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Research Article

Secondary Metabolites Analysis of *Saccharomyces cerievisiae* and Evaluation of Antibacterial Activity

Mohammad J. Al-Jassani[1], Ghaidaa Jihadi Mohammed[2], Imad Hadi Hameed[3*]

[1]*DNA Research Centre, University of Babylon, Iraq*
[2]*College of Science, Al-Qadisiya University, Iraq*
[3]*College of Nursing, University of Babylon, Iraq*

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ABSTRACT

The objectives of this study were analysis of the secondary metabolite products and evaluation antibacterial activity. Bioactives are chemical compounds often referred to as secondary metabolites. Twenty one bioactive compounds were identified in the methanolic extract of *Saccharomyces cerevisiae*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of *Saccharomyces cerevisiae* revealed the existence of the Thieno [2,3-c] furan-3-carbonitrile, 2-amino-4,6-dihydro-4,4,6,6-, Oxime-, methoxy-phenyl-,Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl)phenyl] hydrazide, 1-Aminononadecane, N-trifluoroacetyl, Androstane-11,17-dione,3- [(trimethylsilyl)oxy]-,17-[O-(phenylme, Benzeneacetamide,α-ethyl-, 4-Benzyloxy-6-hydroxymethyl-tetrahydropyran-2,3,5-triol, 1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-,[S-(R*,R*)], Erythritol, 3,6,9,12,-Tetraoxatetradecan-1-ol,14- [4-(1,1,3,3- tetramethylbutyl , Urea,N,N'-bis(2-hydroxyethyl)- , Ergosta-5,22-dien-3-ol,acetate,(3β,22E)-, Ethyl iso-allocholate, (5β)Pregnane-3,20β-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azal,5,5'-Dimethoxy-3,3',7,7'-tetramethyl-2,2'-binaphthalene-1,1',4,4',N-(4,6-Dimethyl2pyrimidinyl)-4-(4 nitrobenzylideneamino) benzene, 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9 (2H,10H) , 2-Methyl-9-β-d-ribofuranosylhypoxanthine, Dodecane,1-chloro-, 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo [4',5'-d] pyridazin and 2-Bromotetradecanoic acid. *Evernia punastri* was very highly antifungal activity (7.00±0.25) mm. The results of anti-bacterial activity produced by *Saccharomyces cerevisiae* showed that the volatile compounds were highly effective to suppress the growth of *Proteus mirabilis*.

Keywords: *Saccharomyces cerevisiae*, Antibacterial activity, Antifungal activity, FT-IR, GC/MS, Secondary metabolites.

INTRODUCTION

Saccharomyces cerevisiae, also known as Baker's yeast is a single-celled fungus[1]. Metabolomics aims to measure the dynamic metabolic response of living complex multicellular systems to biological stimuli or genetic manipulation[2-4]. By identifying biochemical compounds whose concentrations have varied due to a biological stimulus, metabolomics allows uncovering new possible targets (biomarkers) for biochemical interpretation of biological changes[5-7]. S. cerevisiae has been used as a model for higher eukaryote species in biology because its similar metabolism[8-10]. Metabolomics as a term, it includes quantification of extracellular and intracellular metabolite concentrations[11-13]. Currently, a range of analytical platforms are used for metabolomic analysis, including direct infusion mass spectrometry (MS)[14], gas chromatography coupled to mass spectrometry (GC–MS)[15,16], two-dimensional GC coupled to MS (GC 9 GC–MS), liquid chromatography coupled to MS (LC–MS), capillary electrophoresis coupled to MS (CE–MS), and proton nuclear magnetic resonance (1H NMR) spectroscopy and Fourier transform infrared (FT-IR) spectroscopy[17,18]. For metabolite analysis, a large number of metabolites must be extracted, the extract must be representative for the whole culture, the metabolites must be separated from other cell components and the metabolic activity must be stopped immediately after sampling[19,20]. The aims of this research were screening of the metabolite products and evaluation antibacterial activity.

MATERIALS AND METHODS

Growth conditions and determination of metabolites

S. cerevisiae was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants[21,22]. 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. 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

**Author for Correspondence*

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[23,24].

Analysis of chemical compounds using gas chromatography-mass spectrometry (GC/MS)

Analytes are carried on mobile phase and interacts with stationary phase in the column. Interactions between mobile phase and stationary phase determine the retention time of analyte. Retention time is the time taken for an anlayte to pass through a column[25-28]. Any compound carrying charge, or which can be charged and evaporated can be analyzed for mass to charge ratio (m/z), which is the core principle in MS. Fragmentation is reproducible and characteristic for single metabolites, and can be compared to reference spectra for identification[29,30]. Under ionization moleculemolecule collision is avoided by applying high vacuum. The newly made ions are lead to the analyzer by an acceleration plate with higher potential. A repeller plate inside the ionization source controls the electric field[31-33]. A mass spectrum shows the abundance of each ion mass of an ionized and fragmented analyte as a function of its mass to charge ratio. 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 (250 Cº). Ionization voltage was 70 eV and ion source temperature was 230 Cº. Scan range was 41- 450 amu. The constituents were identified after compared with available data in the GC-MS library in the literatures.

Determination of antibacterial activity

The test pathogens (*Klebsiella pneumonia*, *Proteus mirabilis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus pneumonia* and *Pseudomonas eurogenosa*) were swabbed in Muller Hinton agar plates. 90µl of fungal extracts was loaded on the bored wells. The wells were bored in 0.5cm in diameter. The plates were incubated at 37C° for 24 hrs and examined[34]. After the incubation the diameter of inhibition zones around the discs was measured.

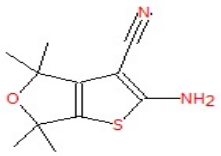
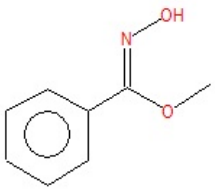
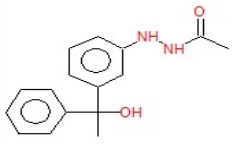
Determination of antifungal activity

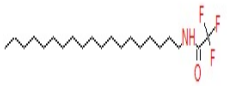
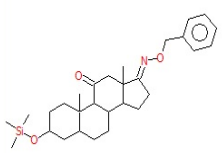
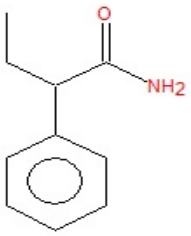
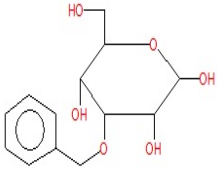
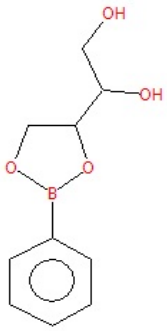
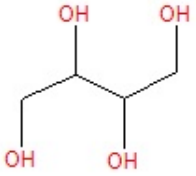
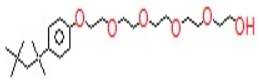
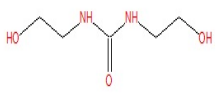
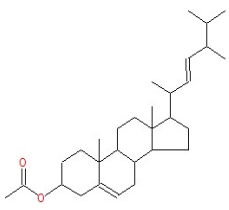
S. cerevisiae isolate was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed[35-37]. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Borrago Officinalis*, *Adiantum capillus- veneris*, *Arbutus unedo*, *Echium vulgare*, *Fumaria officinalis*, *Evernia punastri*, *Iris germanica*, *Adonis vernalis*, *Anchusa officinalis*, *Cassia accutifolia*, *Dipsacus fullonum*, *Juniperus phoenicia*, *Atropa belladone*, *Convulvus arvensis*, *Quercus infectoria*, *Citrullus colocynthis*, *Althaea rosea*, *Anastatica hierochuntica*, *Melia azedarach*, *Origanum vulgare*, *Lawsonia alba*, *Glaucium flavum*, *Nigella sativa*, *Ocimum basilicum*, *Artemisia campestris* and *Erythraea centaurium*) 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[38,39]. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $P < 0.05$ using Duncan's multiple range test (by SPSS software) Version 9.1

Table 1: Secondary metabolites identified in methanolic extract of *Saccharomyces cerevisiae*

S No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass	Chemical structure	MS Fragments
1.	Thieno[2,3-c]furan-3-carbonitrile , 2-amino-4,6-dihydro-4,4,6,6-	3.436	222	222.0826845		60,96,165,207, 222
2.	Oxime-, methoxy-phenyl-	3.830	151	151.063329		55,73,105,133, 151
3.	Acetic acid , N'-[3-(1-hydroxy-1-phenylethyl)phenyl]hydrazide	4.013	270	270.136827		65,77,91,133,165,193,209,237,252

4.	1-Aminononadecane,N-trifluoroacetyl-	4.385	379	379.3062		55,69,79,126,191,209,249,283,310
5.	Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenylme	4.546	481	481.30122		55,73,91,147,207,281,299,360,453
6.	Benzeneacetamide,α-ethyl-	4.729	163	163.099714		51,78,91,105,119,136,163
7.	4-Benzyloxy-6-hydroxymethyl-tetrahydropyran-2,3,5-triol	4.780	270	270.110338		65,91,107,133,163,191,221
8.	1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-,[S-(R*,R*)]	4.992	208	208.09069		61,73,91,104,147,177,208
9.	Erythritol	5.255	122	122.057909		61,74,91
10.	3,6,9,12,-Tetraoxatetradecan-1-ol,14-[4-(1,1,3,3-tetramethylbutyl	5.415	426	426.29814		57,69,89,99,113,135,149,161,175,207,223,249,267,281,295,311,325,355
11.	Urea,N,N'-bis(2-hydroxyethyl)-	5.810	148	148.084792		61,81,132
12.	Ergosta-5,22-dien-3-ol,acetate,(3β,22E)-	5.970	440	440.36543		55,67,91,105,145,159,213,227,255,281,327,365,380

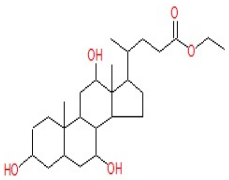
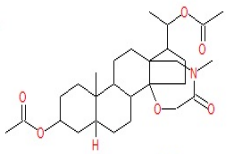
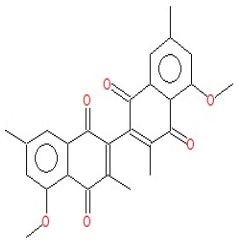
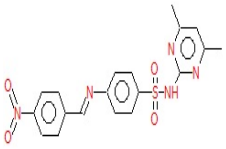
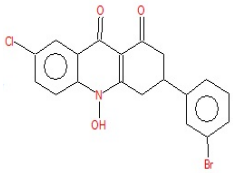
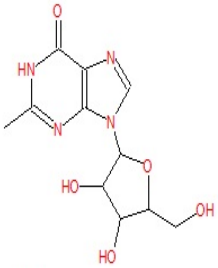
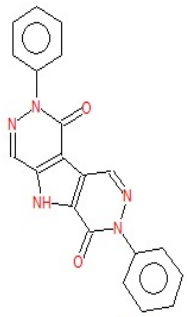
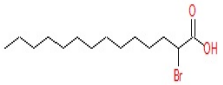
13.	Ethyl iso-allocholate	6.285	436	436.318874		55,69,81,95,145,253,400,418
14.	(5β)Pregnane-3,20β-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azal	6.365	489	489.309038		57,73,133,161,177,223,267,281,328,360,399,429
15.	5,5'-Dimethoxy-3,3',7,7'-tetramethyl-2,2'-binaphthalene-1,1',4,4'	6.525	430	430.141638		57,90,111,149,191,255,400,415,430
16.	N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino)benzene	7.475	411	411.100124		51,65,77,104,120,151,171,214
17.	3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H)	7.710	416	416.976732		57,69,97,147,219,246,321,401,419
18.	2-Methyl-9-β-d-ribofuranosylhypoxanthine	7.841	282	282.09642		57,73,86,114,150,179,206,282
19.	Dodecane,1-chloro-	8.585	204	204.164478		57,69,91,161,204
20.	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazin	10.113	355	355.106924		51,77,93,120,149,165,187,224,267,327,355
21.	2-Bromotetradecanoic acid	13.335	306	306.119442		55,73,83,99,111,138,201,227,249,306

Table 2: Antibacterial activity of bioactive compounds of *Saccharomyces cerevisiae* against bacterial strains.

Bacteria	Antibiotics / Fungal products				
	Streptomycin	Cefotoxime	Kanamycin	Rifambin	Fungal metabolites
Klebsiella pneumonia	0.84±0.21	1.31±0.19	0.99±0.10	2.00±0.11	*5.99±0.12*
Proteus mirabilis	2.00±0.16	1.76±0.13	2.20±0.13	1.50±0.21	*6.96±0.22*
Staphylococcus epidermidis	1.77±0.10	0.55±0.18	1.84±0.20	1.00±0.10	*5.33±0.27*
Escherichia coli	0.63±0.14	2.00±0.100	2.00±0.21	0.38±0.11	*5.76±0.12*
Proteus mirabilis	1.46±0.12	1.21±0.27	1.74±0.39	1.19±0.20	*6.49±0.23*
Streptococcus pyogenes	0.52±0.21	1.99±0.27	2.00±0.11	0.10±0.12	*6.00±0.11*
Staphylococcus aureus	0.89±0.20	0.77±0.20	0.97±0.15	0.19±0.16	*5.10±0.28*
Streptococcus pneumonia	1.66±0.23	1.63±0.18	0.82±0.10	1.00±0.13	*4.00±0.20*
Pseudomonas eurogenosa	0.79±0.13	0.76±0.28	1.77±0.31	0.99±0.19	*4.64±0.19*

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

S. No.	Plant	*Zone of inhibition (mm)*
1.	*Borrago Officinalis*	*5.24±0.17*
2.	*Adiantum capillus-veneris (L)*	*5.32±0.28*
3.	*Arbutus unedo(L)*	*7.05±0.26*
4.	*Echium vulgare(L)*	*5.00±0.20*
5.	*Fumaria officinalis (L)*	*6.09±0.14*
6.	*Evernia punastri(L)*	*7.00±0.25*
7.	*Iris germanica(L)*	*5.35±0.18*
8.	*Adonis vernalis (L)*	*5.44±0.26*
9.	*Anchusa officinalis(L)*	*5.01±0.19*
10.	*Cassia accutifolia*	*5.92±0.17*
11.	*Dipsacus fullonum(L)*	*5.74±0.15*
12.	*Juniperus phoenicia (L)*	*4.00±0.20*
13.	*Atropa belladone (L)*	*3.06±0.17*
14.	*Convulvus arvensis(L)*	*5.09±0.12*
15.	*Quercus infectoria*	*5.03±0.18*
16.	*Citrullus colocynthis*	*3.77±0.22*
17.	*Althaea rosea*	*6.09±0.16*
18.	*Anastatica hierochuntica (L)*	*4.44±0.17*
19.	*Melia azedarach*	*4.08±0.26*
20.	*Origanum vulgare*	*5.71±0.28*
21.	*Lawsonia alba(L)*	*5.11±0.27*
22.	*Glaucium flavum(L)*	*5.39±0.17*
23.	*Anagalis arvensis(L)*	*6.00±0.15*
24.	*Nigella sativa*	*3.74±0.12*
25.	*Ocimum basilicum*	*5.98±0.18*
26.	*Artemisia campestris(L)*	*4.39±0.27*
27.	*Erythraea centaurium(L)*	*4.91±0.17*
28.	Amphotericin B	*6.10±0.48*
29.	Fluconazol	*7.19±0.19*
30.	Control	*0.00*

RESULTS AND DISCUSSION

Based on morphological characteristics of fungi was isolated in selective media of potato dextrose agar media. Morphological, Microscopical and microscopical characteristics of fungal strains were determined using specific media light and compound microscope Fig. 1. The 400ml of fermentation broth (PDA broth) which

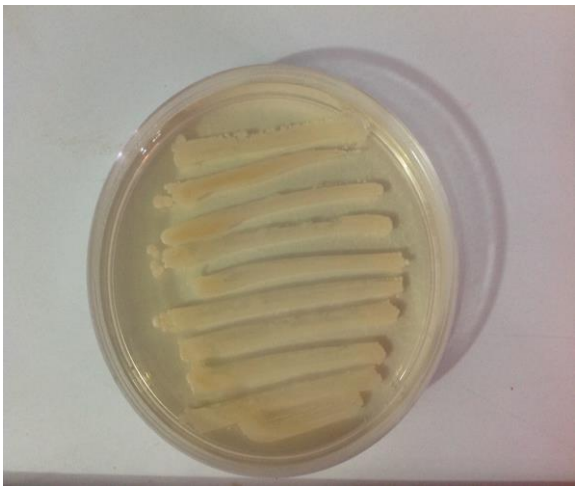


Figure 1: Morphological characterization of *S. cerevisiae* colony.

contain 200µl of the standardized fugal suspensions were used to inoculate the flasks and incubated at 37°C on a shaker at 90 rpm for 7 days. After fermentation, the secondary metabolites were produced by isolated microorganisms.

Identify the secondary metabolites from S. cerevisiae

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *S. cerevisiae*, shown in Table 1. The GC-MS chromatogram of the thirty one peaks of the compounds detected was shown in Fig. 2. 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, Fig. 3. The second peak indicated to be 2-Furancarboxaldehyde,5-methyl, Fig. 4. The next peaks considered to be 2(5H)-Furanone, 6-Hydroxymethyl-5-methyl-bicyclo[3.1.0]hexan-2-one, D-Glucose,6-O-α-D-galactopyranosyl,2-(3-Hydroxy-propyl)-cyclohexane-1,3-dione,9-Oxa-bicyclo[3.3.1]nonane-1,4-diol, Benzenemethanol,2-(2-aminopropoxy)-3-methyl, 1,2-Cyclopentanedione,3-methyl, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-ß-D-fruc, 1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol, Desulphosinigrin, Orcinol, Bicyclo[2.2.1]heptane-2-carboxylic acid isobutyl-amide, 2H-Oxecin-2-one.3.4.7.8.9.10-hexahydro-4-hydroxy-10-methyl-.[4, 2H-Pyran,tetrahydro-2-(12-pentadecynyloxy), Maltol, 2-Tridecyl-5-(acetylamino)tetrahydro-γ-pyrone, Cycloundecanone , oxime, D-Glucose,6-O-α-D-

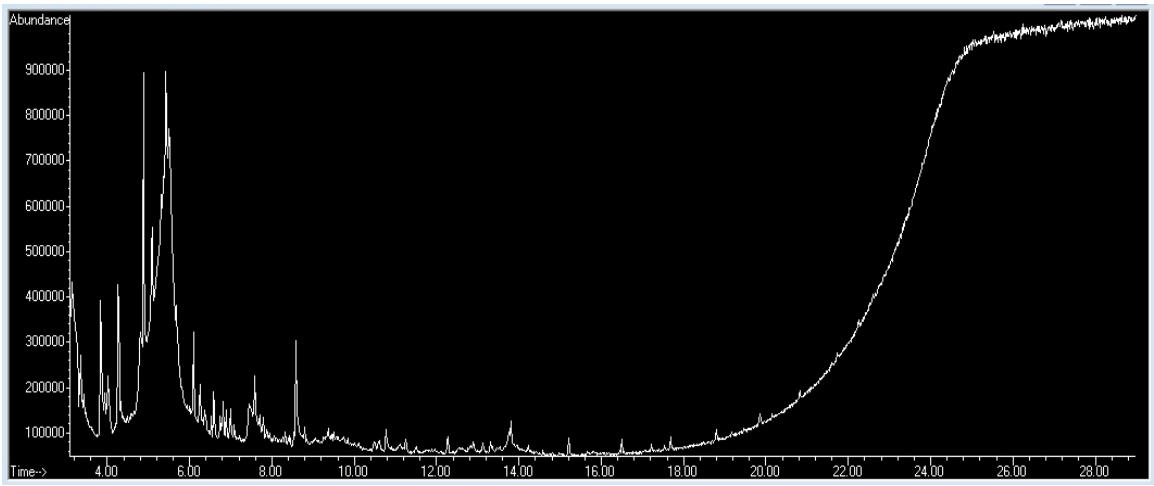


Figure 2: GC-MS chromatogram of methanolic extract of *S. cerevisiae*.

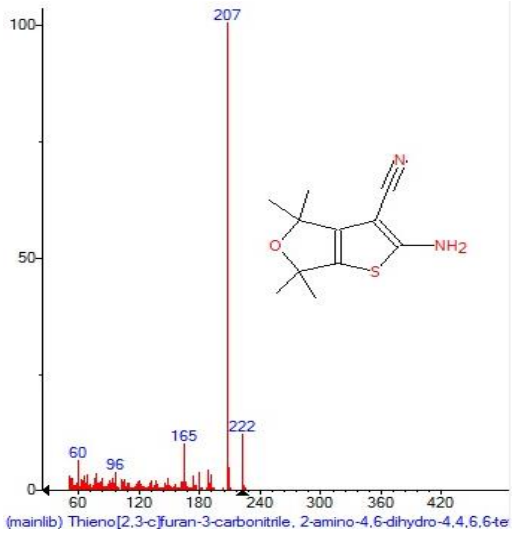


Figure 3: Mass spectrum of Thieno[2,3-c]furan-3-carbonitrile , 2-amino-4,6-dihydro-4,4,6,6- with Retention Time (RT)= 3.436

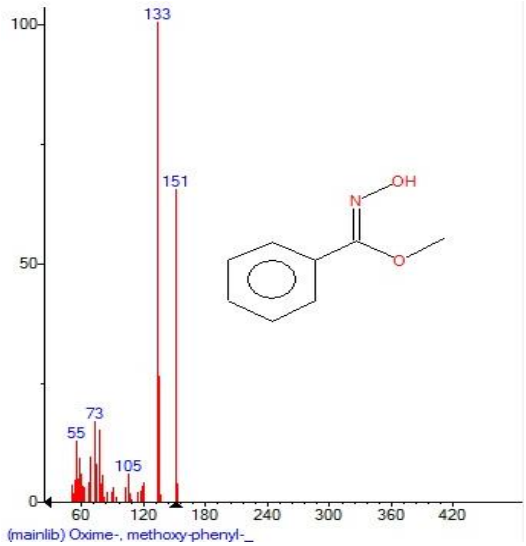


Figure 4: Mass spectrum of Oxime-, methoxy-phenyl- with Retention Time (RT)= 3.830

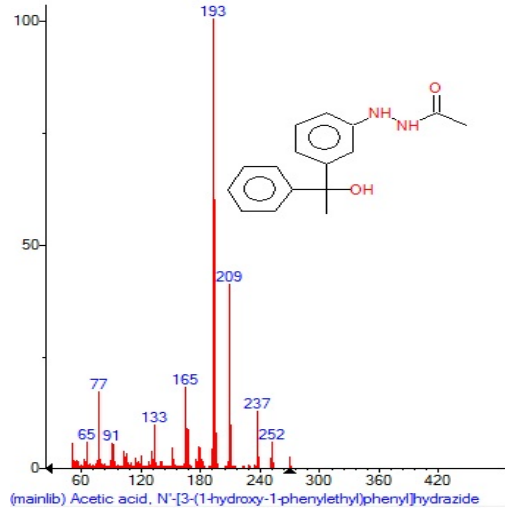


Figure 5: Mass spectrum of Acetic acid, N'-[3-(1-hydroxy-1-phenylethyl) phenyl] hydrazide with Retention Time (RT)= 4.013

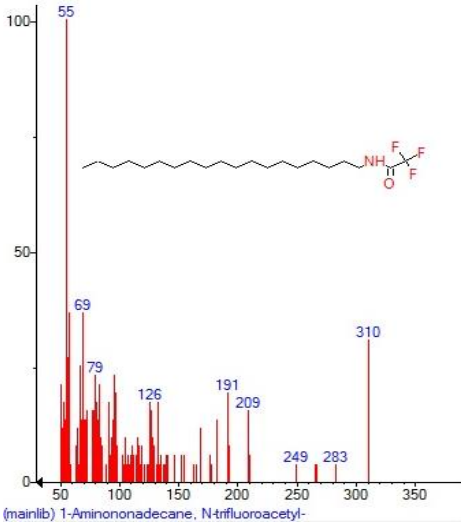


Figure 6: Mass spectrum of 1-Aminononadecane, N-trifluoroacetyl- with Retention Time (RT)= 4.385

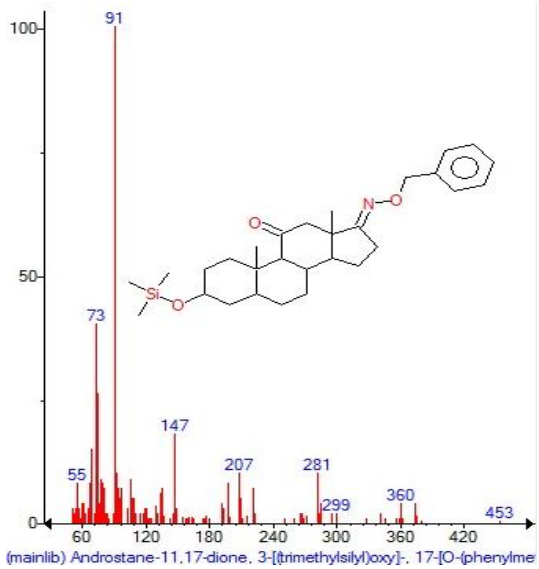


Figure 7: Mass spectrum of Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17- [O-(phenylme with Retention Time (RT)= 4.546

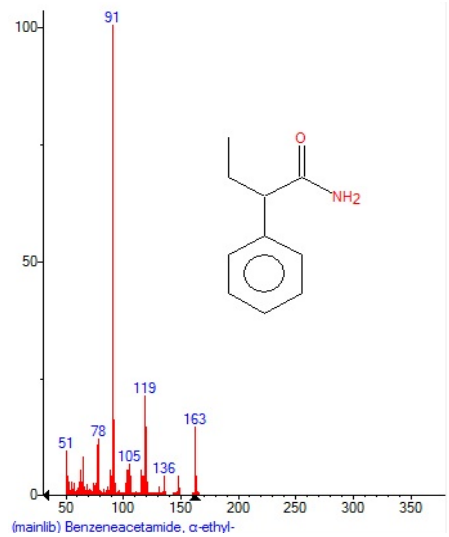


Figure 8: Mass spectrum of Benzeneacetamide, α-ethyl- with Retention Time (RT)= 4.729

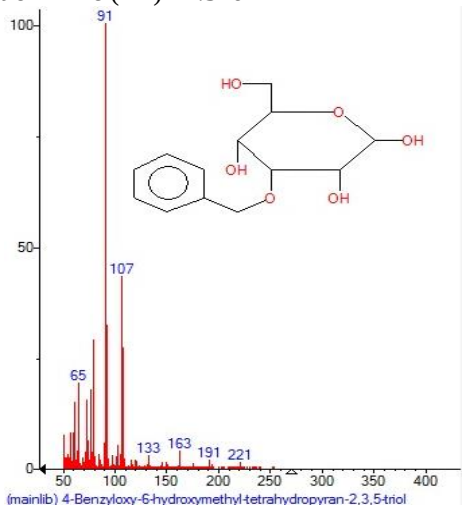


Figure 9: Mass spectrum of 4-Benzyloxy-6-hydroxymethyl-tetrahydropyran-2,3,5-triol with Retention Time (RT)= 4.780

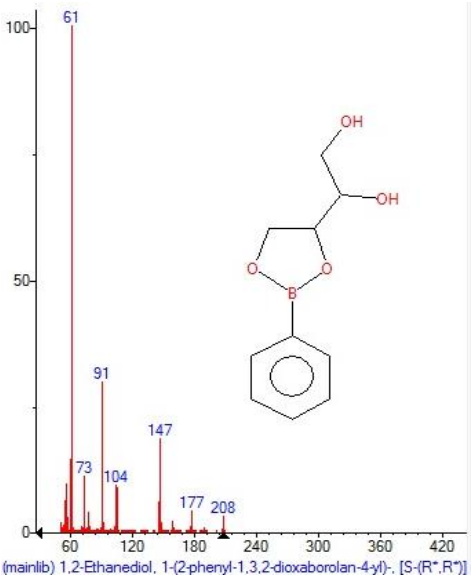
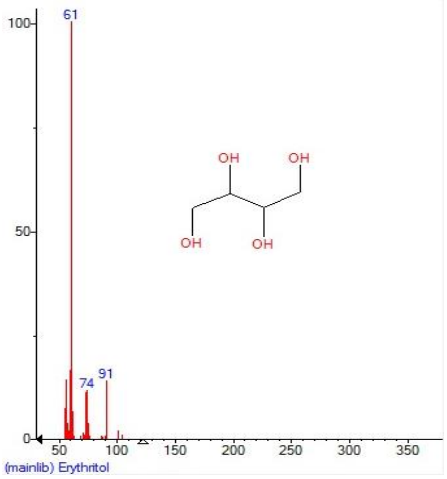


Figure 10: Mass spectrum of 1,2-Ethanediol, 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-, [S-(R*, R*)] with Retention Time (RT)= 4.992



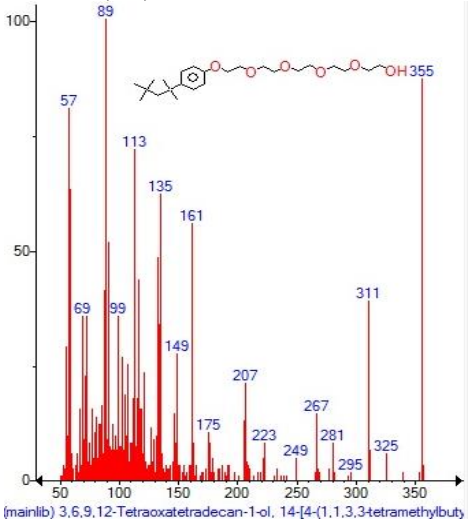


Figure 11: Mass spectrum of Erythritol with Retention Time (RT)= 5.255

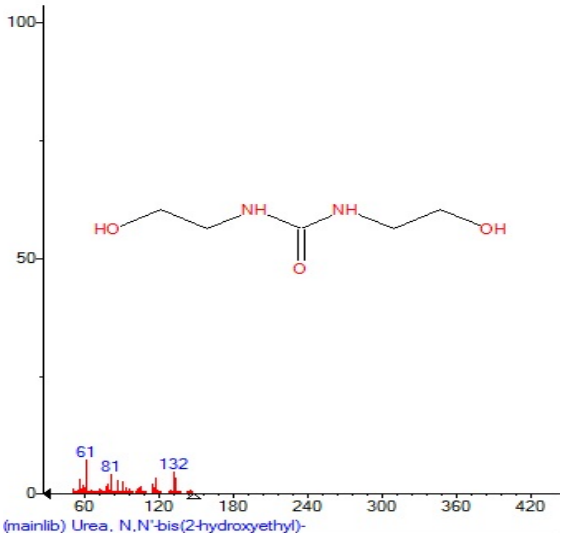


Figure 12: Mass spectrum of 3,6,9,12,-Tetraoxatetradecan-1-ol,14-[4-(1,1,3,3-tetramethylbutyl with Retention Time (RT)= 5.415

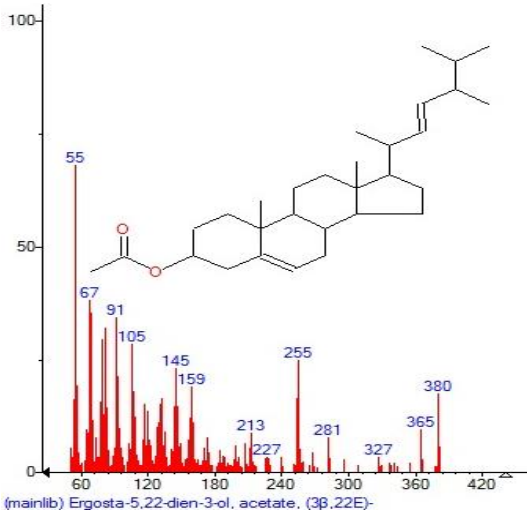


Figure 13: Mass spectrum of Urea,N,N'-bis (2-hydroxyethyl)- with Retention Time (RT)= 5.810

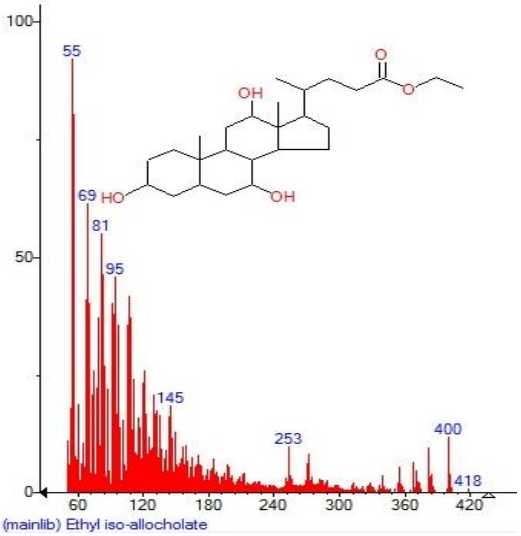


Figure 14: Mass spectrum of Ergosta-5,22-dien-3-ol,acetate,(3β,22E)- with Retention Time (RT)= 5.970

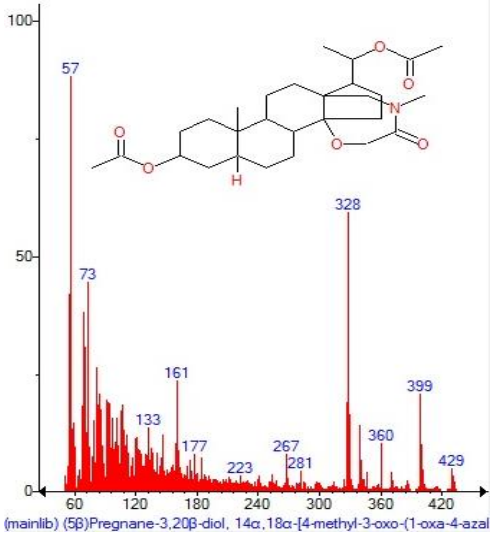


Figure 15: Mass spectrum of Ethyl iso-allocholate with Retention Time (RT)= 6.285

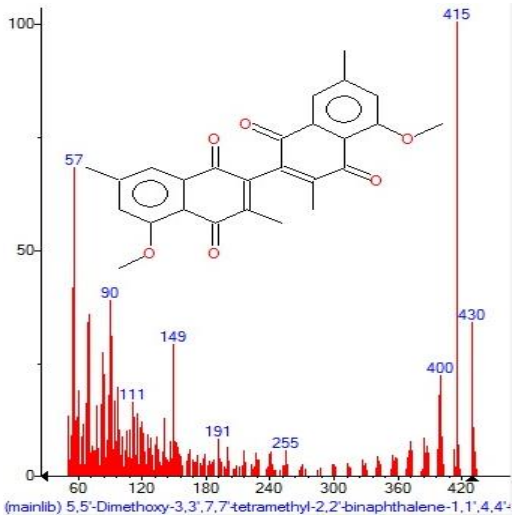


Figure 16: Mass spectrum of (5β)Pregnane-3,20β-diol,14α,18α-[4-methyl-3-oxo-(1-oxa-4-azal with Retention Time (RT)= 6.365

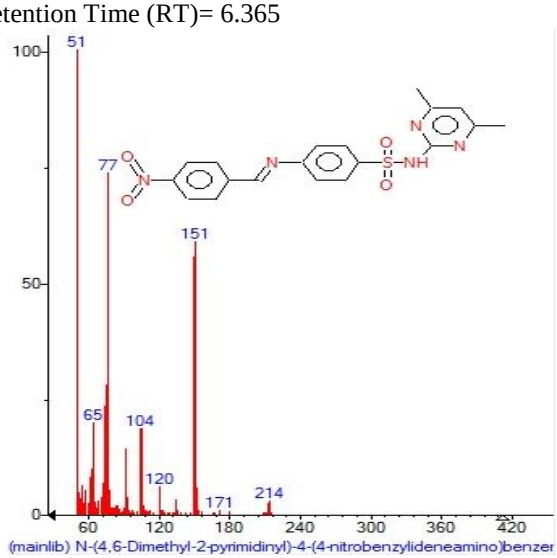


Figure 17: Mass spectrum of 5,5'-Dimethoxy-3,3',7,7'-tetramethyl-2,2'-binaphthalene-1,1',4,4' with Retention Time (RT)= 6.525

Figure 18: Mass spectrum of N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino) benzene with Retention Time (RT)= 7.475

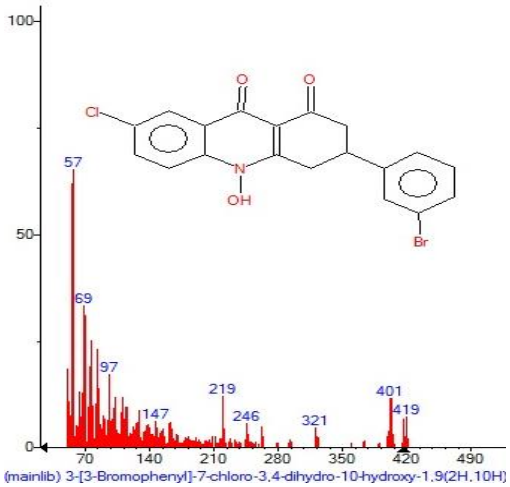


Figure 19: Mass spectrum of 3-[3-Bromophenyl]-7-chloro-3,4-dihydro-10-hydroxy-1,9(2H,10H) with Retention Time (RT)= 7.710

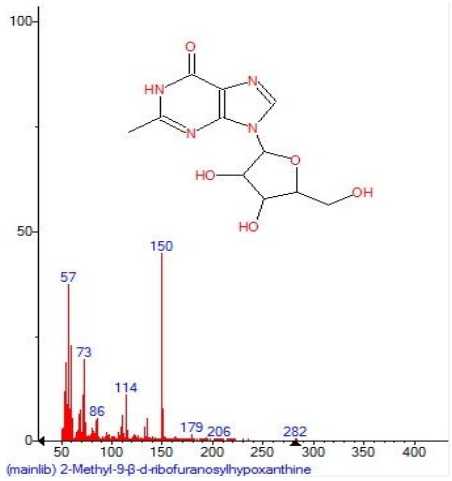


Figure 20: Mass spectrum of 2-Methyl-9-β-d-ribofuranosylhypoxanthine with Retention Time (RT)= 7.841

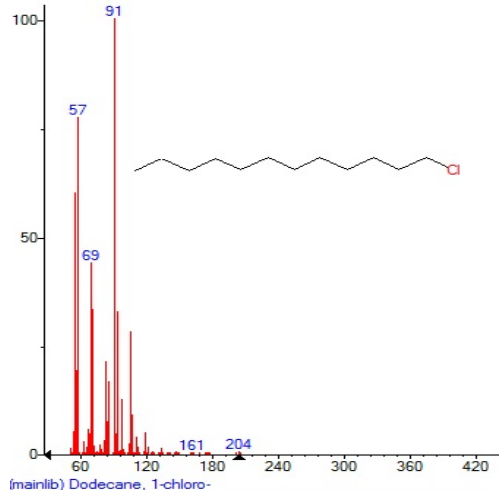


Figure 21: Mass spectrum of Dodecane,1-chloro- with Retention Time (RT)= 8.585

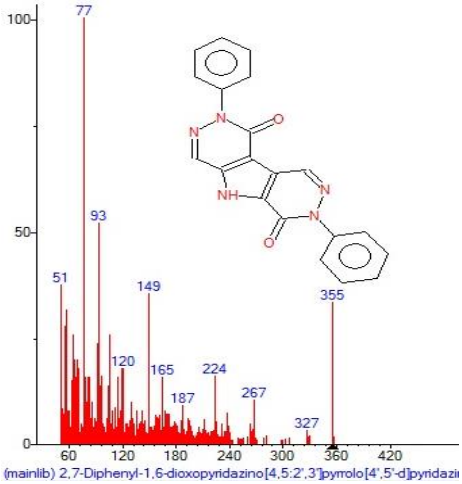


Figure 22: Mass spectrum of 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazin with Retention Time (RT)= 10.113

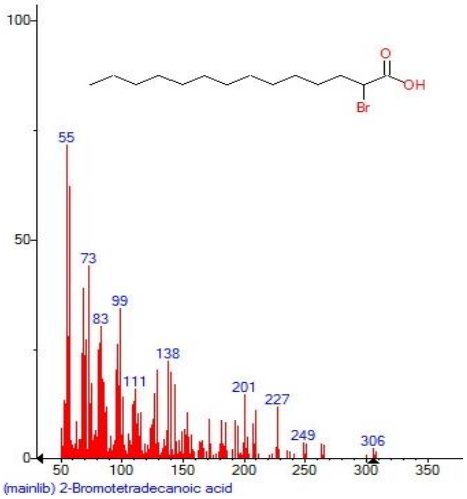


Figure 23: Mass spectrum of 2-Bromotetradecanoic acid with Retention Time (RT)= 13.335

galactopyranosyl, 6-Acetyl-ß-d-mannose, 5-Hydroxymethylfurfural, 1-Gala-l-ido-octonic lactone, Pterin-6-carboxylic acid, Uric acid, Acetamide, N-methyl -N-[4-[2-acetoxymethyl-1-pyrrolidyl]-2-butynyl], l-(+)-Ascorbic acid 2,6-dihexadecanoate, D-fructose, diethyl mercaptal, pentaacetate, 2-Bromotetradecanoic acid, Octadecanal ,2 –bromo, L-Ascorbic acid, 6-octadecanoate, 18,19-Secoyohimban-19- oic acid,16,17,20,21-tetradehydro-16. (Fig. 5-35). Many compounds are identified in the present study. Some of them are biological compounds with antimicrobial activities. Grotkjaer et al. (2004)[40] developed a detailed dynamic model describing carbon atom transitions in the central metabolism of S. cerevisiae to study the rate at which 13C is incorporated into biomass. The mass isotopomer distributions of the intracellular metabolites were measured as described by Van et al. (2005)[41].

Antibacterial and antifungal activity

Clinical pathogens selected for antibacterial activity namely, *Klebsiella pneumonia*, *Proteus mirabilis, Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumonia* and *Pseudomonas eurogenosa,* maximum zone formation against *Proteus mirabilis* (6.96±0.22) mm, Table 2. In agar well diffusion method the selected medicinal plants (*Borrago Officinalis, Adiantum capillus- veneris, Arbutus unedo, Echium vulgare, Fumaria officinalis, Evernia punastri, Iris germanica, Adonis vernalis, Anchusa officinalis, Cassia accutifolia, Dipsacus fullonum, Juniperus phoenicia, Atropa belladone, Convulvus arvensis, Quercus infectoria, Citrullus colocynthis, Althaea rosea, Anastatica hierochuntica, Melia azedarach, Origanum vulgare, Lawsonia alba, Glaucium flavum, Nigella sativa, Ocimum basilicum, Artemisia campestris* and *Erythraea centaurium*) were effective against *S. cerevisiae*, Table 3. *Evernia punastri* was very highly antifungal activity (7.00±0.25) mm against *S. cerevisiae*. *Saccharomyces cerevisiae* 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. In conclusion, this study provides new scientific information about *S. cerevisiae*, based on its secondary metabolites, antibacterial potential and chemical. The antibacterial activity of *S. cerevisiae* may be attributed to the various phytochemical constituents present in the extract. Further work on the types of chemical constituents and purification of individual groups of bioactive components could reveal the full potential of the *S. cerevisiae* extract to inhibit several pathogenic microbes.

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