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Detection of volatile compounds emitted by *Proteus mirabilis* isolated from UTI patients and its anti-fungal potential

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ABSTRACT

The aims of this research were analysis of the bioactive chemical products and evaluation of antibacterial and antifungal activity. Bioactives (chemical compounds often referred to as secondary metabolites) were analyzed using gas chromatography-mass spectroscopy (GC-MS) techniques, then the in vitro antibacterial and antifungal activity of the methanolic extract was evaluated. Proteus mirabilis bioactive compounds were identified in the methanolic extract of Proteus mirabilis. GC-MS analysis of Proteus mirabilis revealed the existence of the 1-Cyclopropyl-3,4-epoxyhex -5-en-1-yne, Benzene , (ethylsulfonyl), Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl , 1-benzylindole , L-Proline , N-(cyclohexanecarbonyl)-,propyl , 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide , (+)-trans-3,4-Dimethyl-2 -phenyltetrahydro-1,4-thiazine , Figure: Isophthalic acid ,di(2-methoxyethyl) ester , Isophthalic acid ,di(2-methoxyethyl) ester , 4-Benzyloxy-N-methylamphetamine, 1-(2-Benzyloxy-4-methyl- cyclohex-3-enyl)-1 -methyl-ethylamine , Ethyl 4-([(E)-(2-nitrophenyl) methylidene]amino) benzoate , Tolpropamine , Methcathinone , 4-Dehydroxy-N-(4,5-methylenedioxy -2-nitrobenzylidene)tyram. The results of anti-fungal activity produced by Staphylococcus aureus showed that the volatile compounds were highly effective to suppress the growth of Aspergillus terreus.

Keywords: Antifungal activity, *Proteus mirabilis*, GC-MS, Secondary metabolites.

INTRODUCTION

Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis and Enterobacter cloacae [1], are The most common bacterial uropathogens in UTI. *Proteus mirabilis* is a rod-shaped, Gram-negative facultative anaerobe that causes 4% of UTIs [2]. Several potential *P. mirabilis* virulence factors related to UTI have been described, including fimbrial-mediated adherence to the uroepithelium, swarming motility mediated by flagella, outer-membrane protein (OMP) expression, cell invasiveness, urease production, hemolysin production, and iron acquisition [3,4]. *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 [5]. P. mirabilis initiates the colonization of the urinary tract colonizing the periurethral region. Then, this microorganism passes through the urethra and access to the bladder. *P. mirabilis* expresses the enzyme urease that hydrolyzes urea to ammonia, leading to alkaline urine and the formation of kidney stones. These stones can cause obstruction and renal failure, and bacteria can persist within them to survive antibiotic therapy. P. mirabilis expresses several virulence factor involved in uropathogenesis like adhesins, flagella, toxins, quorum-sensing, enzymes and immune invasion [6]. This microorganism presents swarming motility ability. This phenomenon occurs on 1.5% of agar surface and describes flagellum-dependent movement across the surface, resulting a characteristic bull's eyes pattern [6].

MATERIALS AND METHODS

Growth conditions and determination of metabolites

Proteus mirabilis 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 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 [7-22].

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 [23-31].

Spectral analysis of bioactive chemical compounds using gas chromatography-mass spectrometry (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). 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 [32-38].

RESULTS AND DISCUSSION

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Proteus mirabilis*. The GC-MS chromatogram of the thirty one peaks of the compounds detected was shown in Figure 1. The First set up peak were determined to be 1,2-cis-1,5-trans-2,5-dihydroxy-4-methyl-1-(1-htdroxy-1-isopropyl)cy, Figure 2. The second peak indicated to be 2-Furancarboxaldehyde, 5-methyl, Figure 3. The next peaks considered to be 1-Cyclopropyl-3,4-epoxyhex -5-en-1-yne, Benzene , (ethylsulfonyl), Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl , 1-benzylindole , L-Proline , N-(cyclohexanecarbonyl)-,propyl , 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide , (+)-trans-3,4-Dimethyl-2 -phenyltetrahydro-1,4-thiazine , Figure: Isophthalic acid ,di(2-methoxyethyl) ester , Isophthalic acid ,di(2-methoxyethyl) ester , 4-Benzyloxy-N-methylamphetamine, 1-(2-Benzyloxy-4-methyl-cyclohex-3-enyl)-1 -methyl-ethylamine , Ethyl 4-([(E)-(2-nitrophenyl) methylidene]amino) benzoate , Tolpropamine , Methcathinone , 4-Dehydroxy-N-(4,5-methylenedioxy -2-nitrobenzylidene)tyram (Figure 4-16). The results of anti-fungal activity produced by *Proteus mirabilis* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus terreus*. *Proteus mirabilis* 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 *Proteus mirabilis* can be useful.

Table 1. Antifungal activity of *Proteus mirabilis* metabolite products.

Fungi	***Proteus mirabilis* metabolite products / Antibiotics**		
	***Proteus mirabilis* metabolite products**	**Amphotericin B**	**Fluconazol**
Aspergillus terreus	7.00±0.32 [a]	3.66±0.13	2.03±0.18
Aspergillus flavus	5.99±0.27	2.86±0.20	3.97±0.26
Candida albicans	6.13±0.21	3.00±0.17	2.95.±0.11
Microsporum canis	4.00±0.18	2.93±0.19	2.82±0.10
Trichoderma viride	4.27±0.25	2.22±0.13	2.52±0.13
Trichophyton mentagrophytes	3.99±0.19	1.98±0.11	1.86±0.10

[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 and Fluconazol).*

Maximum zone formation against *Aspergillus terreus* (7.00±0.32) mm, Table 2. Antibiotic resistance is increasing worldwide in both outpatients as well as hospitalized patients. It varies according to geographic locales and is directly proportional to the use and misuse of antibiotics. Despite newer antibiotic, continued selective antibiotic pressure and bacterial adaptation have resulted in a problem that can no longer be ignored. Resistance can now be demonstrated against all available classes of antibiotics [39-41]. Multiple drug-resistant organisms used in the current study are becoming common causes of infections in the acute and long-term care units in hospitals. *P.*

aeruginosa is responsible for 16%of nosocomial pneumonia cases, 12% of hospital- acquire durinary tract infections, 8% of surgical wound infections, and 10% of blood stream infections.

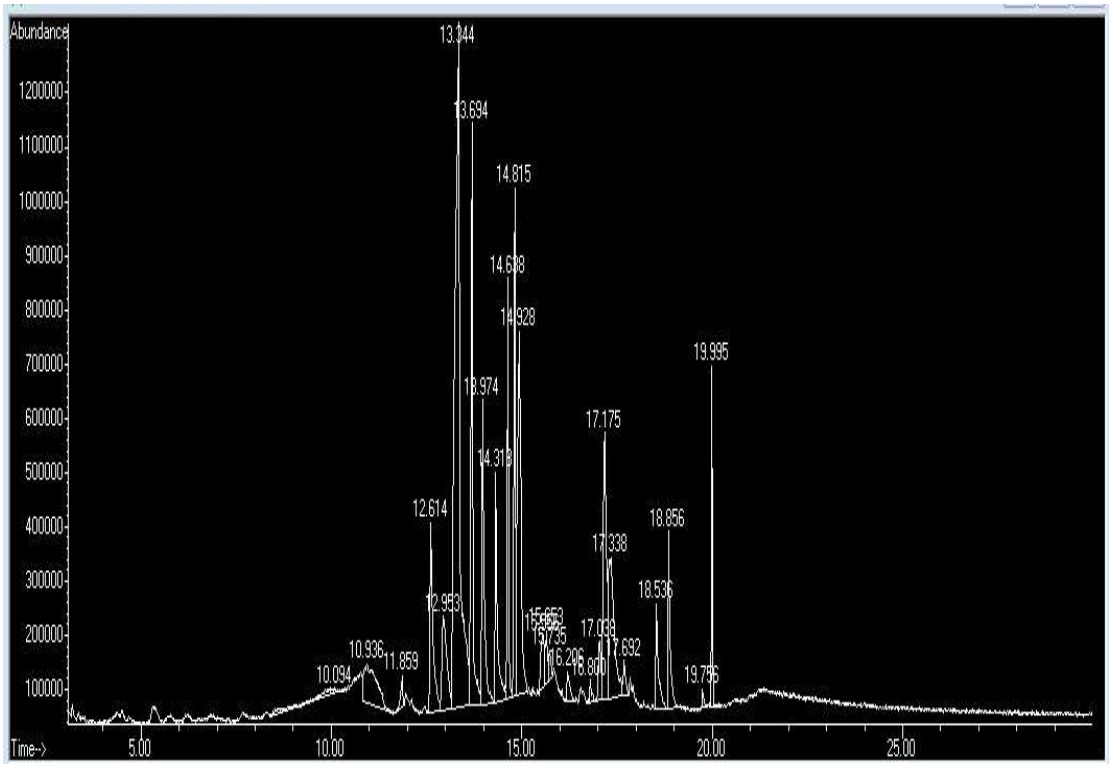

Figure 1: GC-MS chromatogram of methanolic extract of *Proteus mirabilis*.

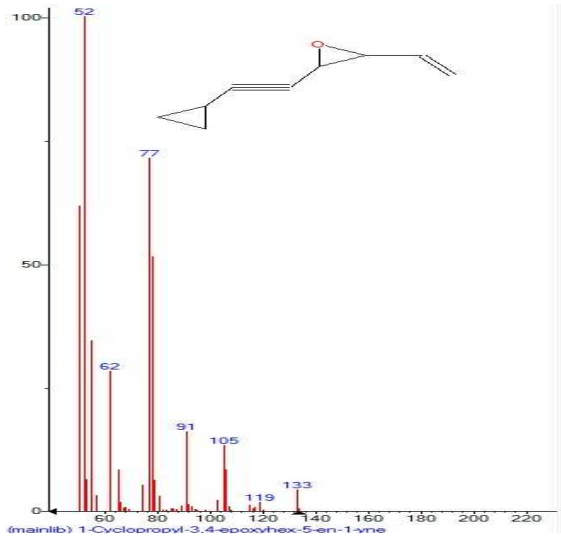

Figure 2: 1-Cyclopropyl-3,4-epoxyhex -5-en-1-yne
RT=3.098
Mw=134.073165

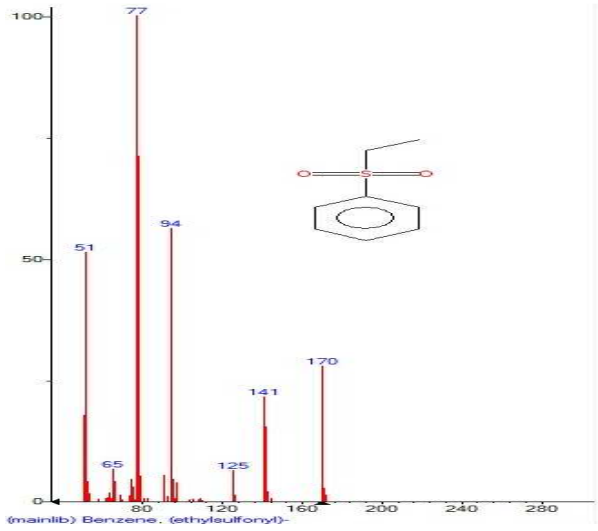

Figure 4: Benzene , (ethylsulfonyl)
RT= 3.115
Mw= 170.040151

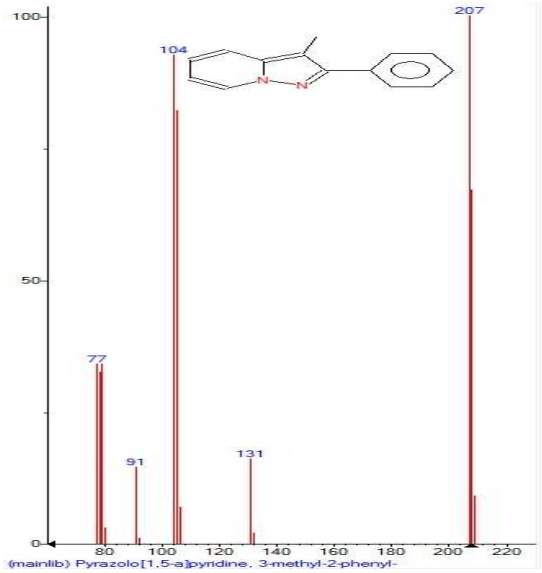

Figure 3: Pyrazolo[1.5-a]pyridine , 3-methyl-2-phenyl-
RT= 3.396
Mw= 208.100048

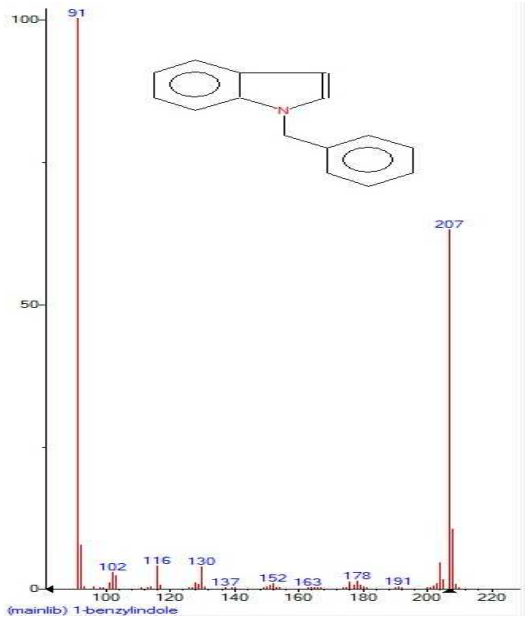

Figure 5: 1-benzylindole
RT= 3.991
Mw= 207.104799

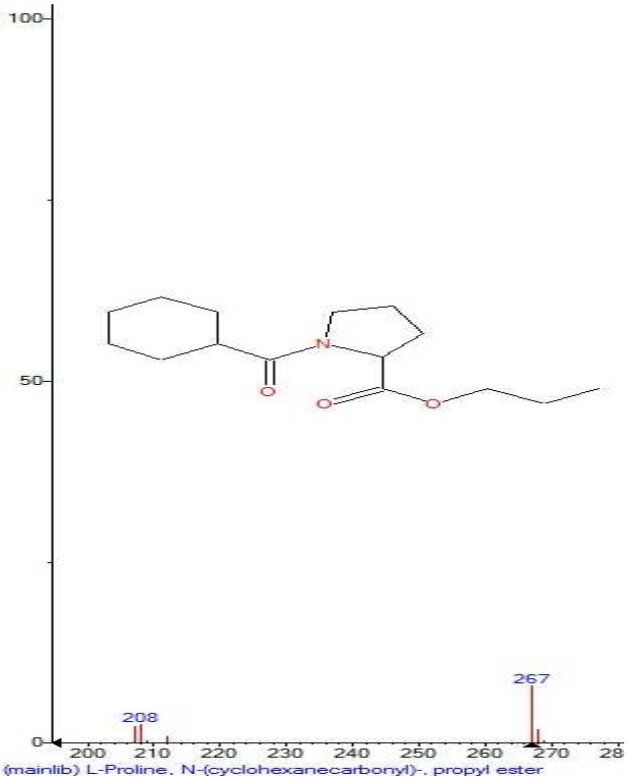

Figure 6: L-Proline , N-(cyclohexanecarbonyl)-,propyl ester
RT= 4.906
Mw= 267.183443

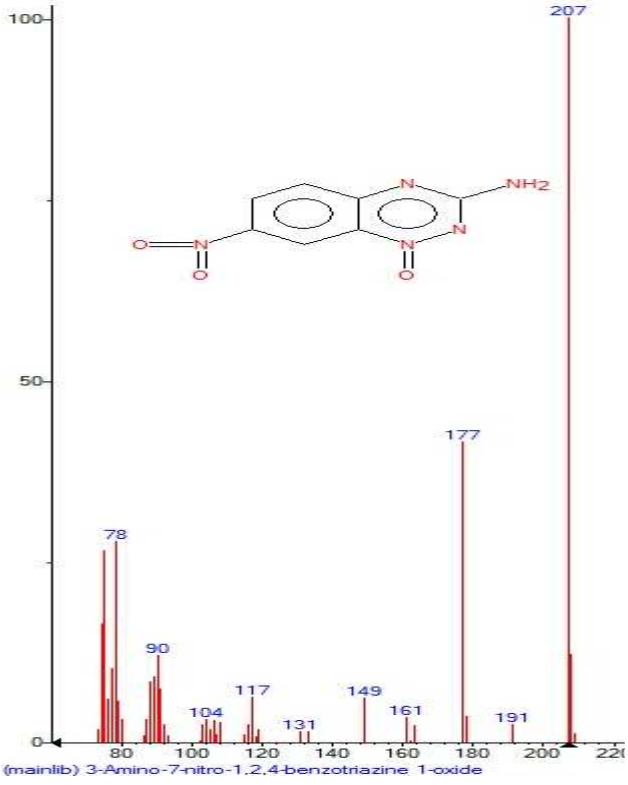

Figure 8: 3-Amino-7-nitro-1,2,4-benzotriazine 1-oxide
RT= 5.009
Mw= 207.039239

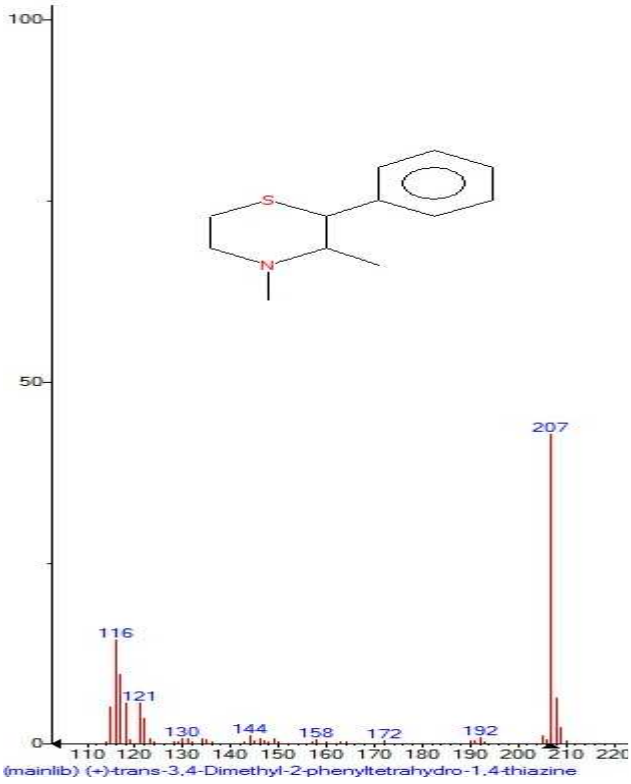

Figure 7: (+)-trans-3,4-Dimethyl-2 -phenyltetrahydro-1,4-thiazine
RT= 7.384
Mw= 207.1081705

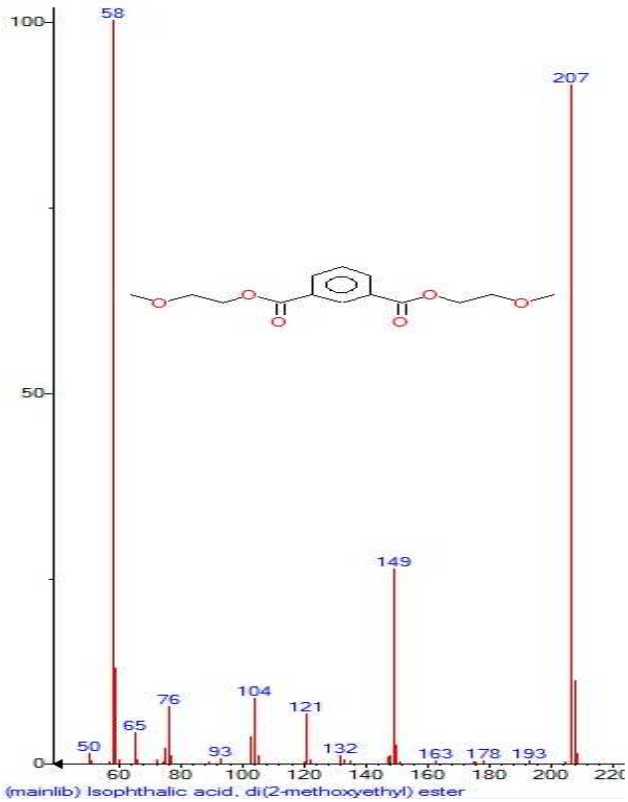

Figure 9: Isophthalic acid ,di(2-methoxyethyl) ester
RT= 9.307
Mw= 282.110338

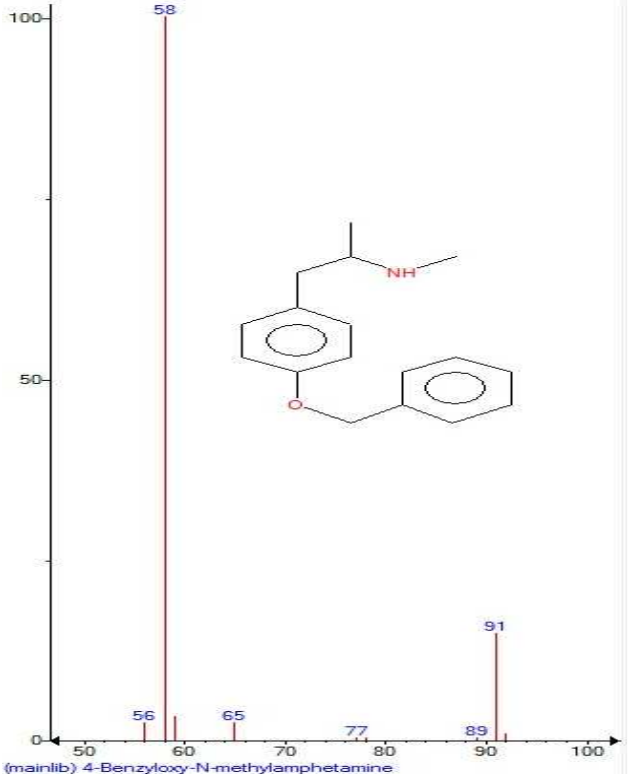

Figure 10: 4-Benzyloxy-N-methylamphetamine
RT= 9.741
Mw= 255.162314

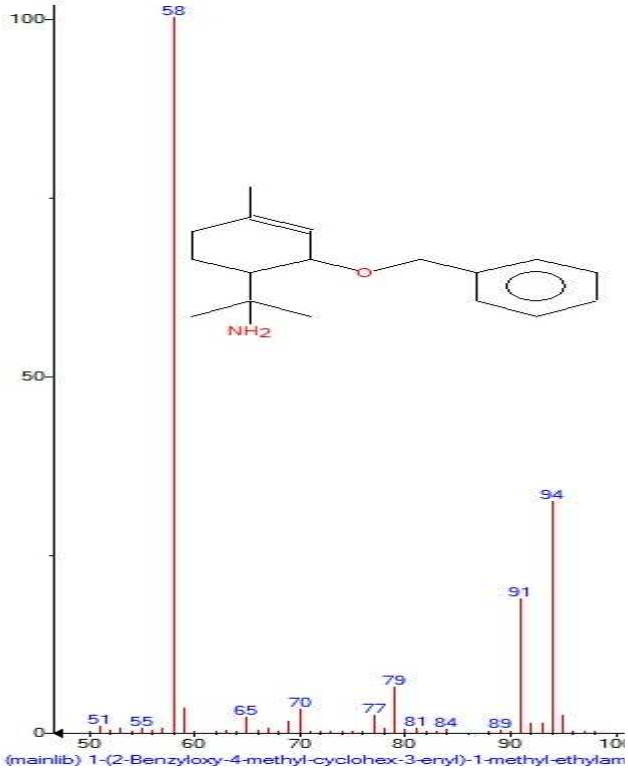

Figure 13: 1-(2-Benzyloxy-4-methyl- cyclohex-3-enyl)-1 -methyl-ethylamine
RT= 9.518
Mw= 259.193615

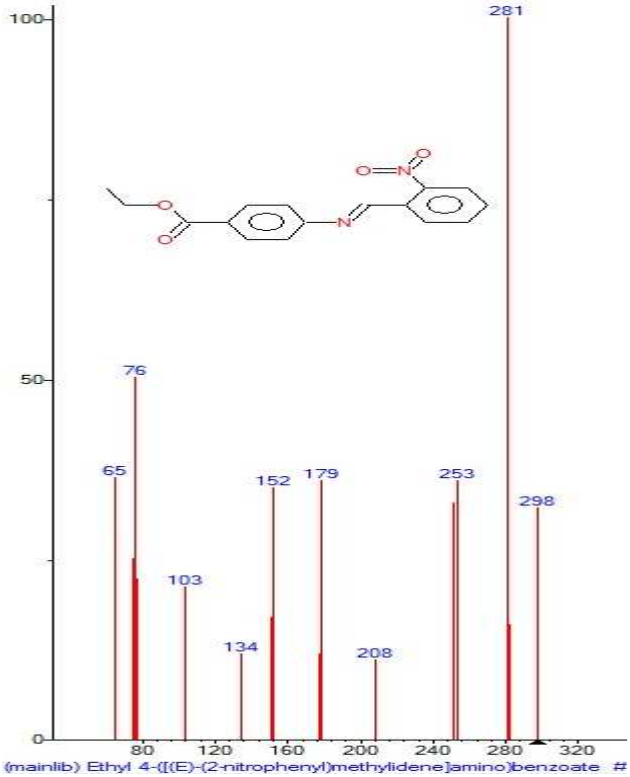

Figure 12: Ethyl 4-([(E)-(2-nitrophenyl) methylidene]amino) benzoate
RT= 9.867
Mw= 298.095356

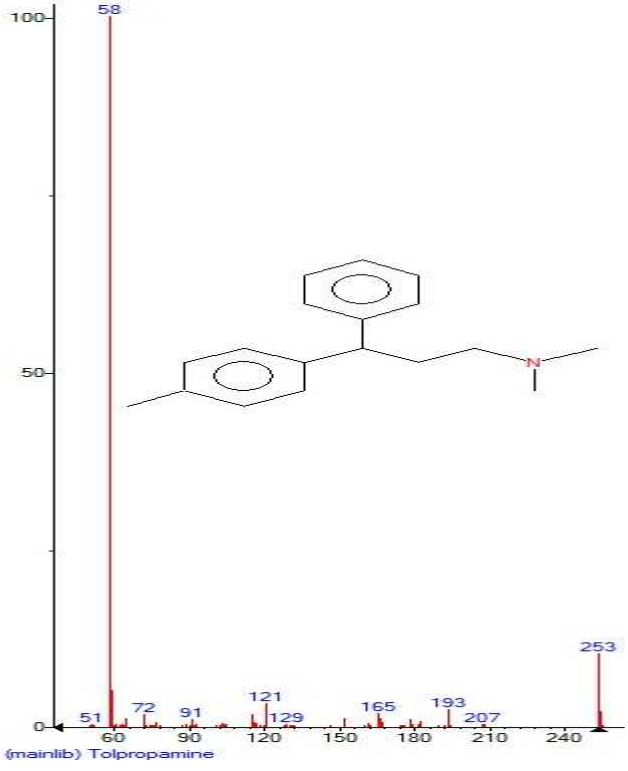

Figure 14: Tolpropamine
RT= 10.823
Mw= 253.18305

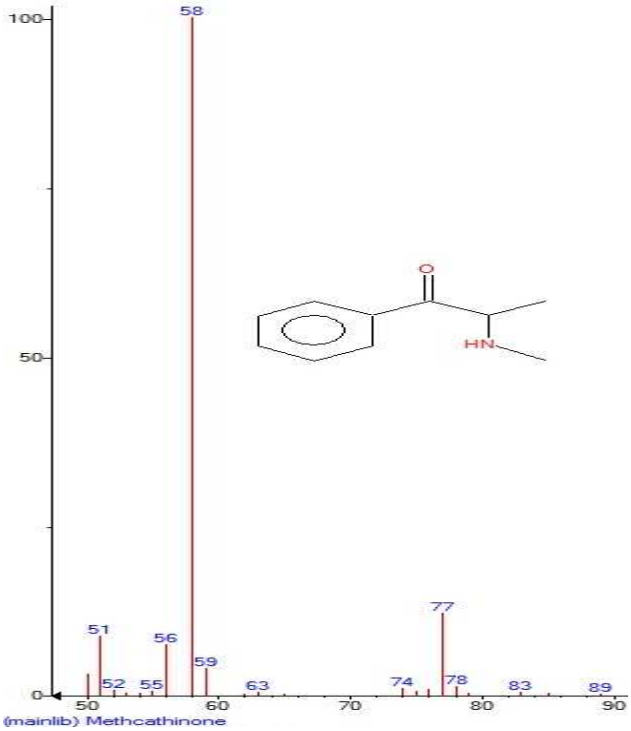

Figure 15: Methcathinone
RT= 12.133
Mw= 163.099714

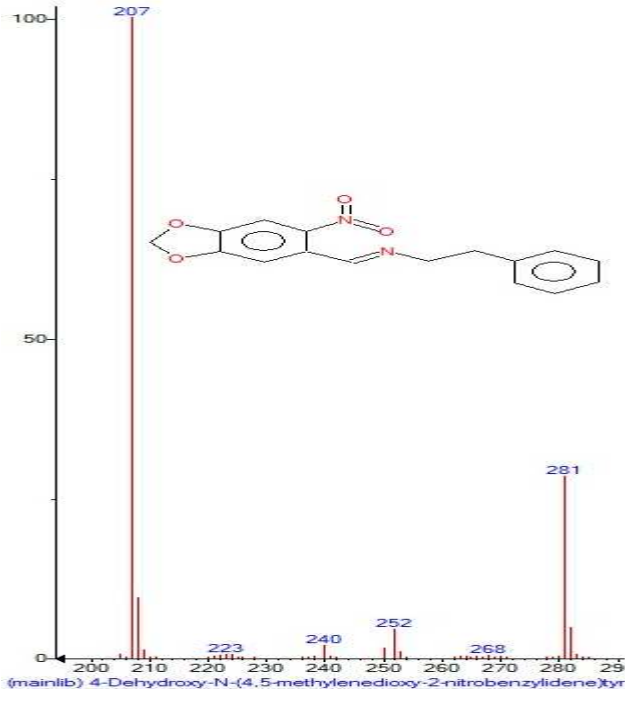

Figure 16: 4-Dehydroxy-N-(4,5-methylenedioxy -2-nitrobenzylidene)tyram
RT= 15.280
Mw= 298.095356

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