (2010).

It has been observed the enrichment of the analyzed volatiles of leaves of *Mangifera* with Chloroform, 2,3-Benzofluorene, 1-bromo-2-chloro-1,1- Difluoro-2-tridecene,

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and Formyl-2-(2-hydroxy ethyl)-2devinylorin e6 trimethyl ester.as the major compounds .our results agreed with the results of Husain et al. (2017)

GC-MS analysis of the extract had confirmed the presence of Esters (Formyl-2-(2-hydroxy ethyl)-2-devinylorin e6 trimethyl ester), phenols (hexadecanol), Bacterioruberin, Eucalyptol, Chloroform, and Flavonoids. Each of the isolated fractions is known to possess antibacterial activity due to various bioactivities.

In our study we found that lantana camara the highest value in DPPH and reducing power, but in the total phenolic Eucalyptus is the highest value. Based on the findings, we propose in a conclusion that Lantana, Eucalyptus, mentha, and Mangifera extracts can be used as an immunization strategy or a line of defense against dangerous bacteria as Pseudomonas aeruginosa.

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