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In Vitro antifungal potential of *Acinetobacter baumannii* and determination of its chemical composition by gas chromatography-mass spectrometry

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ABSTRACT

The aims of this research were analysis of the bioactive chemical products and evaluation of antibacterial and antifungal activity. Fifty one bioactive compounds were identified in the methanolic extract of Acinetobacter baumannii. GC-MS analysis of Acinetobacter baumannii revealed the existence of the Malonic acid ,mononitrile , monothioamide, Oxime-,methoxy, 1,2,3-Propatriol, 1-indol-4-yl ; Propenoic acid , 3-[(phenylmethyl)sulfonyl]- , methyl ester, Cis-Pinen-3-ol, 3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone, 3-(1-Cyclopentenyl)furan , 3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone, 2-Phenylethanamidine, Cyclohexane, 1,3-butadienylidene- , Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, 4H-1-Benzopyran-4-one, 6,7-dimethoxy-3-phenyl-, Cyclopentolate, Levmetamfetamine, Ethanamine,2-(2,6-dimethylphenoxy)-N-methyl, (3-Ethoxy-4,5-dihydro-isoxazol-5-ylmethyl)-amine, DL-Leucine , N-glycyl- , 3,6,9,12-Tetraoxatetradecan-1-ol , 14-[4-(1,1,3-tetramethyl , Phenethylamine , N-hexyl , Dithiocarbamate ,S-methyl-,N-(2-methyl-3-oxobuty , N-Methyl-4-phenyl-butyramide , Hydrazinecarboxamide ,2-[3-cyclohex-3-en- , Hydrazinecarboxamide,2-[3-cyclohex-3-en-, Pterin-6-carboxylic acid , Benzofuran-2-one , 4-amino-2,3-dihydro-, Cyclopentanecarboxylic acid , 1-phenyl -,2-(diethylamino)ethy, Ethanamine , 2-(2,6-dimethylphenoxy)-N-methyl-, Diaziridinone, (1,1-dimethylethyl)(1,1-dimethyl-2-phenylethyl)-, dl-Alanyl-dl-serine, 4-Ethoxy-6-methylhexahydropyrimidin-2-thione, 4H,5H-Pyrano[4,3-d]-1,3-dioxin , tetrahydro-8a-methyl-, Eicosanoic acid, phenylmethyl ester, Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-, Dibutyl phthalate, Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-3-(2-methylpropy, Pterin-6-carboxylic acid, Cytidine , 5-methyl-, 12-Methyl-oxa-cyclododecan-2-one, 3,7-Diazabicyclo[3,3,1]nonane, 9,9-dimethyl-, Actinomycin C2, 4-Azepan-1-yl-oxazolidin-2-one, 4-Carbamoyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl, i-Propyl 9-tetradecenoate, 4,4,6-Trimethyl-2-(N-methyl-m-chlorophenylamino)-5,6-dihydro, 2-Butenoic acid , 4-(morpholin-4-yl)-, methyl ester, 2,5-Piperazinedione , 3,6-bis(2-methylpropyl)-, l-f Leucyl -d-leucine, 3H-3a-Azacyclopenta[a]undene -2-carbonitrile ,3-oxo-1-(piper, and Phthalic acid , di(2-propylpentyl) ester. The results of anti-fungal activity produced by Acinetobacter baumannii showed that the volatile compounds were highly effective to suppress the growth of Aspergillus flavus. Acinetobacter baumannii produce many important secondary metabolites with high biological activities.

Keywords: *Acinetobacter baumannii*, Antifungal activity, gas chromatography-mass spectrometry, Metabolites.

INTRODUCTION

Acinetobacter baumannii is an opportunistic, Gram-negative, cocco-bacillus which is known to cause nosocomial infections like septicaemia, bacteraemia, pneumonia, wound sepsis, endocarditis, meningitis, and urinary tract infections [1, 2]. Acinetobacter are usually found in the hospital environment and infect patients who are hospitalized from a long period of time with severe underlying diseases, are immunosuppressed, or subjected to invasive procedures and treated with broad-spectrum antibiotics [3]. During outbreaks along with one or more epidemic Acinetobacter clones, there exists anendemic strain which makes it difficult to identify and control their transmission [4]. Reports on resistance mechanisms in *A. baumannii* mainly focused on outer membrane impermeability, production of beta lactamases and production of efflux pump. However, equally important for

bacterial survival are penicillin-binding proteins (PBPs) which play a crucial role in the synthesis of peptidoglycan, an essential component of the bacterial cell wall [5, 6]. *A. baumannii* is resistant to desiccation and thus hard to eradicate once established in wards. Carbapenem group, which includes imipenem and meropenem are last resort of β-lactams with highest efficacy and have broad spectrum against several Gram-negative bacteria including *A. baumannii* [7-10]. In the imipenem resistant (I^R) clone of *A. baumannii*, they found a diminished expression of all the PBPs except 24 kDa PBP which is increased in its amount and showed low affinity for imipenem. Cuenca *et al* [11] from University Hospital in Spain found large variations in the PBP patterns (analyzed by ^{125} I Ampicillin reagent) and found only 6 PBPs (93, 84, 73, 64, 49 and 38 kDa) with the decreased expression of 73 kDa PBP in *A. baumannii*. Both the reports utilized the radioactive material for identification of PBPs. In the present study, an attempt was made to identify bioactive natural compounds of *A. baumannii*

MATERIALS AND METHODS

Growth conditions and determination of metabolites

Acinetobacter baumannii strain was isolated from 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 GC-MS. 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, *Aspergillus terreus, Aspergillus flavus, Candida albicans, Microsporum canis, Trichophyton mentagrophytes* 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 to 100 ml liquid culture in an Erlenmeyer flask after the infiltration of the culture.

Spectral analysis of bioactive compounds using gas chromatography-mass spectrometry

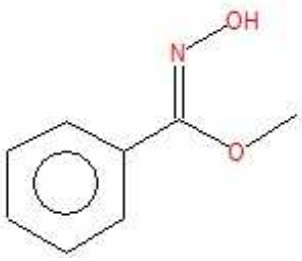
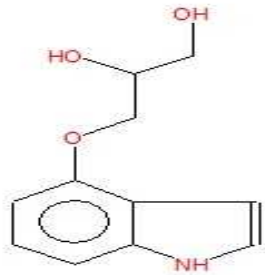
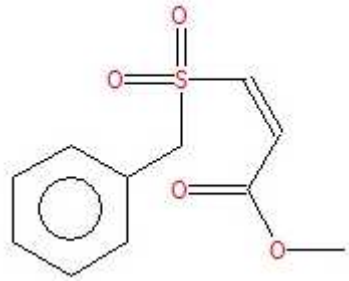
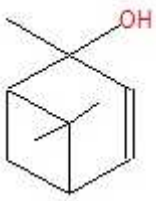
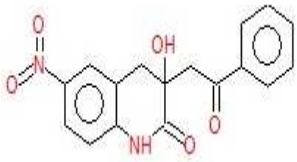
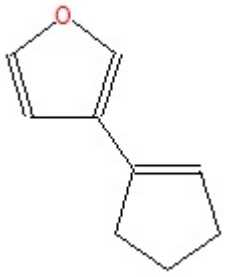
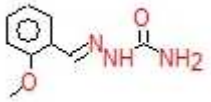
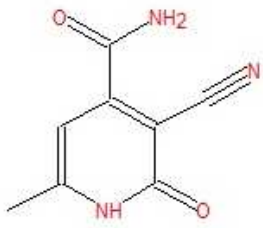
Analysis was conducted using GC-MS (Agilent 789 A) equipped with a DB-5MS column (30 m×0.25 mm i.d., 0.25 um film thickness, J&W Scientific, Folsom, CA). The oven temperature was programmed as for the previous analysis. Helium was used as the carrier gas at the rate of 1.0 mL/min. Effluent of the GC column was introduced directly into the source of the MS via a transfer line (250oC). Ionization voltage was 70 eV and ion source temperature was 230oC. Scan range was 41- 450 amu. The components were identified by comparing their retention times to those of authentic samples of WILEY MASS SPECTRAL DATA BASE Library.

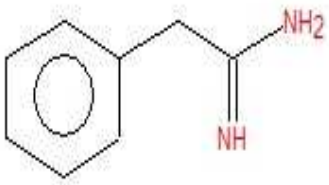
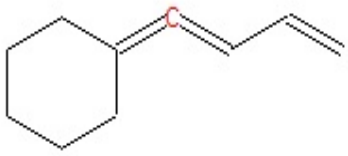
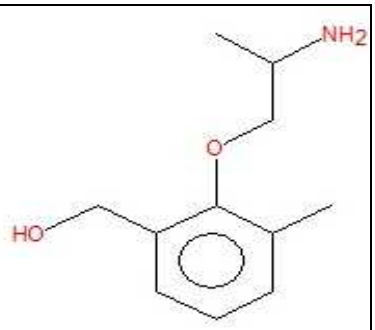
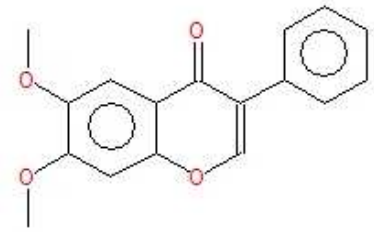
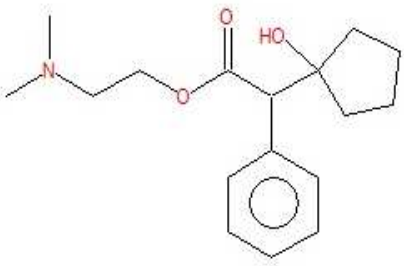
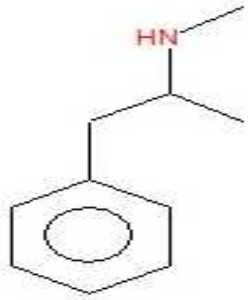
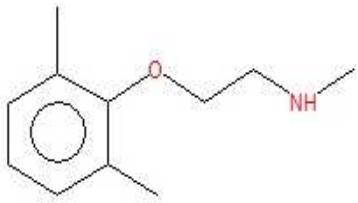
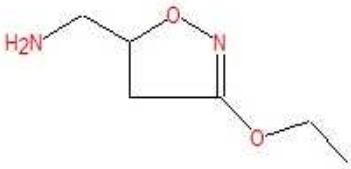
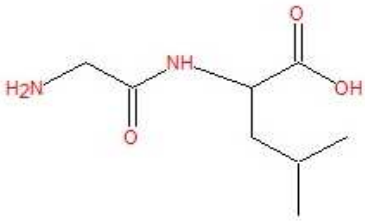
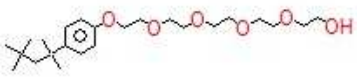
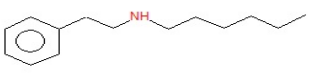
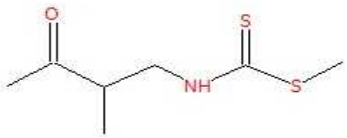
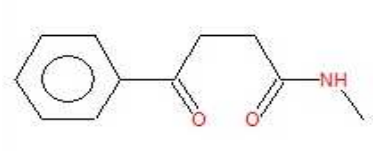
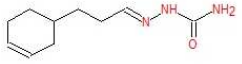
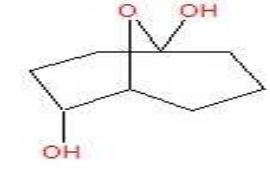
RESULTS AND DISCUSSION

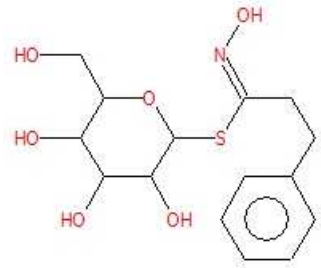
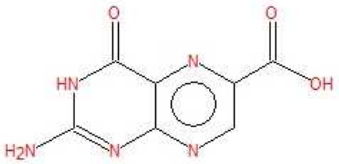
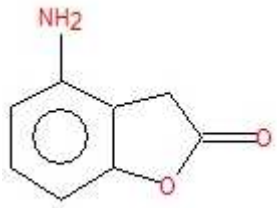
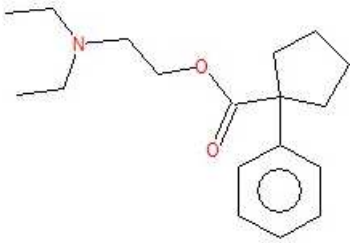
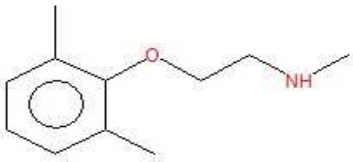
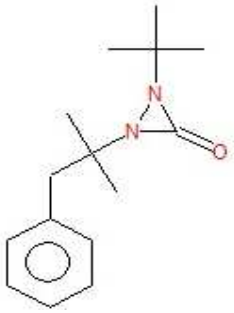
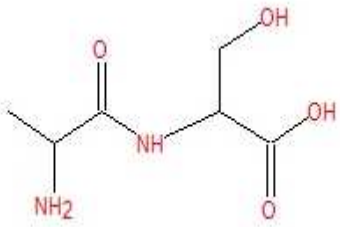
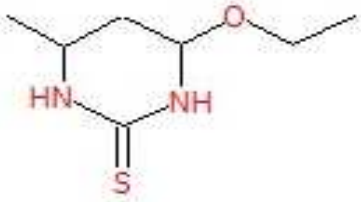
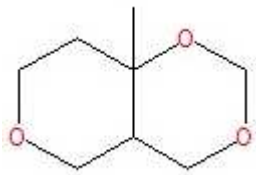
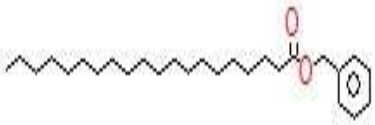
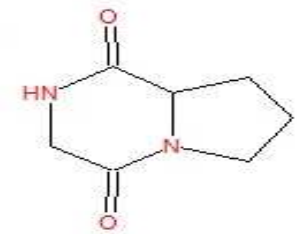
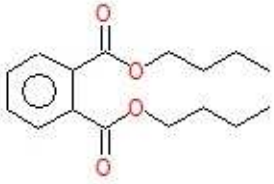
Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Acinetobacter baumannii*, shown in **Table 1.** The GC-MS chromatogram of the Fifty one peaks of the compounds detected was shown in **Figure 1.** The First set up peak were determined to be Malonic acid ,mononitrile , monothioamide, Oxime-,methoxy, 1,2,3-Propatriol, 1-indol-4-yl ; Propenoic acid , 3-[(phenylmethyl)sulfonyl]- , methyl ester, Cis-Pinen-3-ol , 3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone, 3-(1-Cyclopentenyl)furan , 3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone, 2-Phenylethanamidine, Cyclohexane, 1,3-butadienylidene- , Benzenemethanol, 2-(2-aminopropoxy)-3-methyl, 4H-1-Benzopyran-4-one, 6,7-dimethoxy-3-phenyl-, Cyclopentolate, Levmetamfetamine, Ethanamine,2-(2,6-dimethylphenoxy)-N-methyl, (3-Ethoxy-4,5-dihydro-isoxazol-5-ylmethyl)-amine, DL-Leucine , N-glycyl- , 3,6,9,12-Tetraoxatetradecan-1-ol , 14-[4-(1,1,3-tetramethyl , Phenethylamine , N-hexyl , Dithiocarbamate ,S-methyl-,N-(2-methyl-3-oxobuty , N-Methyl-4-phenyl-butyramide , Hydrazinecarboxamide ,2-[3-cyclohex-3-en- , Hydrazinecarboxamide ,2-[3-cyclohex-3-en-, Pterin-6-carboxylic acid, Benzofuran-2-one , 4-amino-2,3-dihydro-, Cyclopentanecarboxylic acid, 1-phenyl -,2-(diethylamino)ethy, Ethanamine , 2-(2,6-dimethylphenoxy)-N-methyl-, Diaziridinone , (1,1-dimethylethyl)(1,1-dimethyl-2-phenylethyl), dl-Alanyl-dl-serine, 4-Ethoxy-6-methylhexahydropyrimidin-2-thione, 4H,5H-Pyrano[4,3-d]-1,3-dioxin , tetrahydro-8a-methyl-, Eicosanoic acid, phenylmethyl ester, Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-, Dibutyl phthalate, Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-3-(2-methylpropy, Pterin-6-carboxylic acid, Cytidine , 5-methyl-, 12-Methyl-oxa-cyclododecan-2-one, 3,7-Diazabicyclo[3,3,1]nonane , 9,9-dimethyl-, Actinomycin C2, 4-Azepan-1-yl-oxazolidin-2-one, 4-Carbamoyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl, i-Propyl 9-tetradecenoate, 4,4,6-Trimethyl-2-(N-methyl-m-chlorophenylamino)-5,6-dihydro, 2-Butenoic acid , 4-(morpholin-4-yl)-, methyl ester, 2,5-Piperazinedione , 3,6-bis(2-methylpropyl)-, l-f Leucyl -d-leucine, 3H-3a-Azacyclopenta[a]undene -2-carbonitrile ,3-oxo-1-(piper, and Phthalic acid , di(2-propylpentyl) ester. *Acinetobacter baumannii* produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive

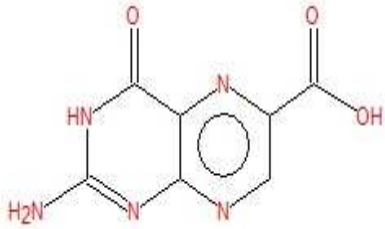
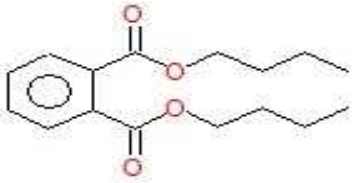
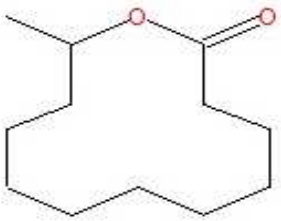
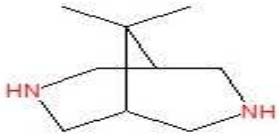
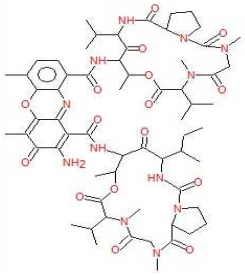
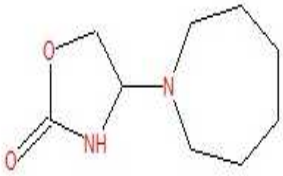
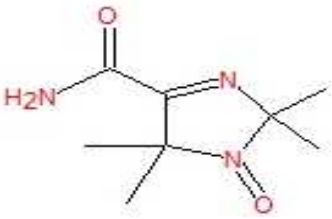
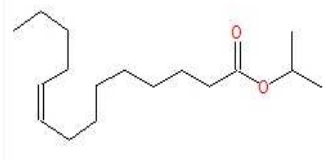
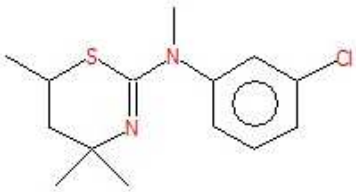
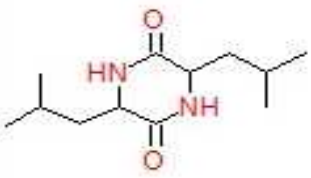
compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by *Acinetobacter baumannii* can be useful. Maximum zone formation against *Aspergillus flavus* (7.07±0.26) mm, **Table 2**. Many microbes, including *A. baumannii*, have several properties that allow them to be more successful as pathogens. These properties may be virulence factors such as toxins or toxin delivery systems which directly affect the host cell. They may also be virulence determinants, which are qualities contributing to a microbe's fitness and allow it to survive the host environment, but that do not affect the host directly [44-46]. Multidrug resistance in *A. baumannii* is a common clinical problem which further complicates the therapy. The first known *A. baumannii* resistant to carbapenem was reported as early as in 1985 in Scotland. The nosocomial outbreaks began in 1990s in all around the world and since then, incidence of carbapenem resistant isolates from hospitals worldwide has become a subject of concern. In the past, multiple outbreaks of such infections caused by *A. baumannii* have been reported from different regions of India [47-49].

Table 1. Bioactive chemical compounds identified in methanolic extract of *Acinetobacter baumannii*.

 Mal onic acid ,mononitrile , monothioamide , **RT**=3.121 **Mw**=208.01289 **Pharmacological activity:** Unknown	 Oxime- ,methoxy-phenyl **RT**=3.396 **Mw**=151.063329 **Pharmacological activity:** Anti-Bacteria	 1,2,3-Propatriol, 1-indol-4-yl **RT**=4.300 **Mw**=207.089543 **Pharmacological activity:** anti-inflammatory
 2-Propenoic acid , 3-[(phenylmethyl)sulfonyl]- , methyl ester . **RT**=4.489 **Mw**=240.0456295 **Pharmacological activity:** antibacterial, antioxidant,	 Cis-Pinen-3-ol **RT**=5.587 **Mw**=152.120115 **Pharmacological activity:** anti-microbial, anti-inflammatory	 3-Benzoylmethyl-3-hydroxy-5-nitro-2-indolinone **RT**=5.473 **Mw**=326.090271 **Pharmacological activity:** anti-inflammatory
 3-(1-Cyclopentenyl)furan **RT**=5.553 **Mw**=134.073165 **Pharmacological activity:** analgesic and anti-inflammatory activities	 o-Anisaldehyde , semicarbazone **RT**=5.828 **Mw**=193.085127 **Pharmacological activity:** antimicrobial activities	 4-Pyridinecarboxamide , 3-cyano-1,2-dihydro-6-methyl-2- xo-o **RT**=5.885 **Mw**=177.053826 **Pharmacological activity:** anticancer activity

 2-Phenylethanamidine **RT**=6.034 **Mw**=134.084398 **Pharmacological activity:** Unknown	 Cyclohexane , 1,3-butadienylidene- **RT**=6.154 **Mw**=134.10955 **Pharmacological activity:** anti-Candida, anti-inflammatory, antidiabetic	 Benzenemethanol , 2-(2-aminopropoxy)-3-methyl- **RT**=6.377 **Mw**=195.125929 **Pharmacological activity:** anti-inflammatory activity
 4H-1-Benzopyran-4-one , 6,7-dimethoxy-3-phenyl- **RT**=6.955 **Mw**=282.089209 **Pharmacological activity:** Anti- inflammatory *activity*	 Cyclopentolate **RT**=7.487 **Mw**=291.183443 **Pharmacological activity:** anti-hypertensive	 Levmetamfetamine **RT**=7.773 **Mw**=149.120449 **Pharmacological activity:** Anti-inflammatories
 Ethanamine ,2-(2,6-dimethylphenoxy)-N-methyl- **RT**=7.813 **Mw**=179.131014 **Pharmacological activity:** Anti-rrhythmic	 (3-Ethoxy-4,5-dihydro-isoxazol-5-ylmethyl)-amine **RT**=8.197 **Mw**=144.089878 **Pharmacological activity:** Anti-inflammatory effect	 DL-Leucine , N-glycyl- **RT**=8.345 **Mw**=188.116093 **Pharmacological activity:** Unknown
 3,6,9,12-Tetraoxatetradecan-1-ol , 14-[4-(1,1,3-tetramethyl **RT**=8.483 **Mw**=426.29814 **Pharmacological activity:** anti-bacterial effect	 Phenethylamine , N-hexyl- **RT**=8.540 **Mw**=205.18305 **Pharmacological activity:** Anti-inflammatory effect	 Dithiocarbamate ,S-methyl-,N-(2-methyl-3-oxobuty **RT**=8.883 **Mw**=191.043856 **Pharmacological activity:** Anti-bacterial and antifungal activity
	1-Hydrazinecarboxamide ,2-[3-cyclohex-3-en-1-yl)propyliden 	

- N Methyl-4-phenyl-butyramide **RT**=8.906 **Mw**=191.094628 **Pharmacological activity:** anti-epileptic and anti-microbial agents	**RT**=8.998 **Mw**=195.137162 **Pharmacological activity:** anti-parasitic activity	9-Oxa-bicyclo[3.3.1]nonane-1,4-diol **RT**=9.089 **Mw**=158.094295 **Pharmacological activity:** anti-inflammatory activity
 β-D-Glucopyranose , 1-thio , 1- (N-hydroxybenzenepropanimid **RT**=9.330 **Mw**=343.10896 **Pharmacological activity:** Unknown	 Pterin-6-carboxylic acid **RT**=11.035 **Mw**=207.039239 **Pharmacological activity:** antioxidant and anti- inflammatory activity	 Benzofuran-2-one , 4-amino-2,3-dihydro- **RT**=11.229 **Mw**=149.047678 **Pharmacological activity:** anticancer, antiviral and anti-inflammatory
 Cyclopentanecarboxylic acid , 1-phenyl -,2-(diethylamino)ethy **RT**=11.487 **Mw**=289.20418 **Pharmacological activity:** antipsychotic, anti-ischemic	 Ethanamine , 2-(2,6-dimethylphenoxy)-N-methyl- **RT**=11.670 179.131014 **Pharmacological activity:** anti-inflammatory	 Diaziridinone , (1,1-dimethylethyl)(1,1-dimethyl-2-phenylethyl)- **RT**=11.779 **Mw**=246.173213 **Pharmacological activity:** Unknown
 dl-Alanyl-dl-serine **RT**=12.070 **Mw**=176.079707 **Pharmacological activity:** antimicrobial agents	 4-Ethoxy-6-methylhexahydropyrimidin-2-thione **RT**=12.099 **Mw**=174.0826845 **Pharmacological activity:** Unknown	 4H,5H-Pyrano[4,3-d]-1,3-dioxin , tetrahydro-8a-methyl- **RT**=12.219 **Mw**=158.094295 **Pharmacological activity:** Unknown
 Eicosanoic acid, phenylmethyl ester **RT**=12.511 **Mw**=402.349781 **Pharmacological activity:** anti-inflammatory activity	 Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro- **RT**=13.266	 Dibutyl phthalate **RT**=14.062 **Mw**=278.15181 **Pharmacological activity:** antimicrobial activity

	Mw=154.074227 **Pharmacological activity:** Unknown	
 Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-3-(2-methylpropy **RT**=15.023 **Mw**=210.136827 **Pharmacological activity:** antibacterial activity	 Pterin-6-carboxylic acid **RT**=15.687 **Mw**=207.039239 **Pharmacological activity:** antioxidant and anti-inflammatory activity	 Cytidine , 5-methyl- **RT**=15.841 **Mw**=257.101171 **Pharmacological activity:** Anti-hepatitis C
 12-Methyl-oxa-cyclododecan-2-one **RT**=15.887 **Mw**=198.16198 **Pharmacological activity:** anti-inflammatory	 3,7-Diazabicyclo[3,3,1]nonane , 9,9-dimethyl- **RT**=16.196 **Mw**=154.146998 **Pharmacological activity:** Unknown	 Actinomycin C2 **RT**=16.259 **Mw**=1268.64413 **Pharmacological activity:** Anti-Infective
 4-Azepan-1-yl-oxazolidin-2-one **RT**=16.076 **Mw**=184.121178 **Pharmacological activity:** Anti-Infective	 4-Carbamoyl-2,2,5,5-tetramethyl-3-imidazoline-1-oxyl **RT**=16.877 **Mw**=184.108602 **Pharmacological activity:** anti-oxidative	 i-Propyl 9-tetradecenoate **RT**=16.928 **Mw**=268.24023 **Pharmacological activity:** Antimicrobial activity
 4,4,6-Trimethyl-2-(N-methyl-m-chlorophenylamino)-5,6-dihydro **RT**=17.295 **Mw**=282.095747 **Pharmacological activity:** antimicrobial, anti-viral and anticancer agents	 2-Butenoic acid , 4-(morpholin-4-yl)-, methyl ester **RT**=17.415 **Mw**=185.105193 **Pharmacological activity:** Unknown	 2,5-Piperazinedione , 3,6-bis(2-methylpropyl)- **RT**=17.586 **Mw**=226.168128 **Pharmacological activity:** Unknown

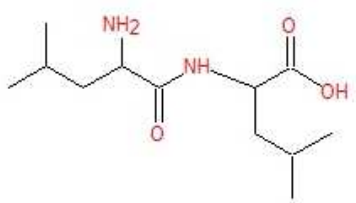
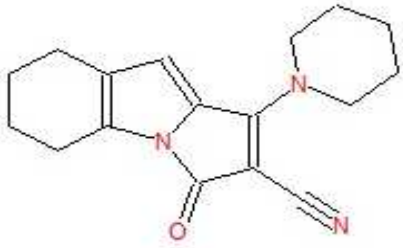
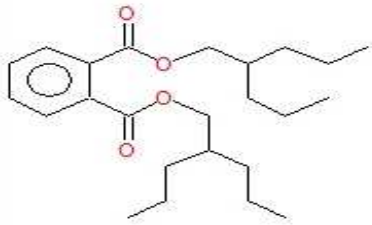
 l-f Leucyl -d-leucine **RT**=17.832 **Mw**=244.178693 **Pharmacological activity:** Anti-malarial activity	 3H-3a-Azacyclopenta[a]undene -2-carbonitrile ,3-oxo-1-(piper **RT**=18.250 **Mw**=281.152813 **Pharmacological activity:** Unknown	 Phthalic acid , di(2-propylpentyl) ester **RT**=20.007 **Mw**=390.27701 **Pharmacological activity:** anti-inflammatory activity

Table 2. Antifungal activity of *Acinetobacter baumannii* metabolite products.

Fungi	*Acinetobacter baumannii* metabolite products / Antibiotics			
	***Acinetobacter baumannii* metabolite products**	**Amphotericin B**	**Fluconazol**	**Miconazole nitrate**
Aspergillus niger	5.14±0.21 [a]	2.10±0.10	3.09±0.14	3.00±0.14
Aspergillus terreus	6.05±0.22	3.63±0.13	1.93±0.11	3.00±0.14
Aspergillus flavus	7.07±0.26	3.00±0.18	4.07±0.21	3.05±0.16
Aspergillus fumigatus	6.02±0.20	2.00±0.16	2.90±0.18	1.99±0.12
Candida albicans	5.79±0.21	3.28±0.17	2.95.±0.19	1.79±0.11
Saccharomyces cerevisiae	3.89±0.17	1.98±0.15	2.11±0.15	2.87±0.15
Microsporum canis	4.00±0.19	2.00±0.10	3.02±0.19	2.00±0.11
Streptococcus faecalis	4.02±0.20	2.98±0.13	2.00±0.11	2.09±0.11
Penicillium expansum	3.64±0.19	3.90±0.19	3.02±0.16	2.47±0.13
Trichoderma viride	5.86±0.21	1.19±0.12	1.99±0.10	3.01±0.18

[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).*

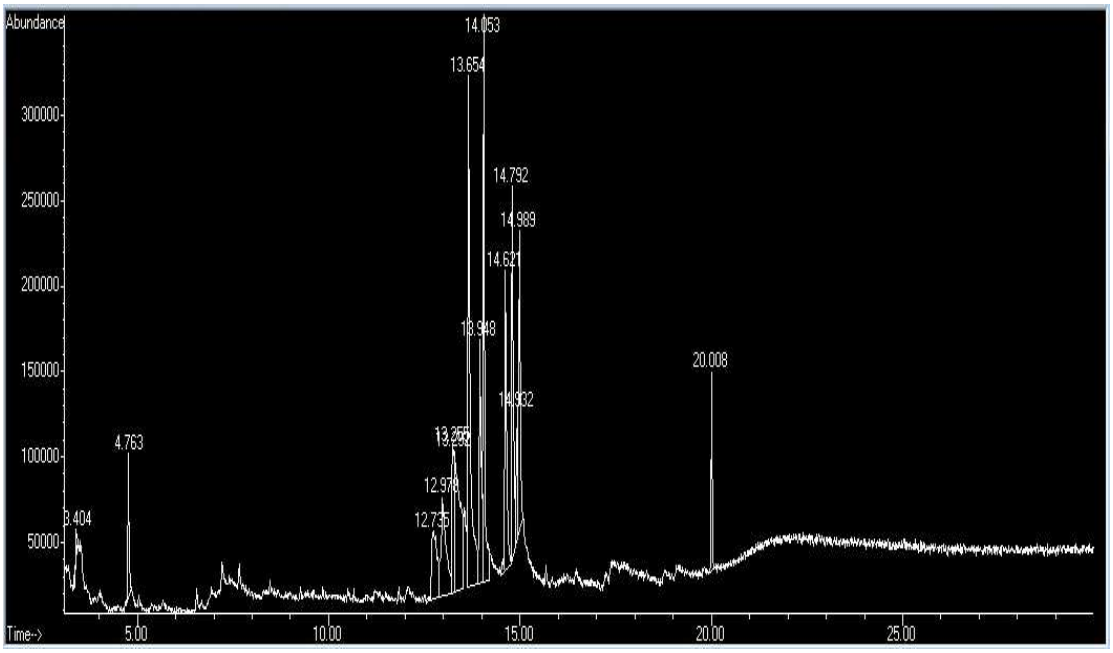


Figure 1: GC-MS chromatogram of methanolic extract of *Acinetobacter baumannii*.

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