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Research Article

Detection of Volatile Compounds Produced by *Pseudomonas aeruginosa* Isolated from UTI Patients by Gas Chromatography-Mass Spectrometry

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ABSTRACT

Pseudomonas aeruginosa bacteremia has become an important cause of mortality and morbidity over the past few decades. Bioactives were analyzed using gas chromatography-mass spectroscopy (GC-MS) techniques, then the in vitro antibacterial and antifungal activity of the methanolic extract was evaluated. Twenty nine bioactive compounds were identified in the methanolic extract of *Pseudomonas aeruginosa*. GC-MS analysis of *Pseudomonas aeruginosa* revealed the existence of the Oxime-,methoxy-phenyl, Edulan II, Methyl-4-[nitromethyl] -4- piperidinol, Acetamide, N-methyl-N- [4-[2-fluoromethyl-1 -pyrrolidyl-2-buty, Oxaspiro[4,4] nonane -4-one, 2-isopropyl, Octahydrochromen-2-one, 3,7-Diazabicyclo[3.3.1] nonane, 9,9-dimethyl, N-[3-[N-Aziridyl] propylidene] tetrahydrofurfurylamine, N-[3-t-Butylimino-1, 2-dimethylpropyl] aziridine, Benzenemethanol-aminopropoxy)-3-methyl, Borabicyclo[3.3.1] nonane, 9-methylthio, Butanamide, 3-propionylhydrazono-N-(2-ethylphenyl), Dithiocarbamate, S-methyl-, N- (2-methyl-3-oxobutyl)- , dl-Allo-cystathionine, Deoxyspergualin, dl-2,6-Diaminoheptanedioic acid, Glycyl-D-asparagine, Hydroxyphenazine, 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-, Cyclohexen-3-ol-1-one, 2-[11-tetradecenoyl]-, Ergotaman -3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'methyl, 3',8,8'-Trimethoxy-3-piperidyl -2,2'-binaphthalene-1,1',4,4'-tetr, Z-10-Methyl-11-tetradecen -1-ol propionate, -Cyclohex-3-enyl -propionic acid, Benzyl methyl ketone, Urea, N,N'-bis (2-hydroxyethyl, Cystine, Butanamide, N-(5-methyl- 3-isoxazoly)-2-[[4-methyl-5-(2-methyl-, Dasycarpidan-1-methanol, acetate (ester). *Punica granatum* (Crude) was very highly active (6.71±0.25) mm. The results of anti-fungal activity produced by *Pseudomonas aeruginosa* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus fumigatus* (6.000±0.32). *Pseudomonas aeruginosa* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Pseudomonas aeruginosa* can be useful.

Keywords: Antifungal activity, *Pseudomonas aeruginosa*, GC-MS, Secondary metabolites.

INTRODUCTION

The Gram-negative bacterium *Pseudomonas aeruginosa* is the most prevalent Gram-negative biofilm forming medical device associated pathogens[1,2]. *Pseudomonas aeruginosa* is an opportunistic pathogen that normally inhabits the soil and surfaces in aqueous environments. *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis* and *Enterobacter cloacae* are the most common bacterial uropathogens in UTI[3]. *Pseudomonas aeruginosa*, a ubiquitous gram-negative bacterium, has been intensively studied as the dominant pathogen infecting the lungs of cystic fibrosis patients[4], and an opportunistic human pathogen[5]. *Pseudomonas aeruginosa* accounts for 10%–20% of all hospital-acquired infections[6]. Nosocomial infections are estimated to occur annually in 1.75 million hospitalized patients throughout Europe, resulting in 175,000 deaths[7]. Serious *P. aeruginosa* infections are often nosocomial, and nearly all are associated with compromised host defenses such as in neutropenia, severe burns, or cystic fibrosis[8]. *Pseudomonas aeruginosa* is notoriously difficult to eradicate when colonizing the lungs of cystic fibrosis patients, forming thick antibiotic resistant biofilms that also guard from host immune defenses, lowering of the long-term prognosis of the infected patient[9]. *P. mirabilis* is a common cause of UTI in the complicated urinary tract, most frequently in patients with indwelling catheters or structural abnormalities of the urinary tract[10]. There are also a few sporadic reports describing human clinical isolates of *P. aeruginosa* that are capable of eliciting soft- rotsymptoms when infiltrated into a variety of plants including tomato, lettuce, onion, and tobacco[11]. Urinary catheter related infections are the most common form of nosocomial infection with over one million cases a year in the United

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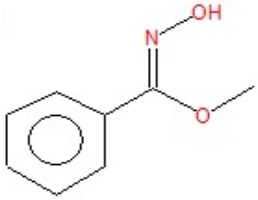
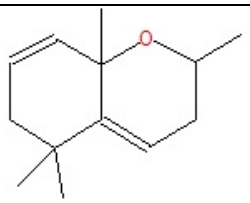
States alone[12]. In both *Pseudomonas aeruginosa* and *Escherichia coli* the flagellum-associated hook protein 1 is encoded by the *flgK* gene with a 40% correlation between the nucleotide sequences of the two species[13,14]. The presence of functional flagella enables the bacterium to swim and overcome repulsive electrostatic forces that

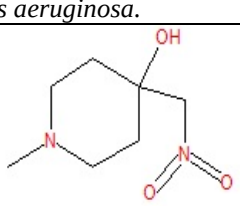
Table 1: Bioactive chemical compounds identified in methanolic extract of *Pseudomonas aeruginosa*.



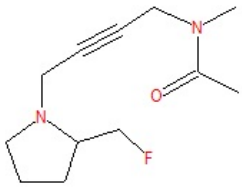
Oxime-,methoxy-phenyl
RT= 3.195
Mw= 151.063329
Pharmacological activity:
Anti-Bacteria



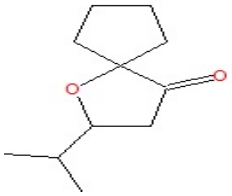
Edulan II
RT= 3.367
Mw= 192.151415
Pharmacological activity:
anti-bacterial *activity*



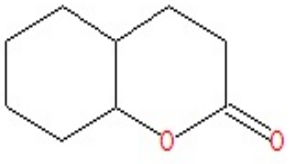
1-Methyl-4-[nitromethyl] -4-piperidinol
RT= 3.413
Mw= 174.100442
Pharmacological activity:
anti-cancer agents



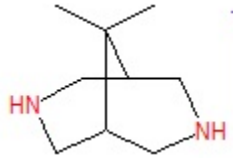
Acetamide,N-methyl-N- [4-[2-fluoromethyl-1 -pyrrolidyl-2-buty
RT= 3.521
Mw= 226.148142
Pharmacological activity:
antimicrobial



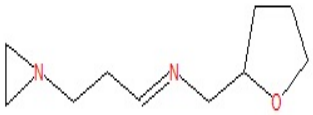
1-Oxaspiro[4,4] nonane -4-one , 2-isopropyl
RT= 3.590
Mw= 182.13068
Pharmacological activity:
anti-inflammatory



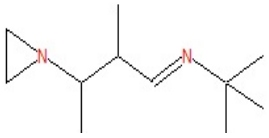
Octahydrochromen-2-one
RT= 3.653
Mw= 154.09938
Pharmacological activity:
Unknown



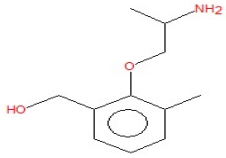
3,7-Diazabicyclo[3.3.1] nonane , 9,9-dimethyl
RT= 4.345
Mw= 154.146998
Pharmacological activity:
Unknown



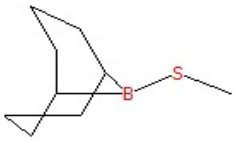
N-[3-[N-Aziridyl] propylidene] tetrahydrofurfurylamine
RT= 4.500
Mw= 182.141913
Pharmacological activity:
anti-inflammatory, antioxidant



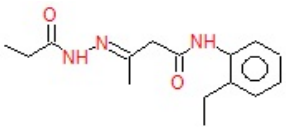
N-[3-t-Butylimino-1, 2-dimethylpropyl] aziridine
RT= 5.038
Mw= 182.178299
Pharmacological activity:
anti-bacterial *activity*



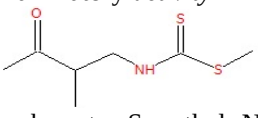
Benzenemethanol-aminopropoxy)-3-methyl
RT= 5.782
Mw= 195.125929
Pharmacological activity:
anti-inflammatory *activity*



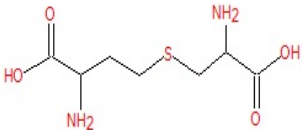
9-Borabicyclo[3.3.1] nonane , 9-methylthio
RT= 6.102
Mw= 168.114402
Pharmacological activity:
antibacteria



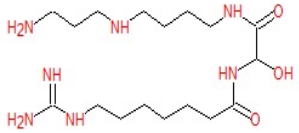
Butanamide, 3-propionylhydrazono-N-(2-ethylphenyl)
RT= 6.514
Mw= 275.163378
Pharmacological activity:
Antitumor



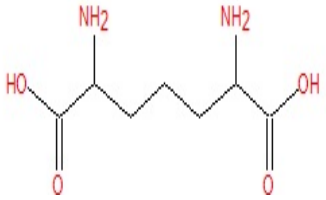
Dithiocarbamate , S-methyl-,N- (2-methyl-3-oxobutyl)-
RT= 7.046
Mw= 191.043856
Pharmacological activity:
Anti-bacterial, antifungal *activity*



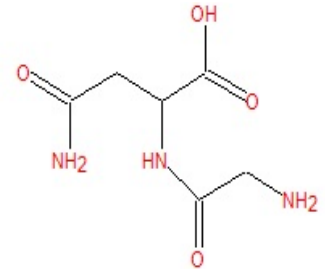
dl-Allo-cystathionine
RT= 7.681
Mw= 222.067428
Pharmacological activity:
anti- inflammatory



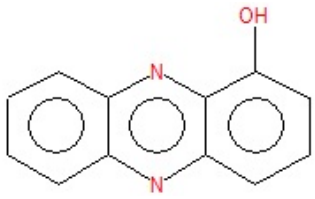
Deoxyspergualin
RT= 9.856
Mw= 387.295788
Pharmacological activity:
anti- tumor *activity*



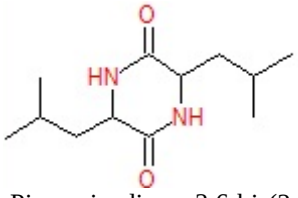
dl-2,6-Diaminoheptanedioic acid
RT= 11.967
Mw= 190.095357
Pharmacological activity:
Unknown



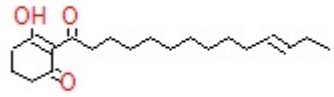
Glycyl-D-asparagine
RT= 12.453
Mw= 189.074956
Pharmacological activity:
analgesic activity



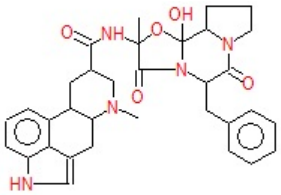
1-Hydroxyphenazine
RT= 14.336
Mw= 196.063663
Pharmacological activity:
anti-bacterial activity



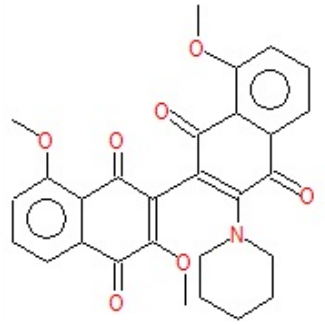
2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-
RT= 17.243
Mw= 226.168128
Pharmacological activity:
Unknown



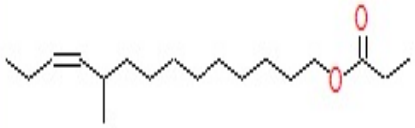
2-Cyclohexen-3-ol-1-one, 2-[11-tetradecenoyl]-
RT= 17.706
Mw= 320.235146
Pharmacological activity:
Unknown



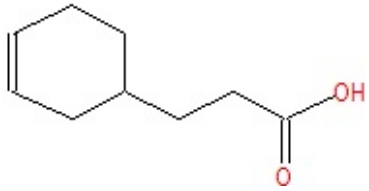
Ergotaman -3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'methyl
RT= 18.559
Mw= 583.27947
Pharmacological activity:
New chemical compound



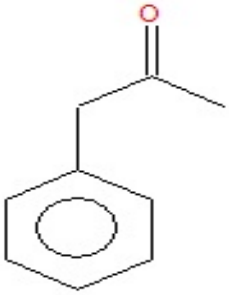
3',8,8'-Trimethoxy-3-piperidyl -2,2'-binaphthalene-1,1',4,4'-tetr
RT= 20.012
Mw= 487.163101
Pharmacological activity:
New chemical compound



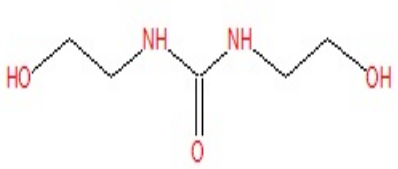
Z-10-Methyl-11-tetradecen -1-ol propionate
RT= 3.493
Mw= 282.25588
Pharmacological activity:
antiviral activity



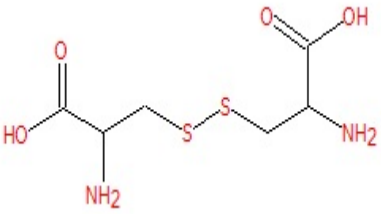
3-Cyclohex-3-enyl -propionic acid
RT= 3.916
Mw= 154.09938
Pharmacological activity:
anti-inflammatory *effect*



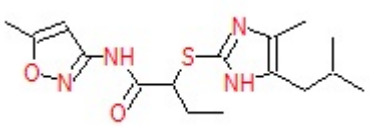
Benzyl methyl ketone
RT= 5.341
Mw= 134.073165
Pharmacological activity:
antibacterial and antifungal, antiviral, antiparasitic, *anti*-tubercular and insecticidal



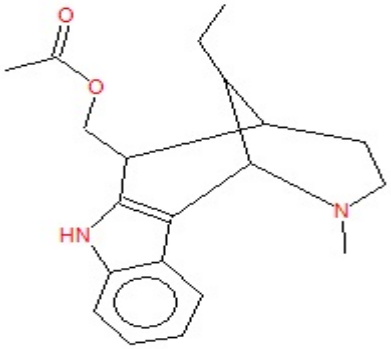
Urea, N,N'-bis (2-hydroxyethyl)
RT= 7.018
Mw= 148.084792
Pharmacological activity:
Unknown



Cystine
RT= 10.960
Mw= 240.023849
Pharmacological activity:
anti-inflammatory properties



Butanamide, N-(5-methyl- 3-isoxazoly)-2-[[4-methyl-5-(2-methyl-
RT= 16.556
Mw= 336.161997
Pharmacological activity: *anti*-tubercular activity



Dasycarpidan-1-methanol, acetate (ester)
RT= 19.760
Mw= 326.199429
Pharmacological activity: antimicrobial, antioxidant and *anti*-inflammatory

Table 2: Antifungal activity of *Pseudomonas aeruginosa* metabolite products.

Fungi	*Pseudomonas aeruginosa* metabolite products / Antibiotics			
	Pseudomonas aeruginosa metabolite products	Amphotericin B	Fluconazol	Miconazole nitrate
Microsporum canis	3.007±0.19 [a]	1.991±0.10	2.904±0.11	3.008±0.19
Streptococcus faecalis	3.000±0.20	2.903±0.15	3.007±0.14	2.000±0.10
Aspergillus flav	5.839±0.23	2.866±0.14	4.000±0.27	3.004±0.17
Aspergillus fumigatus	6.000±0.32	2.490±0.12	2.870±0.15	3.010±0.19
Candida albicans	5.608±0.19	3.718±0.20	3.009±0.12	2.090±0.19
Saccharomyces cerevisiae	3.971±0.20	2.195±0.14	1.994±0.10	3.000±0.20
Fusarium sp.	5.000±0.22	2.000±0.15	2.994±0.18	2.800±0.19
Mucor sp.	4.630±0.19	2.008±0.17	2.009±0.14	1.070±0.10
Penicillium expansum	3.900±0.17	2.910±0.19	3.000±0.19	2.001±0.16
Trichoderma viride	5.119±0.22	2.000±0.12	1.855±0.10	3.391±0.24
Trichoderma horzianum	4.002±0.19	0.991±0.02	3.999±0.19	3.001±0.21
Aspergillus niger	4.837±0.20	2.050±0.11	3.609±0.20	2.00±0.17
Aspergillus terreus	5.220±0.21	2.950±0.14	3.003±0.17	2.981±0.19

[a] The values (average of triplicate) are diameter of zone of inhibition at 100 mg/mL crude extract and 30 µg/mL of (Amphotericin B; Fluconazol and Miconazole nitrate).

may exist between the cell surface and the surface of material or the host's conditioning film[15]. This increased mortality may be reflective of more severe underlying illness or could be related to the greater inherent virulence of the organism. The aims of this research were analysis of the bioactive chemical products and evaluation of antibacterial and antifungal activity.

MATERIALS AND METHODS

Detection of secondary metabolites

Pseudomonas aeruginosa strain was isolated from bronchitis patients and obtained from Maternity and children hospital. Subcultures were obtained on the nutrient agar for 48 hrs. at 22°C. The mixture was incubated at 4ºC for 10 min and then shook for 10 min at 130 rpm. Metabolites was separated from the liquid culture and evaporated to dryness with a rotary evaporator at 45ºC. The residue was dissolved in 1 ml methanol, filtered through a 0.2 µm syringe filter, and stored at 4ºC for 24 h before being used for gas chromatography mass spectrometry[16-23]. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library as well as on comparison of their retention indices either with those of authentic compounds or with literature values. The studied fungi, *Candida albicans*, *Aspergillus niger*, *Aspergillus terreus*, *Penicillium expansum*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Trichoderma horzianum* and *Trichoderma viride* were isolated and maintained in potato dextrose agar slants. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for 16 days at 130 rpm. The extraction was performed by adding 25 ml methanol

Table 3: Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of medicinal plants to *Pseudomonas aeruginosa*.

S. No.	Plant	Zone of inhibition (mm)
1.	*Zingiber officinale* (Crude)	4.90±0.21
2.	*Nerium olender* (Alkaloids)	3.89±0.20
3.	*Ricinus communis* (Alkaloids)	3.00±0.19
4.	*Datura stramonium*(Alkaloids)	3.63±0.21
5.	*Linum usitatissimum* (Crude)	5.00±0.23
6.	*Anastatica hierochuntica* (Crude)	5.88±0.22
7.	*Cassia angustifolia* (Crude)	5.71±0.24
8.	*Euphorbia lathyrus* (Crude)	6.30±0.25
9.	*Rosmarinus oficinalis* (Crude)	5.54±0.23
10.	*Mentha viridis* (Crude)	6.00±0.24
11.	*Quercus infectoria* (Crude)	5.79±0.24
12.	*Citrullus colocynthis* (Crude)	4.00±0.17
13.	*Althaea rosea* (Crude)	5.01±0.20
14.	*Coriandrum sativum* (Crude)	6.01±0.26
15.	*Origanum vulgare* (Crude)	5.99±0.25
16.	*Urtica dioica* (Crude)	4.08±0.21
17.	*Foeniculum vulgare* (Crude)	3.05±0.19
18.	*Ocimum basilicum* (Crude)	4.77±0.23
19.	*Punica granatum* (Crude)	6.71±0.25
22.	Control	0.00

to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture[24-29].

Materials of Plants Collection and Preparation

In this study, the leaves were dried at room temperature for ten days and when properly dried the leaves were powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve[30-40]. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature.

Spectral analysis of bioactive natural chemical compounds of Pseudomonas aeruginosa using (GC/MS)

Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250oC). The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library[41-47].

Determination of antibacterial and antifungal activity

Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Quercus infectoria, Piper nigrum*, *Mentha viridis, Zingiber officinale, Gramineae poaceae*, *Ocimum basilicum*, *Nerium olender*, *Ricinus communis*, *Datura stramonium*, *Linum usitatissimum, Anastatica hierochuntica*, *Cassia angustifolia*, *Citrullus colocynthis, Euphorbia lathyrus, Rosmarinus oficinalis, Artemisia annua*, *Althaea rosea, Coriandrum sativum, Origanum vulgare, Urtica dioica*, *Equisetum arvense, Foeniculum vulgare, Nigella sativa*, *Punica granatum, Punica granatum* and *Cinnamomum zeylanicum*) were delivered into the wells. The plates were incubated for 48 h at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent[48-58]. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Data analysis

All the measurements were replicated three times for each assay and the results are presented as mean ± SD and mean ± SE. IBM SPSS 20 version statistical software package was used for statistical analysis of percentage inhibition and disease incidence and disease severity in each case.

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Pseudomonas aeruginosa*, shown in Table 1. The GC-MS chromatogram of the twenty nine peaks of the compounds detected was shown in Figure 1. Peaks were determined to be Oxime-,methoxy-phenyl, Edulan II, Methyl-4-[nitromethyl] -4- piperidinol , Acetamide ,N-methyl-N-[4-[2-fluoromethyl-1 -pyrrolidyl-2-buty, Oxaspiro[4,4] nonane -4-one, 2-isopropyl, Octahydrochromen-2-one, 3,7-Diazabicyclo[3.3.1] nonane, 9,9-dimethyl, N-[3-[N-Aziridyl] propylidene] tetrahydrofurfurylamine, N-[3-t-Butylimino-1, 2-dimethylpropyl] aziridine, Benzenemethanol-aminopropoxy)-3-methyl, Borabicyclo[3.3.1] nonane, 9-methylthio, Butanamide, 3-propionylhydrazono-N-(2-ethylphenyl), Dithiocarbamate, S-methyl-,N- (2-methyl-3-oxobutyl)-, dl-Allo-cystathionine, Deoxyspergualin, dl-2,6-Diaminoheptanedioic acid, Glycyl-D-asparagine,

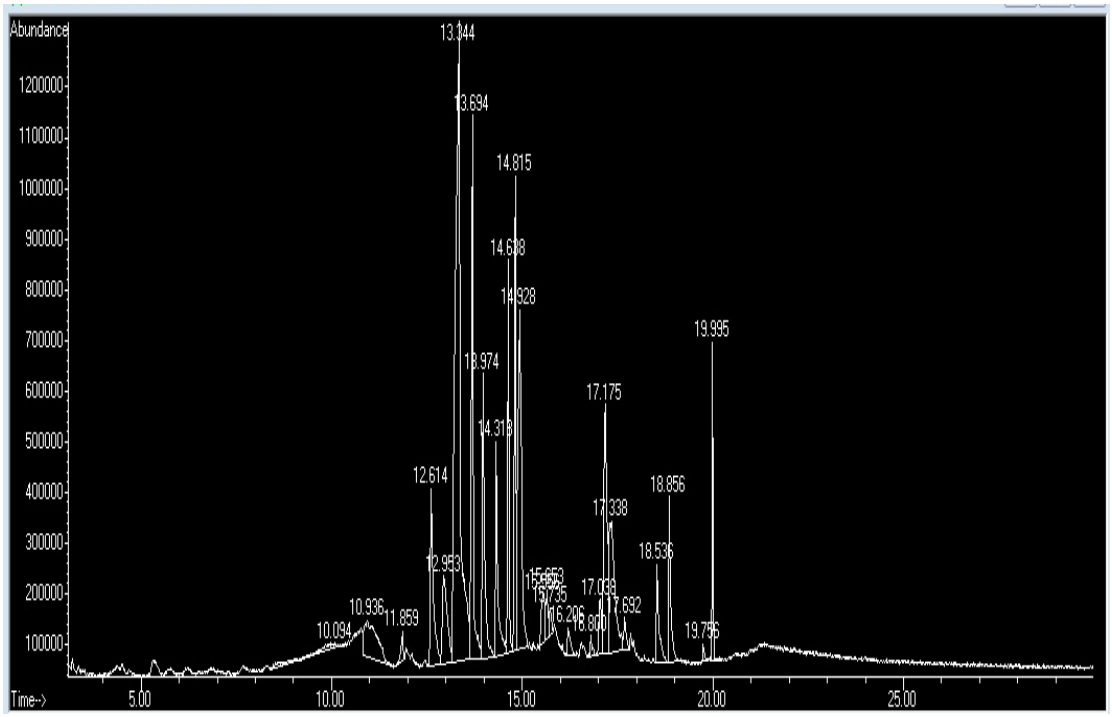


Figure 1: GC-MS chromatogram of methanolic extract of *Pseudomonas aeruginosa*.

Hydroxyphenazine, 2,5-Piperazinedione, 3,6-bis(2-methylpropyl)-, Cyclohexen-3-ol-1-one, 2-[11-tetradecenoyl]-, Ergotaman -3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'methyl, 3',8,8'-Trimethoxy-3-piperidyl - 2,2'-binaphthalene-1,1',4,4'-tetr, Z-10-Methyl-11-tetradecen -1-ol propionate, -Cyclohex-3-enyl - propionic acid, Benzyl methyl ketone, Urea, N,N'-bis (2-hydroxyethyl, Cystine, Butanamide, N-(5-methyl- 3-isoxazoly)-2-[[4-methyl-5-(2-methyl-, Dasycarpidan-1-methanol, acetate (ester). The results of anti-fungal activity produced by *Pseudomonas aeruginosa* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus fumigatus*. *Pseudomonas aeruginosa* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Pseudomonas aeruginosa* can be useful. Maximum zone formation against *Aspergillus fumigatus* (6.000±0.32) mm, Table 2. In agar well diffusion method the selected medicinal plants (*Linum usitatissimum, Citrullus colocynthis, Piper nigrum, Punica granatum, Zingiber officinale, Gramineae poaceae, Coriandrum sativum, Nerium olender, Ricinus communis, Datura stramonium, Anastatica hierochuntica, Cassia angustifolia, Euphorbia lathyrus, Rosmarinus oficinalis, Mentha viridis, Artemisia annua, Quercus infectoria, Althaea rosea, Origanum vulgare, Urtica dioica, Equisetum arvense, Foeniculum vulgare, Nigella sativa, Ocimum basilicum, Punica granatum,* and *Cinnamomum zeylanicum*) were effective against *Staphylococcus aureus*, Table 3. *Punica granatum* (Crude) was very highly active (6.71±0.25) mm against *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* was found to be sensitive to all test medicinal plants and mostly comparable to the standard reference antifungal drug Amphotericin B and fluconazole to some extent. Recently, it was demonstrated that volatile organic compounds (VOCs) of bacteria such as terpenoids, phenylpropanoids and fatty acid derivatives can influence the growth of some fungi and, in general, the inter- and intra-organismic communication signals.

CONCLUSION

Twenty nine bioactive chemical constituents have been identified from methanolic extract of the *Pseudomonas aeruginosa* by gas chromatogram mass spectrometry (GC-MS). In vitro antifungal and antibacterial evaluation of secondary metabolite products of *Pseudomonas aeruginosa* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

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REFERENCES

1. Christensen LD, Moser C, Jensen PO, Rasmussen TB, Christophersen L, Kjelleberg S, Kumar N, Hoiby N, Givskov M, Bjarnsholt T. Impact of *Pseudomonas aeruginosa* Quorum Sensing on Biofilm Persistence in an *in Vivo* Intraperitoneal Foreign-Body Infection Model. *Microbiology*. 2007; 153: 2312–2320.
2. Cole SJ, Records AR, Orr MW, Linden SB, Lee VT. Catheter-Associated Urinary Tract Infection by *Pseudomonas aeruginosa* is mediated by

Exopolysaccharide-Independent Biofilms. *Infect. Immun.* 2014; 82: 2048–2058.
3. Foxman B. Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. *Infectious Disease Clinics of North America*. 2014; 28: 1-13.
4. Pier GB, Grout M, Zaidi TS, Olsen JC, Johnson LG, Yankaskas JR, Goldberg JB. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. *Science*. 1996; 271: 64–67.
5. Britigan BE, Rasmussen GT, Cox CD. Augmentation of oxidant injury to human pulmonary epithelial cells by the *Pseudomonas aeruginosa* siderophore pyochelin. *Infect Immun*. 1997; 63: 1071–1076.
6. Guggenbichler, J.P.; Assadian, O.; Boeswald, M.; Kramer, A. Incidence and Clinical Implication of Nosocomial Infections Associated with Implantable Biomaterials—Catheters, Ventilator-Associated Pneumonia, Urinary Tract Infections. *GMS Krankenhhyg. Interdiszip.* 2011; 6: 1-19.
7. Ramos GP, Rocha JL, Tuon FF. Seasonal Humidity may Influence *Pseudomonas aeruginosa* Hospital-Acquired Infection Rates. *Int. J. Infect. Dis*. 2013; 17: e757–e761.
8. Lyczak JB, Cannon CL, Pier GB. Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. *Microbiol Infect*. 2000; 2: 1051–1060.
9. Hoiby N, Ciofu O, Bjarnsholt, T. *Pseudomonas aeruginosa* Biofilms in Cystic Fibrosis. *Future Microbiol.* 2010; 5: 1663–1674.
10. Warren JW, J.H. Tenney, J.M. Hoopes, H.L. Muncie, W.C. Anthony, A prospective microbiologic study of bacteriuria in patients with chronic indwelling catheters. *The Journal of Infectious Diseases*. 1982; 146: 719–723.
11. Cho JJ, Schroth MN, Kominos SD, Green SK. Ornamental plants as carriers of *Pseudomonas aeruginosa*. *Phytopathology*. 1975; 65: 425–431.
12. Foxman B, Brown P. Epidemiology of Urinary Tract Infections: Transmission and Risk Factors, Incidence, and Costs. *Infect. Dis. Clin. N. Am.* 2003; 17: 227–241.
13. Lejeune P. Contamination of Abiotic Surfaces: What a Colonizing Bacterium Sees and how to Blur it. *Trends Microbiol.* 2003; 11: 179–184.
14. O'Toole GA, Kolter R. Flagellar and Twitching Motility are Necessary for *Pseudomonas aeruginosa* Biofilm Development. *Mol. Microbiol.* 1998; 30: 295–304.
15. Dunne, W.M., Jr. Bacterial Adhesion: Seen any Good Biofilms Lately? *Clin. Microbiol. Rev.* 2002; 15: 155–166.
16. Kadhim MJ, Sosa AA, Hameed IH. Evaluation of anti-bacterial activity and bioactive chemical analysis of *Ocimum basilicum* using Fourier transform infrared (FT-IR) and gas chromatography-mass spectrometry (GC-MS) techniques. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 127-146.
17. Mohammed GJ, Kadhim MJ, Hussein HM. Characterization of bioactive chemical compounds from *Aspergillus terreus* and evaluation of antibacterial and antifungal activity. *International Journal of Pharmacognosy and Phytochemical Research.* 2016; 8(6): 889-905.
18. Hameed IH, Altameme HJ, Idan SA. Artemisia annua: Biochemical products analysis of methanolic aerial parts extract and anti-microbial capacity. *Research Journal of Pharmaceutical, Biological and Chemical Sciences.* 2016; 7(2): 1843- 1868.
19. Hussein AO, Mohammed GJ, Hadi MY, Hameed IH. Phytochemical screening of methanolic dried galls extract of *Quercus infectoria* using gas chromatography-mass spectrometry (GC-MS) and Fourier transform-infrared (FT-IR). *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(3): 49-59.
20. Sosa AA, Bagi SH, Hameed IH. Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research.* 2016; 8(5): 109-126.
21. Altameme HJ, Hadi MY, Hameed IH. Phytochemical analysis of *Urtica dioica* leaves by fourier-transform infrared spectroscopy and gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(10): 238-252.
22. Mohammed GJ, Omran AM, Hussein HM. Antibacterial and Phytochemical Analysis of *Piper nigrum* using Gas Chromatography-Mass Spectrum and Fourier-Transform Infrared Spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(6): 977-996.
23. Hamza LF, Kamal SA, Hameed IH. Determination of metabolites products by *Penicillium expansum* and evaluating antimicobial activity. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(9): 194-220.
24. Jasim H, Hussein AO, Hameed IH, Kareem MA. Characterization of alkaloid constitution and evaluation of antimicrobial activity of *Solanum nigrum* using gas chromatography mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(4): 56-72.
25. Hadi MY, Mohammed GJ, Hameed IH. Analysis of bioactive chemical compounds of *Nigella sativa* using gas chromatography-mass spectrometry. *Journal of Pharmacognosy and Phytotherapy.* 2016; 8(2): 8-24.
26. Hameed IH, Ibraheam IA, Kadhim HJ. Gas chromatography mass spectrum and fourier-transform infrared spectroscopy analysis of methanolic extract of *Rosmarinus oficinalis* leaves. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7 (6): 90-106.
27. Shareef HK, Muhammed HJ, Hussein HM, Hameed IH. Antibacterial effect of ginger (*Zingiber officinale*) roscoe and bioactive chemical analysis using gas chromatography mass spectrum. *Oriental Journal of Chemistry*. 2016; 32(2): 20-40.

28. Al-Jassaci MJ, Mohammed GJ, Hameed IH. Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity. International *Journal of Pharmaceutical and Clinical Research*. 2016; 8(5): 304-315.
29. Mohammed GJ, Al-Jassani MJ, Hameed IH. Anti-bacterial, Antifungal Activity and Chemical analysis of *Punica grantanum* (Pomegranate peel) using GC-MS and FTIR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 480-494.
30. Al-Marzoqi AH, Hadi MY, Hameed IH. Determination of metabolites products by *Cassia angustifolia* and evaluate antimicobial activity. *Journal of Pharmacognosy and Phytotherapy.* 2016; 8(2): 25-48.
31. Altameme HJ, Hameed IH, Abu-Serag NA. Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemila valgaris* seed using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Malays. Appl. Biol*. 2015; 44(4): 47–58.
32. Hameed IH, Hamza LF, Kamal SA. Analysis of bioactive chemical compounds of *Aspergillus niger* by using gas chromatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(8): 132-163.
33. Hameed IH, Hussein HJ, Kareem MA, Hamad NS. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7 (7): 107-125.
34. Hussein HM, Hameed IH, Ibraheem OA. Antimicrobial Activity and spectral chemical analysis of methanolic leaves extract of *Adiantum Capillus-Veneris* using GC-MS and FT-IR spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research*. 2016; 8(3): 369-385.
35. Hussein HJ, Hadi MY, Hameed IH. Study of chemical composition of *Foeniculum vulgare* using Fourier transform infrared spectrophotometer and gas chromatography - mass spectrometry. *Journal of Pharmacognosy and Phytotherapy*. 2016; 8(3): 60-89.
36. Kadhim MJ, Mohammed GJ, Hameed IH. In *vitro* antibacterial, antifungal and phytochemical analysis of methanolic fruit extract of *Cassia fistula*. *Oriental Journal of Chemistry*. 2016; 32(2): 10-30.
37. Altameme HJ, Hameed IH, Idan SA, Hadi MY. Biochemical analysis of *Origanum vulgare* seeds by fourier-transform infrared (FT-IR) spectroscopy and gas chromatography-mass spectrometry (GC-MS). *Journal of Pharmacognosy and Phytotherapy*. 2015; 7(9): 221-237.
38. Hussein HM. Determination of phytochemical composition and ten elements content (CD, CA, CR, CO, FE, PB, MG, MN, NI AND ZN) of *CARDARIA DRABA* by GC-MS, FT-IR and AAS technique. *Int. J Pharm Bio Sci.* 2016;7(3): (B) 1009 – 1017.
39. Hussein HM. Analysis of trace heavy metals and volatile chemical compounds of *Lepidium sativum* using atomic absorption spectroscopy, gas chromatography-mass spectrometric and fourier-transform infrared spectroscopy. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2016;7(4): 2529 – 2555.
40. Jaddoa HH, Hameed IH, Mohammed GJ. Analysis of volatile metabolites released by *Staphylococcus aureus* using gas chromatography-Mass spectrometry and determination of its antifungal activity. *Orient J Chem*. 2016; 32(4).
41. Hameed IH, Salman HD, Mohammed GJ. Evaluation of antifungal and antibacterial activity and analysis of bioactive phytochemical compounds of *Cinnamomum zeylanicum* (Cinnamon bark) using gas chromatography-mass spectrometry. *Orient J Chem*. 2016; 32(4).
42. Kadhim MJ, Mohammed GJ, Hussein HM. Analysis of bioactive metabolites from *Candida albicans* using (GC-MS) and evaluation of antibacterial activity. *International Journal of Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
43. Ubaid JM, Hussein HM, Hameed IH. Analysis of bioactive compounds of *Tribolium castaneum* and evaluation of anti-bacterial activity. International Journal of *Pharmaceutical and Clinical Research*. 2016; 8(7): 655-670.
44. Hameed IH, Jebor MA, Ommer AJ, Abdulzahra AI. Haplotype data of mitochondrial DNA coding region encompassing nucleotide positions 11,719–12,184 and evaluate the importance of these positions for forensic genetic purposes in Iraq. *Mitochondrial DNA*. 2016; 27(2): 1324-1327.
45. Hameed IH. A new polymorphic positions discovered in mitochondrial DNA hypervariable region HVIII from central and north-central of Iraq. *Mitochondrial DNA*. 2016; 27(5): 3250-4.
46. Mohammad A, Imad H. Autosomal STR: From locus information to next generation sequencing technology. *Research Journal of Biotechnology*. 2013.
47. Hameed IH, Abdulzahra AI, Jebor MA, Kqueen CY, Ommer AJ. Haplotypes and variable position detection in the mitochondrial DNA coding region encompassing nucleotide positions 10,716-11,184. *Mitochondrial DNA*. 2015.
48. Anesini C, Perez C. Screening of plants used in Argentine folk medicine for antimicrobial activity. *J. Ethnopharmacol*. 1993; 39: 119-128.
49. Rajasekar T, Balaji S, Kumaran S. Isolation and characterization of marine fungi metabolites against clinical pathogens. *Asian. pacific journal of tropical disease*. 2012; S387-S392.
50. Tabaraie B, Ghasemian E, Tabaraie T. Comparitive evolution of cephalosphrinc production in solid state fermentation and submerged liquid culture. *Journal of microbial biotechnology food science*. 2012; 2(1): 83-94.
51. Gebreselema G, Feleke M, Samuel S, Nagappan R. Isolation and characterization of potencial antibiotic

producing actinomycetes from water and sediments of lake Tana, Ethiopa. *Asian pacific journal of Tropical biomedicine*. 2013; 3(6): 426-435.

52. Usha NS and Masilamani SM. Bioactive compound produced by streptomycin strain. *International journal of pharmacy and pharmaceutical science*. 2013; 5(1): 0975-14.
53. Anupama M, Narayana KJ, Vijayalakshmi M. Screening of streptomyces perpuofucus for antimicrobial metabolites. *Res journal of microbiology*. 2007; 2: 992-994.
54. Altaee N, Kadhim MJ, Hameed IH. Characterization of metabolites produced by *E. coli* and analysis of its chemical compounds using GC-MS. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).
55. Hussein J H, Ubaid JM, Hameed IH. Gas chromatography – mass spectrum analysis of volatile components of methanolic leaves extract of *Cordia myxa. International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).
56. Kadhim MJ. *In Vitro* antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry. *Der Pharma Chemica*. 2016; 8(19): 657-665.
57. Al-Yaseri A, Kadhim WA, Hameed IH. Detection of volatile compounds emitted by *Proteus mirabilis* isolated from UTI patients and its anti-fungal potential. *Der Pharma Chemica*. 2016; 8(19): 671-678.
58. Ubaid JM, Kadhim MJ, Hameed IH. Study of bioactive methanolic extract of *Camponotus fellah* using Gas chromatography – mass spectrum. *International Journal of Current Pharmaceutical Review and Research*. 2017; 7(6).