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Research Article

Analysis of Bioactive Metabolites from *Candida albicans* Using (GC-MS) and Evaluation of Antibacterial Activity

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ABSTRACT

The objectives of this research were analysis of the secondary metabolite produced by *Candida albicans* and evaluation antibacterial activity. Bioactives are chemical compounds often referred to as secondary metabolites. Thirtynine bioactive compounds were identified in the methanolic extract of*Candida albicans*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. GC-MS analysis of*Candida albicans* revealed the existence of the 1,4-Benzendiol ,2,6-bis(1,1-dimethylethyl)- , Thieno[2,3-c]furan-3-carboniterile , 2-amino-4,6-dihydro-4,4,6,6-te , Z-8-Methyl-9-tetradecenoic acid , i-Propyl 9-tetradecenoate , 9,12,15,-Octadecatrienoic, 2-[(trimethylsilyl)oxy]-1-[(trimethyl , 17-Octadecynoic acid , Oxime-, methoxy-phenyl- , Edulanll , p-Menth-1-en-3-one ,semicarbazone , 5,7-dodecadiyn-1,12-diol , Methyl 2-O-benzyl-d-arabinofuranoside , Erythritol , d-Glycero-l-gluco-heptose , D-Glucose , 6-O-α-D-galactopyranosyl- , l-Gala-l-ido-octonic lactone , Desulphosingrin , 2(3H)-Furanone , 3-butyldihydro- , β-Hydroxyquebrachamine , 1,4-benzendiol , 2,6-bis(1,1-dimethylethyl)- , 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-,N-(4,6-Dimethyl-2-pyrimi-dinyl)-4-(4-nitrobenzylideneamino)benzel , 2,7-Diphenyl-1,6-dioxopyridazino [4,5:2',3'] pyrrolo[4',5'-d]pyridazin , 2-Methyl-9-β-d-ribofuranosylhypoxanthine , Ergosta-5,22-dien-3-ol,acetate,(3β,22E)- , 10-Heptadecen-8-ynoic acid , methyl ester , (E)- , Chromone , 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl- , 1-Methyl-8-propyl-3,6-diazahomoadamantan-9-ol , 1-(4-Amino-furazan-3-yl)-5-dimethylaminomethyl-1H-[1,2,3]triazole , 5-Bromo-8-[(4-hydroxybenzylidene)amino]quinolone , Carbamic acid , N-methyl-,(6-chloro-2-methyl-1,1-dioxidobenzo) , d-Mannose , α-D-Glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D- , 12-Methyl-oxa-cyclododecan-2-one , Acetamide , N-methyl-N-[4-[2acetoxymethyl-1-pyrrolidyl]-2-butyn , Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2butynyl]- , Estra-1,3,5(10)-triene-17β-ol , Curan-17-oic acid, 19,20-dihydroxy-,methyl ester,(19S)- , 2,5,5,8A-Tetramethyl-6,7,8,8atetrahydro-5H-chromen-8-ol and 6-Octadecenoic acid. *Proteus mirabilis* was very highly antifungal activity (6.19±0.20) mm while *Neriumolender*(Alkaloids) has maximum zone formation (7.67±0.21) mm against *Aspergillus fumigatus*.

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

INTRODUCTION

*Candida albicans*is a fungal yeast involved in candidiasis that ranges from none-life-threatening mucocutaneous illnesses to invasive processes[1,2]. The control of *Candia* infections is proving to be intractable by means of present anti *Candida* agents due to variety of reasons including development of resistance to antimicrobials, expensive nature and undesirable effects on non-target tissues and organisms[3,4]. Antimicrobial agents, particularly antibiotics, have been the standard therapy for managing microbial infections, but in recent years, genetic variation has given to pathogenic microbes a great advantage by creating antibiotic resistance so the search for new antimicrobial substances or drugs continues to be necessary[5-7].The broad range of *Candida* infections requires an equally broad range of diagnostic and therapeutic strategies[8-11]. Major clinical issues arise when pathogenic microbes develop multi-drug resistance intertwined with other problems such as level of toxicity of antimicrobial drugs on host tissues. Further, reports from the scientific community have raised concerns that antibacterial drug development will not be adequately addressing the problems posed by antibiotic resistance among important bacterial pathogens[12-18].Several plants have been reported significant for their anti-fungal activity but only a few botanicals have moved from the laboratory to field use, as they are poorly characterized, in most cases active principals are not determined and most of the works are restricted to preliminary screening. The aims of this study were screening of the bioactive chemical products and evaluation antimicobial activity.

MATERIALS AND METHODS

Growth conditions of C. albicans and determination of metabolites

C. albicans was isolated from dried fruit and the pure colonies were selected, isolated and maintained in potato dextrose agar slants[19-21]. Spores were grown in a liquid

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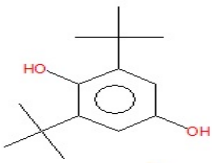
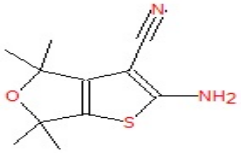
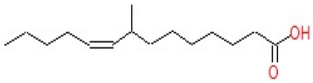
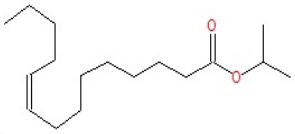
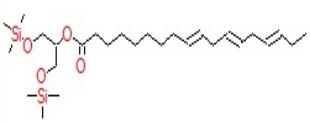
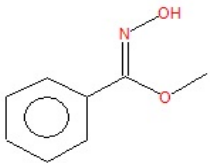
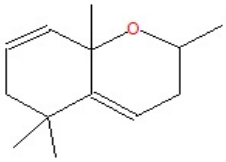
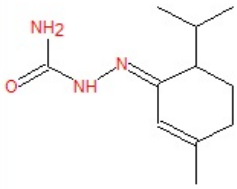
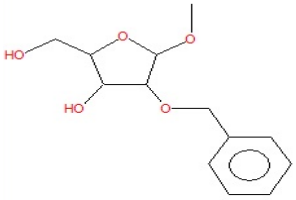
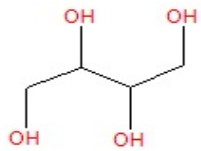
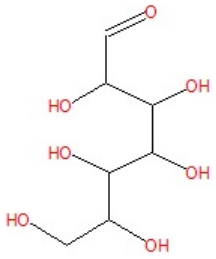
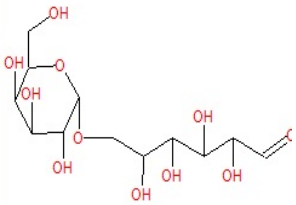
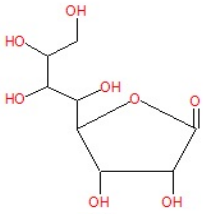
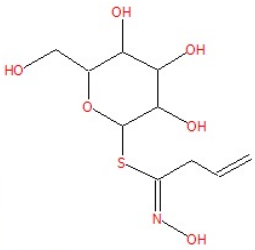
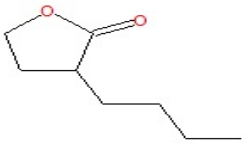
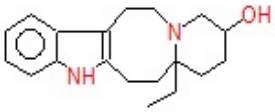
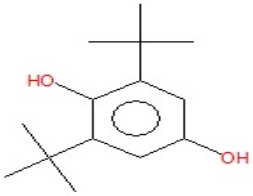
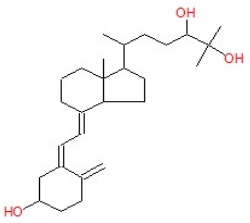
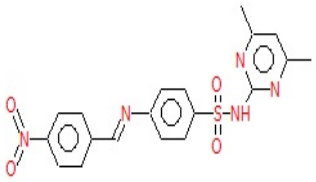
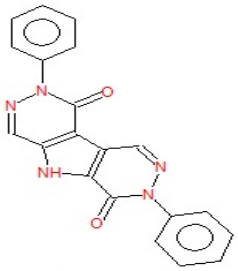
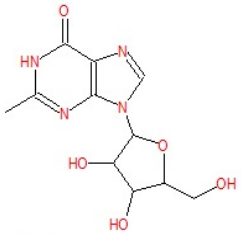
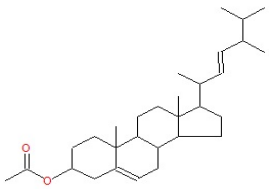
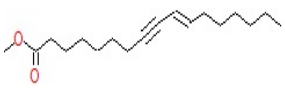
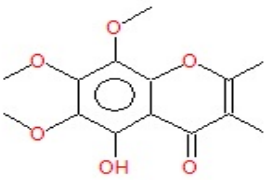
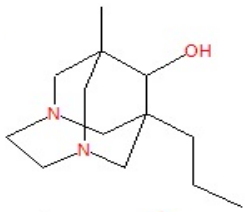
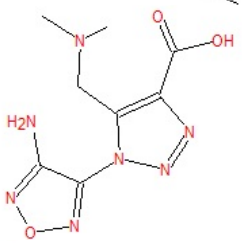
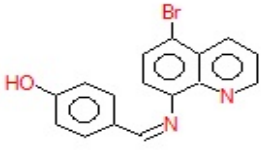
Table 1: Biochemical compounds identified in methanolic extract of *Candida albicans*.

S. No.	Phytochemical compound	Exact Mass	Molecular Weight	RT (min)	Chemical structure	MS Fragment-ions
1.	1,4-Benzendiol ,2,6-bis(1,1-dimethylethyl)-	222.16198	222	3.144		57,68,137,177,207, 222
2.	Thieno[2,3-c]furan-3-carboniterile , 2-amino-4,6-dihydro-4,4,6,6-te	222.0826845	222	3.224		60,96,165,207,222
3.	Z-8-Methyl-9-tetradecenoic acid	240.20893	240	3.304		55,69,97,111,137,208,240
4.	i-Propyl 9-tetradecenoate	268.24023	268	3.384		55,69,83,166,209,226,268
5.	9,12,15,-Octadecatrienoic , 2-[(trimethylsilyl)oxy]-1-[(trimethyl	496.340414	496	3.487		55,73,103,133,149, 191,221,249,281
6.	17-Octadecynoic acid	280.24023	280	3.647		55,67,81,95,201,233,264
7.	Oxime-, methoxy-phenyl-	151.063329	151	3.807		55,73,105,133,151
8.	Edulanll	192.151415	192	3.939		55,77,91,105,133,177,192
9.	p-Menth-1-en-3-one ,semicarbazone	209.152812	209	4.042		55,79,93,108,150,167,193,209
10.	5,7-dodecadiyn-1,12-diol	194.13068	194	4.546		55,79,91,115,163
11.	Methyl 2-O-benzyl-d-arabinofuranoside	254.115423	254	4.780		57,91,163,254

12.	Erythritol	122.057909	122	4.912		61,91
13.	d-Glycero-l-gluco-heptose	210.073953	210	5.518		60,73,85,103,115,133,149,174,210
14.	D-Glucose , 6-O-α-D-galactopyranosyl -	342.11621	342	5.627		60,73,85,110,126,212,261
15.	l-Gala-l-ido-octonic lactone	238.068868	238	5.707		61,73,84,112,127,142,159,238
16.	Desulphosingrin	279.077658	279	5.999		60,73,85,103,127,145,163,213,262
17.	2(3H)-Furanone , 3-butyldihydro-	142.09938	142	6.182		55,73,86,99,142
18.	β-Hydroxyquebrachamine	298.204514	298	6.892		55,77,124,172,185,281,298
19.	1,4-benzendiol , 2,6-bis(1,1-dimethylethyl)-	222.16198	222	7.080		57,68,137,177,207,222
20.	9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)-	416.329044	416	7.212		55,91,118,136,158,176,207,253,383,416

21.	N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino)benzel	411.100124	411	7.395		51,77,104,120,151,171,214
22.	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazin	355.106924	355	7.578		51,77,93,120,149,165,187,224,267,327,355
23.	2-Methyl-9-β-d-ribofuranosylhypoxanthine	282.09642	282	7.790		57,73,114,150,179,216,282
24.	Ergosta-5,22-dien-3-ol,acetate,(3β,22E)-	440.36543	440	8.265		55,67,91,105,145,159,213,227,255,281,327,365,380
25.	10-Heptadecen-8-ynoic acid , methyl ester , (E)-	278.22458	278	8.585		57,67,79,91,150,164,205,247,278
26.	Chromone , 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-	280.094688	280	8.980		57,71,91,119,151,165,193,237,265,280
27.	1-Methyl-8-propyl-3,6-diazahomoadamantan-9-ol	224.188864	224	9.060		55,72,82,96,124,195,224
28.	1-(4-Amino-furazan-3-yl)-5-dimethylaminomethyl-1H-[1,2,3]triazole	253.092338	253	9.215		58,82,151,209,253
29.	5-Bromo-8-[(4-hydroxybenzylidene)amino]quinolone	326.005476	326	9.404		64,77,101,128,143,163,207,222,248,326

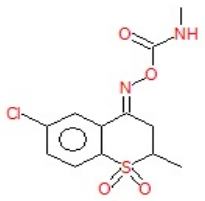
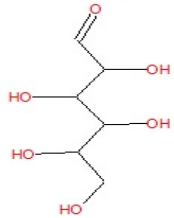
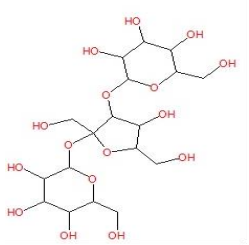
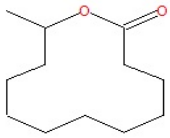
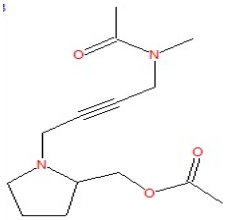
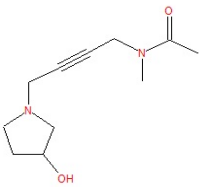
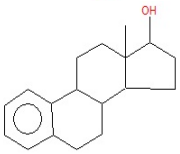
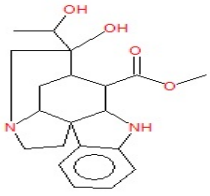
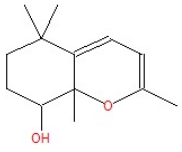
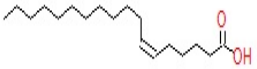
30.	Carbamic acid , N-methyl-,(6-chloro-2-methyl-1,1-dioxidobenzo)	316.028456	316	9.798		59,75,100,150,178,226,259,281
31.	d-Mannose	180.063388	180	10.639		60,73,103,149,163
32.	α-D-Glucopyranoside ,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-	504.169035	504	10.960		60,73,85,97,126,145,199
33.	12-Methyl-oxa-cyclododecan-2-one	198.16198	198	12.224		55,69,84,98,138,154,180,198
34.	Acetamide , N-methyl-N-[4-[2acetoxymethyl-1-pyrrolidyl]-2-butyn	266.163042	266	13.100		55,67,82,124,141,193,251
35.	Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2butynyl]-	210.136827	210	13.598		56,68,124,137,167,192
36.	Estra-1,3,5(10)-triene-17β-ol	256.182714	256	13.678		57,73,85,97,129,185,213,256
37.	Curan-17-oic acid , 19,20-dihydroxy-,methyl ester,(19S)-	358.189257	358	14.336		57,83,97,111,144,199,228,270,326,358
38.	2,5,5,8A-Tetramethyl-6,7,8,8atetrahydro-5H-chromen-8-ol	208.14633	208	15.160		57,91,106,134,175,190,208
39.	6-Octadecenoic acid	282.25588	282	15.343		55,69,97,222,264,282

Table 2: Zone of inhibition (mm) of test bacterial strains to *Candida albicans* bioactive compounds and standard antibiotics.

Bacteria	(*Candida albicans*) products /Antibiotics				
	Fungal metabolites	Cefotoxime	Kanamycin	Rifambin	Streptomycin
Streptococcus pneumonia	5.04±0.10	2.04±0.13	2.00±0.11	0.94±0.10	2.81±0.15
Pseudomonas eurogenosa	6.00±0.21	2.02±0.20	1.29±0.18	1.50±0.71	1.33±0.21
Staphylococcus epidermidis	5.08±0.11	1.06±0.28	0.98±0.19	2.00±0.23	1.40±0.19
Escherichia coli	5.01±0.23	2.00±0.15	1.07±0.23	0.07±0.10	2.00±0.10
Proteus mirabilis	6.19±0.20	2.04±0.20	2.04±0.29	1.91±0.14	1.66±0.11
Streptococcus pyogenes	4.0±0.11	1.07±0.20	1.11±0.20	1.88±0.11	2.00±0.13
Staphylococcus aureus	3.99±0.12	0.99±0.18	0.75±0.10	2.01±0.24	1.50±0.16
Streptococcus faecalis	4.87±0.20	1.97±0.25	0.80±0.10	0.11±0.12	2.70±0.28
Klebsiella pneumonia	5.10±0.13	2.01±0.100	2.00±0.17	1.30±0.22	2.10±0.20

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

S. No.	Plant	Zone of inhibition (mm)
1.	*Gramineaepoaceae*(Crude)	7.00±0.23
2.	*Neriumolender*(Alkaloids)	7.61±0.20
3.	*Datura stramonium*(Alkaloids)	5.95±0.19
4.	*Piper nigrum*(Crude)	6.01±0.27
5.	*Zingiberofficinale*(Crude)	4.71±0.23
6.	*Linumusitatissimum*(Crude)	4.66±0.18
7.	*Cassia angustifolia*(Crude)	5.00±0.29
8.	*Euphorbia lathyrus*(Crude)	5.36±0.10
9.	*Foeniculum vulgare* (Crude)	5.49±0.27
10.	*Quercusinfectoria*(Crude)	5.30±0.18
11.	*Citrulluscolocynthis*(Crude)	3.15±0.24
12.	*Coriandrumsativum*(Crude)	4.09±0.26
13.	*Origanum vulgare*(Crude)	6.48±0.21
14.	*Urticadioica*(Crude)	5.00±0.27
15.	*Equisetum arvense*(Crude)	5.11±0.23
16.	*Artemisia annua*(Crude)	5.09±0.29
17.	*Punicagranatum*(Crude)	5.14±0.19
18.	*Cinnamomumzeylanicum*(Crude)	4.37±0.28
19.	Amphotericin B	5.98±0.27
20.	Fluconazol	7.62±0.15
21.	Control	0.00

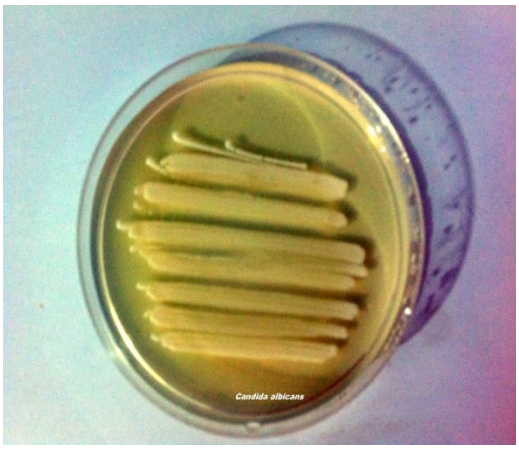


Figure 1: Morphological characterization of *C. albicans* colony.

culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for sixteen days at 150 rpm. The extraction was performed by adding 50 ml methanol to 150 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[22,23]. 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.

Analysis of bioactive compounds

GC-MS analysis was done on a thermo gas chromatography mass spectrometer (Agilent 789 A) equipped with DB-5 capillary column (30 m long, 0.25 mm i.d., filmthickness 0.25 µm). The column temperature program was 50 ℃ for 6 min, with 5 ℃ increases per minto 250 ℃; which was maintained for 30 min. The carrier gas was helium at a flow rate of 1 mL/min.The detector and injector temperatures were both maintained at 250 ℃. The quadrupole massspectrometer scanned over the range 28–400 amu at 1 scan/ sec, with an ionizing voltage of 70 eV, anionization current of 150 Ma and an ion source temperature of 200 ℃. 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[24,25].One-Way ANOVA was used to compare the means of three experimental groups with Tukey'spost-hoc test to calculate least significant differences. The difference between means was consideredsignificant when p was <0.05.

Determination of antibacterial and antifungal activity

The test pathogens (*Streptococcus pneumonia*, *Pseudomonaseurogenosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus faecalis*and*Klebsiella pneumonia*) 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 hr and examined[26-28]. After the incubation the diameter of inhibition zones around the discs was measured.*C. albicans*isolate was suspended in potato dextrose broth

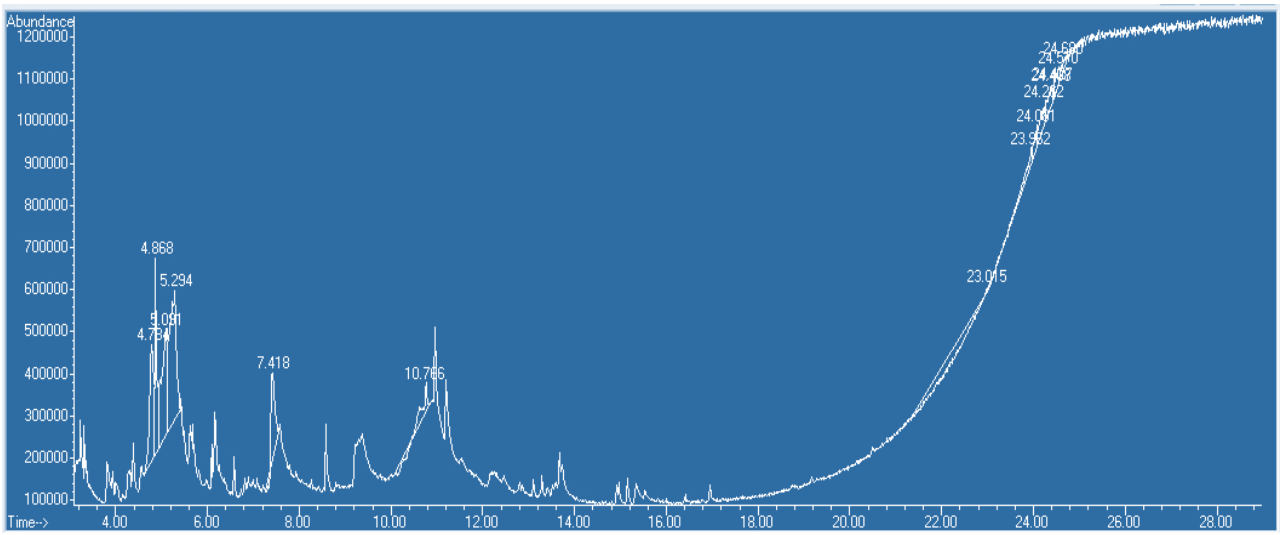


Figure 2: GC-MS chromatogram of methanolic extract of *Candida albicans*.

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[29-33]. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (*Gramineaepoaceae, Neriumolender, Datura stramonium, Piper nigrum, Zingiberofficinale, Linumusitatissimum, Cassia angustifolia, Euphorbia lathyrus, Foeniculum vulgare, Quercusinfectoria, Citrulluscolocynthis, Coriandrumsativum, Origanum vulgare, Urticadioica, Equisetum arvense, Artemisia annua, Punicagranatum* and *Cinnamomumzeylanicum*) 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[34-41]. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

RESULTS AND DISCUSSION

Microscopical characteristics of fungal strains were determined using specific media light and compound microscope Figure 1. The 400ml of fermentation broth (PDA broth) which 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.

Determination of secondary metabolites from Candida albicans

Gas chromatography and mass spectroscopy analysis of compounds was carried out in methanolic extract of *Candida albicans*, shown in Table 1. The GC-MS chromatogram of the thirtyone peaks of the compounds detected was shown in Figure 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, Figure 3. The second peak indicated to be 2-Furancarboxaldehyde,5-methyl, Figure 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-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. (Figure 5-41). Many compounds are identified in the presentstudy. Some of them are biologicalcompounds with antimicrobial activities.

Antibacterial and antifungal activity

Clinical pathogens selected for antibacterial activity namely, *Streptococcus pneumonia*, *Pseudomonaseurogenosa*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus mirabilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Streptococcus faecalis*and*Klebsiella pneumonia*, maximum zone formation against *Proteus mirabilis* (6.19±0.20) mm, Table 2. In agar well diffusion method the selected medicinal plants (*Gramineaepoaceae, Neriumolender, Datura stramonium, Piper nigrum, Zingiberofficinale, Linumusitatissimum, Cassia angustifolia, Euphorbia*

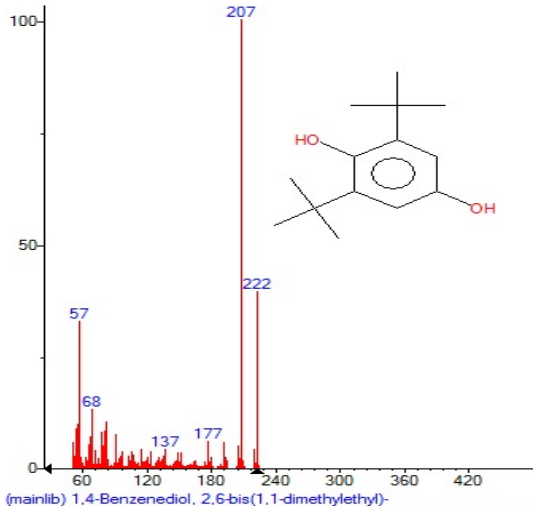


Figure 3: Mass spectrum of 1,4-Benzendiol ,2,6-bis(1,1-dimethylethyl)- with Retention Time (RT)= 3.144.

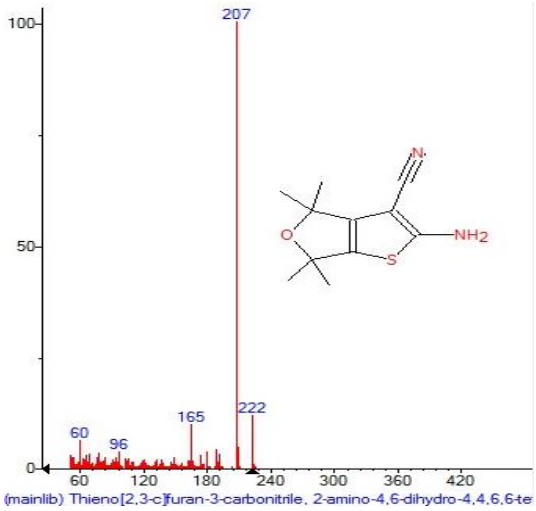


Figure 4: Mass spectrum of Thieno[2,3-c]furan-3-carboniterile , 2-amino-4,6-dihydro-4,4,6,6-te with Retention Time (RT)= 3.224.

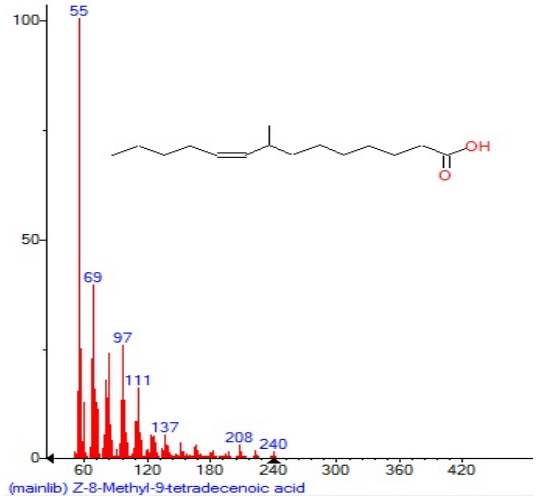


Figure 5: Mass spectrum of Z-8-Methyl-9-tetradecenoic acid with Retention Time (RT)= 3.304.

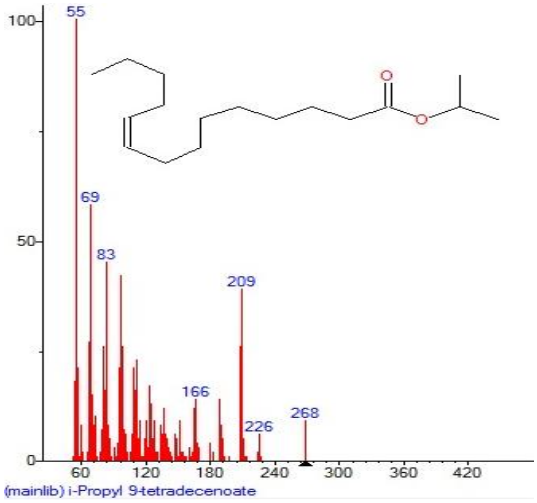


Figure 6: Mass spectrum of i-Propyl 9-tetradecenoate with Retention Time (RT)= 3.384.

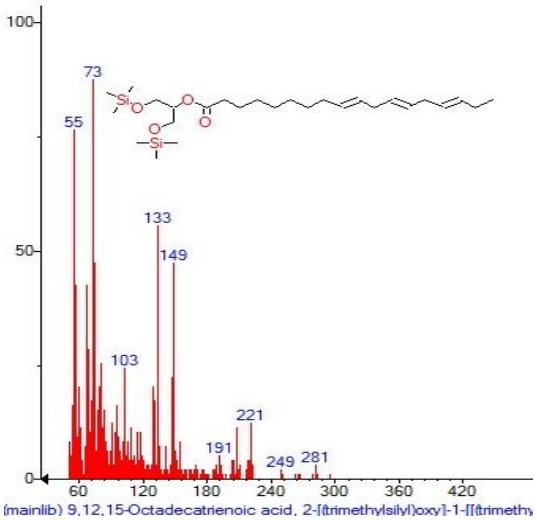


Figure 7: Mass spectrum of 9,12,15,-Octadecatrienoic, 2-[(trimethylsilyl)oxy]-1-[(trimethyl with Retention Time (RT)= 3.487.

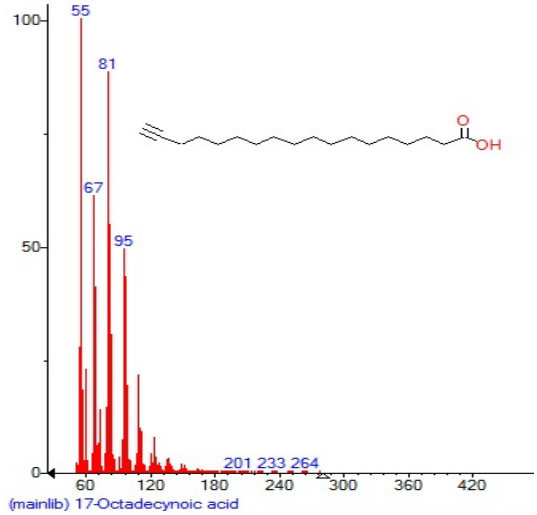


Figure 8: Mass spectrum of 17-Octadecynoic acid with Retention Time (RT)= 3.647.

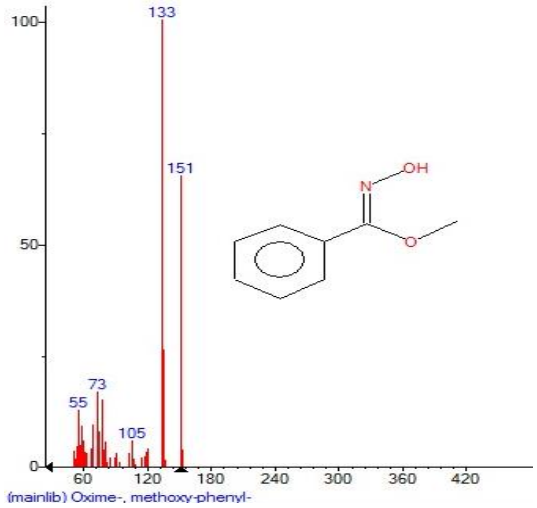


Figure 9: Mass spectrum of Oxime-, methoxy-phenyl- with Retention Time (RT)= 3.807.

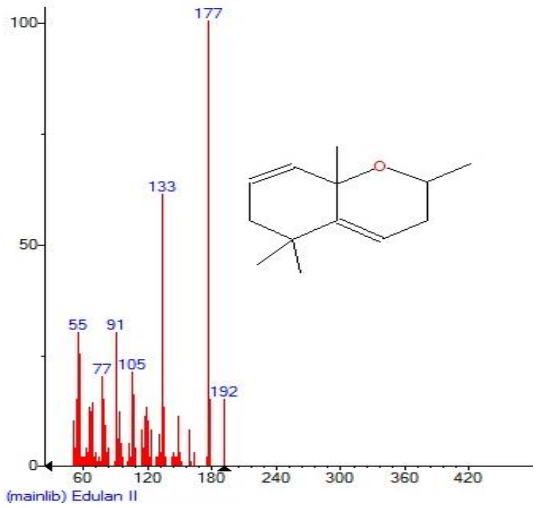


Figure 10: Mass spectrum of Edulanll with Retention Time (RT)= 3.939.

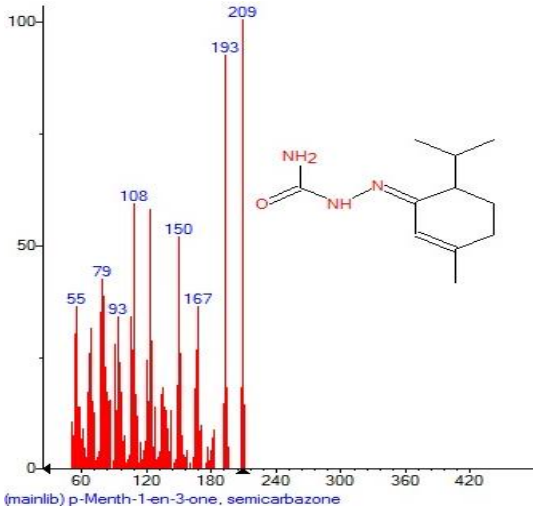


Figure 11: Mass spectrum of p-Menth-1-en-3-one , semicarbazone with Retention Time (RT)= 4.042.

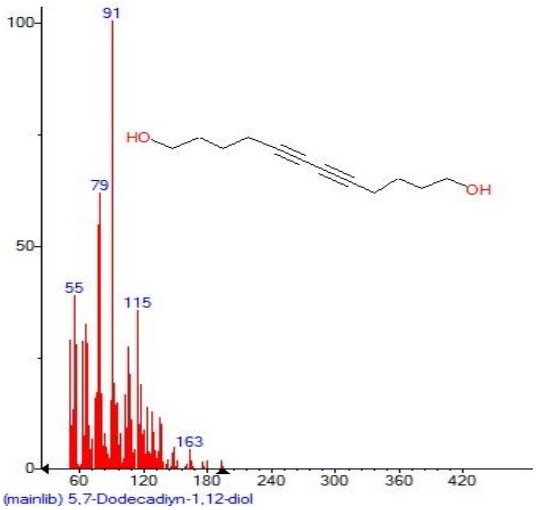


Figure 12: Mass spectrum of 5,7-dodecadiyn-1,12-diol with Retention Time (RT)= 4.546.

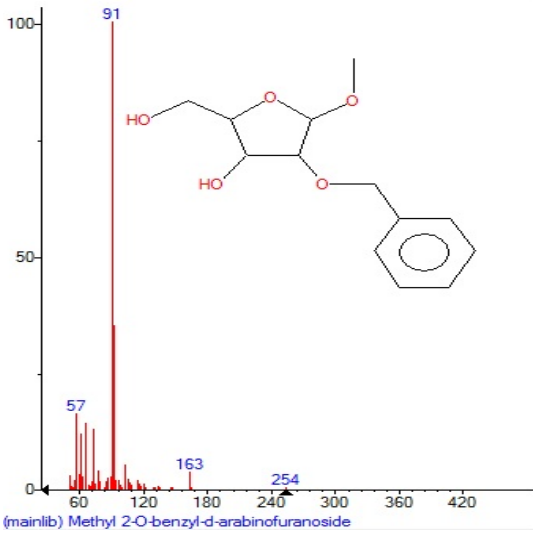


Figure 13: Mass spectrum of Methyl 2-O-benzyl-d-arabinofuranoside with Retention Time (RT)= 4.780.

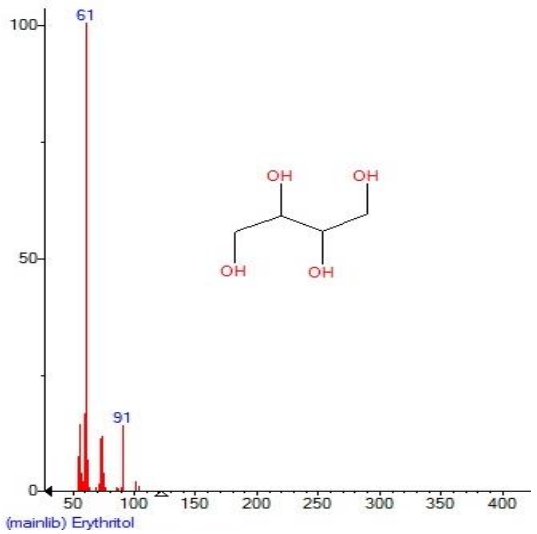


Figure 14: Mass spectrum of Erythritol with Retention Time (RT)= 4.912.

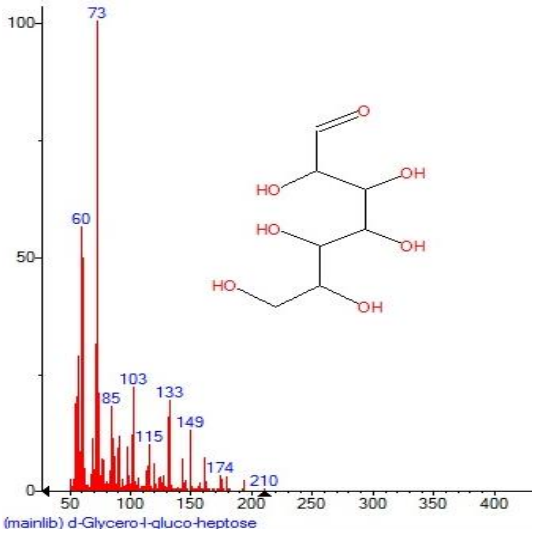


Figure 15: Mass spectrum of d-Glycero-l-gluco-heptose with Retention Time (RT)= 5.518.

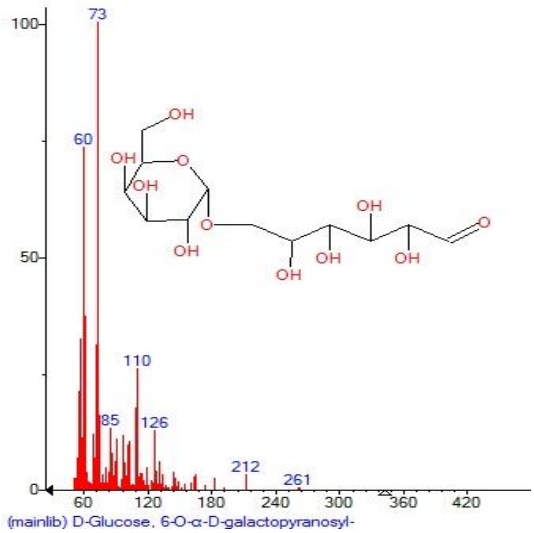


Figure 16: Mass spectrum of D-Glucose , 6-O-α-D-galactopyranosyl- with Retention Time (RT)= 5.627.

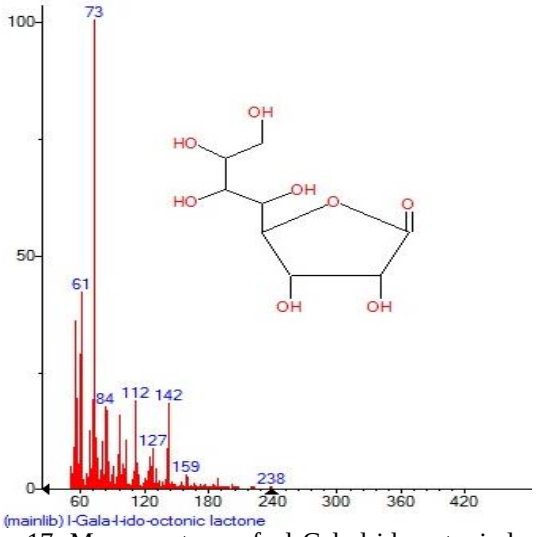


Figure 17: Mass spectrum of l-Gala-l-ido-octonic lactone with Retention Time (RT)= 5.707.

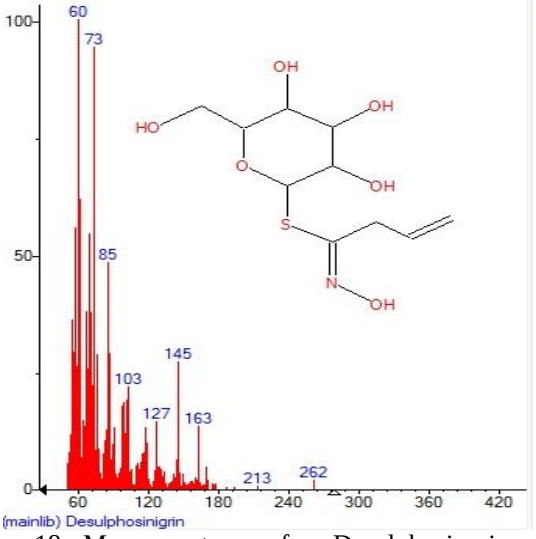


Figure 18: Mass spectrum of Desulphosingrin with Retention Time (RT)= 5.999.

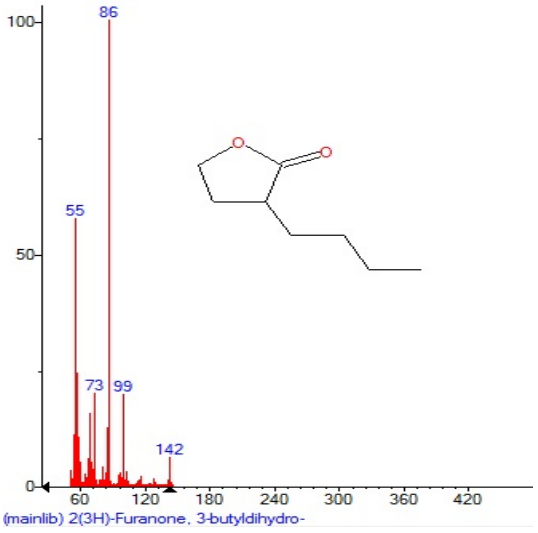


Figure 19: Mass spectrum of 2(3H)-Furanone , 3-butyldihydro- with Retention Time (RT)= 6.182.

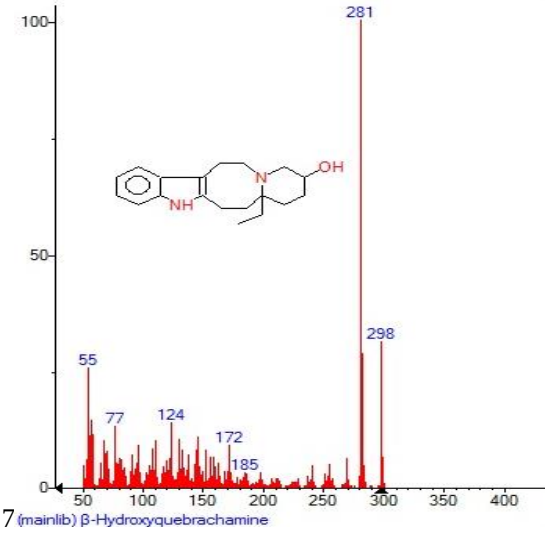


Figure 20: Mass spectrum of β-Hydroxyquebrachamine with Retention Time (RT)= 6.892.

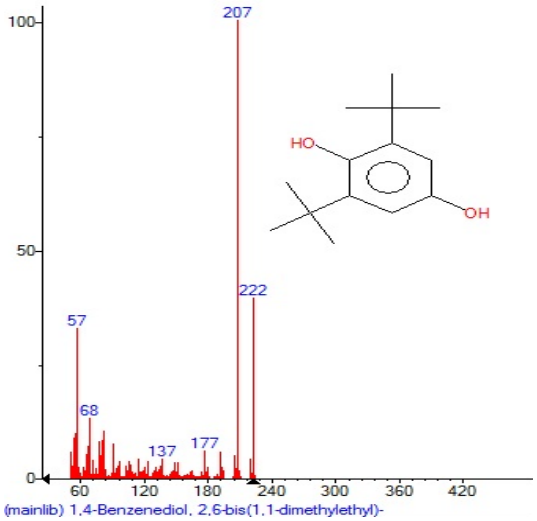


Figure 21: Mass spectrum of 1,4-benzendiol , 2,6-bis(1,1-dimethylethyl)- with Retention Time (RT)= 7.080.

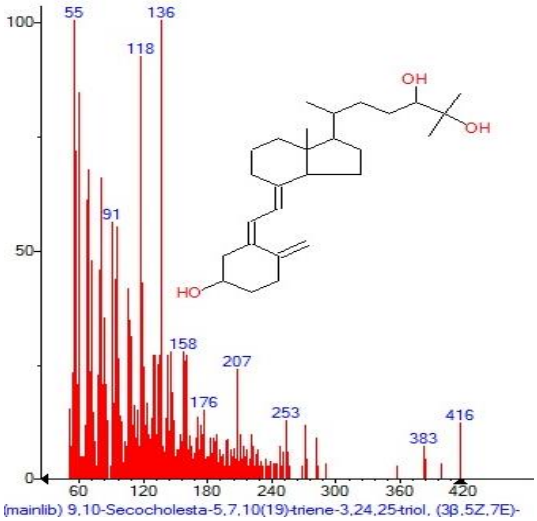


Figure 22: Mass spectrum of 9,10-Secocholesta-5,7,10(19)-triene-3,24,25-triol,(3β,5Z,7E)- with Retention Time (RT)= 7.212.

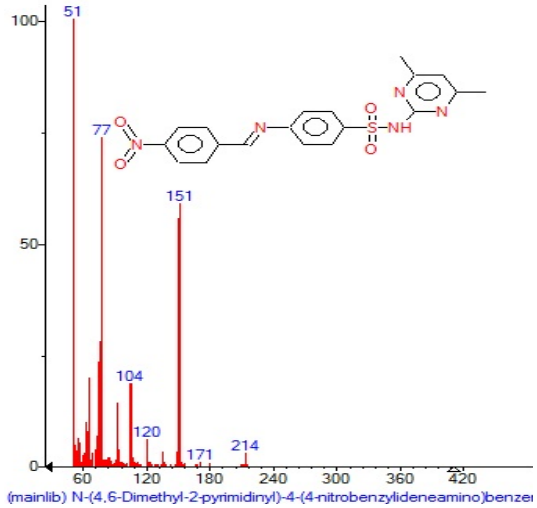


Figure 23: Mass spectrum of N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylideneamino)benzel with Retention Time (RT)= 7.395.

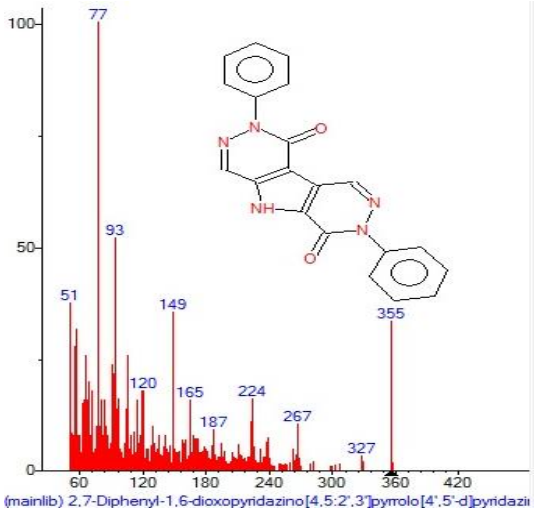


Figure 24: Mass spectrum of 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2',3']pyrrolo[4',5'-d]pyridazin with Retention Time (RT)= 7.578.

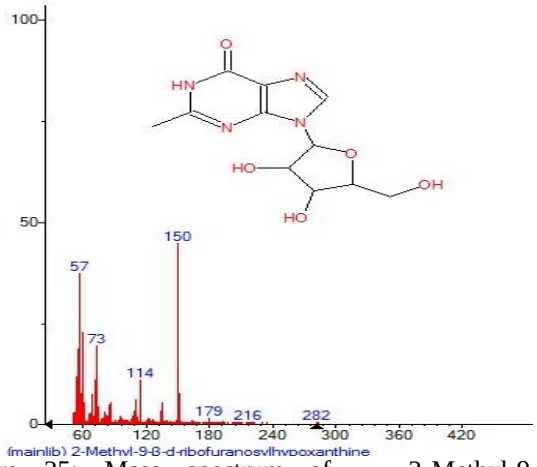


Figure 25: Mass spectrum of 2-Methyl-9-β-d-ribofuranosylhypoxanthine with Retention Time (RT)= 7.790.

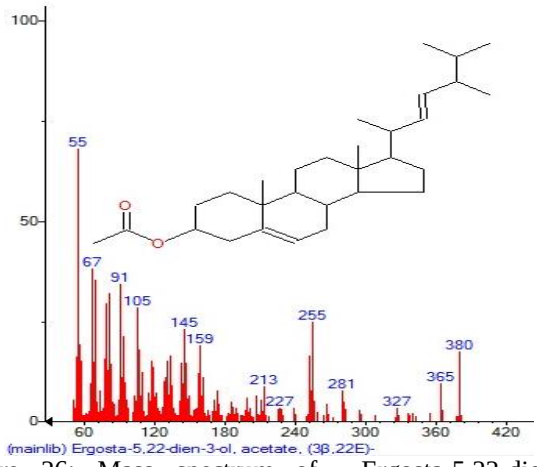


Figure 26: Mass spectrum of Ergosta-5,22-dien-3-ol,acetate,(3β,22E)- with Retention Time (RT)= 8.265.

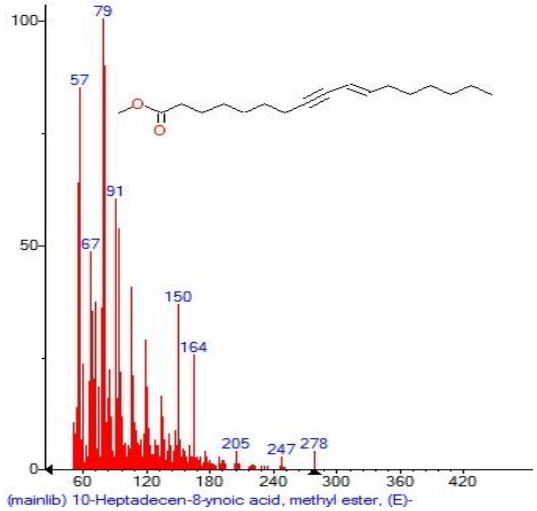


Figure 27: Mass spectrum of 10-Heptadecen-8-ynoic acid , methyl ester , (E)- with Retention Time (RT)= 8.585.

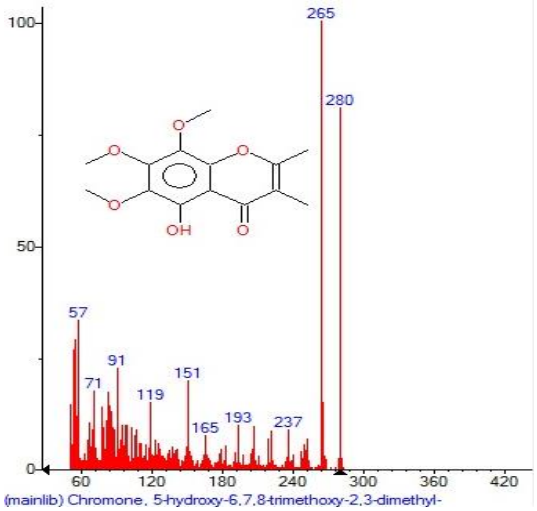


Figure 28: Mass spectrum of Chromone , 5-hydroxy-6,7,8-trimethoxy-2,3-dimethyl-with Retention Time (RT)= 8.980.

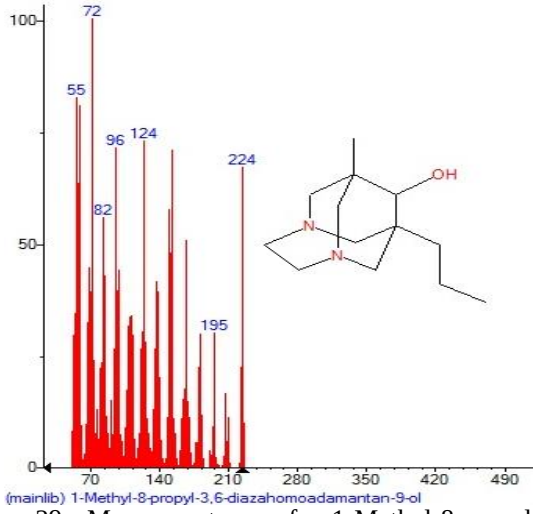


Figure 29: Mass spectrum of 1-Methyl-8-propyl-3,6-diazahomoadamantan-9-ol with Retention Time (RT)= 9.060 .

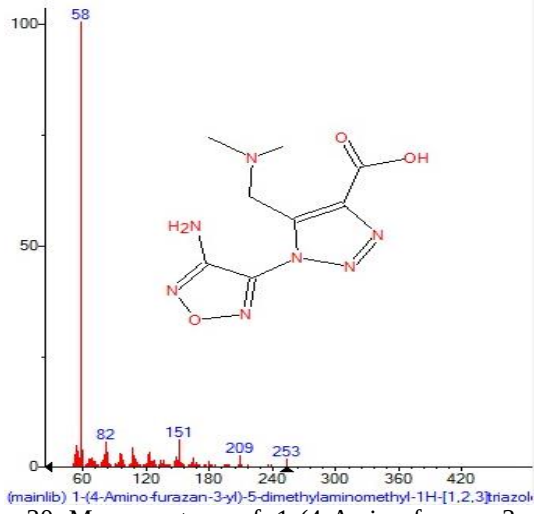


Figure 30: Mass spectrum of 1-(4-Amino-furazan-3-yl)-5-dimethylaminomethyl-1H-[1,2,3]triazole with Retention Time (RT)= 9.215.

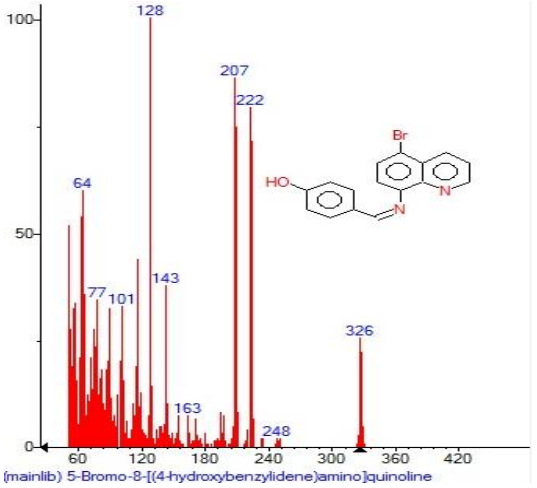


Figure 31: Mass spectrum of 5-Bromo-8-[(4-hydroxybenzylidene)amino]quinolone with Retention Time (RT)= 9.404.

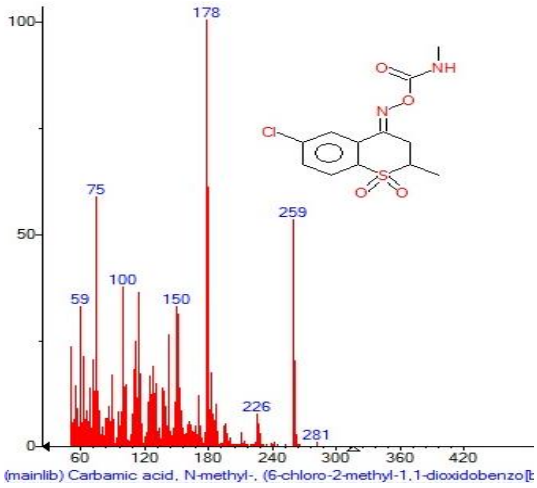


Figure 32: Mass spectrum of Carbamic acid , N-methyl-,(6-chloro-2-methyl-1,1-dioxidobenzo) with Retention Time (RT)= 9.798.

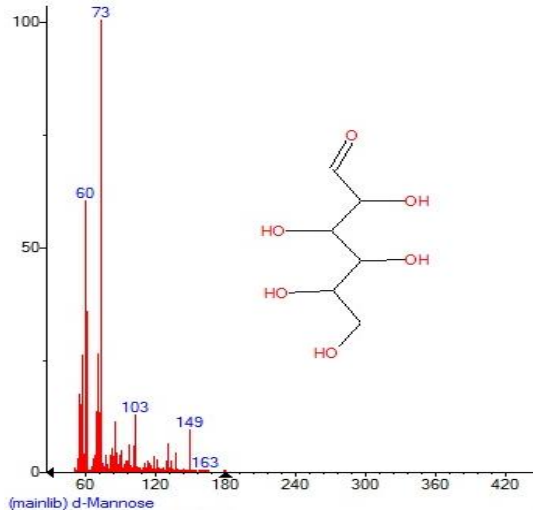


Figure 33: Mass spectrum of d-Mannose with Retention Time (RT)= 10.639.

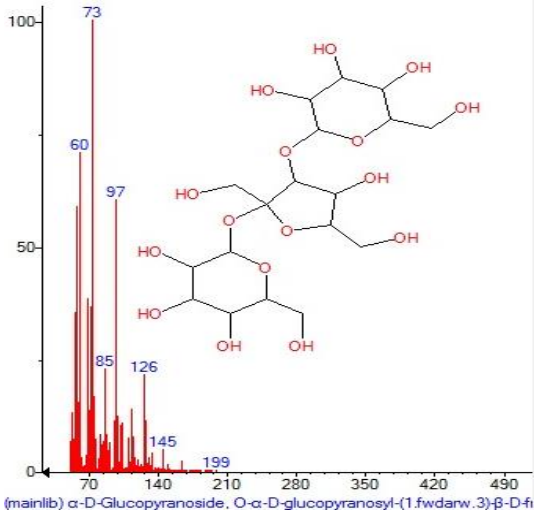


Figure 34: Mass spectrum of α-D-Glucopyranoside,O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D- with Retention Time (RT)= 10.960.

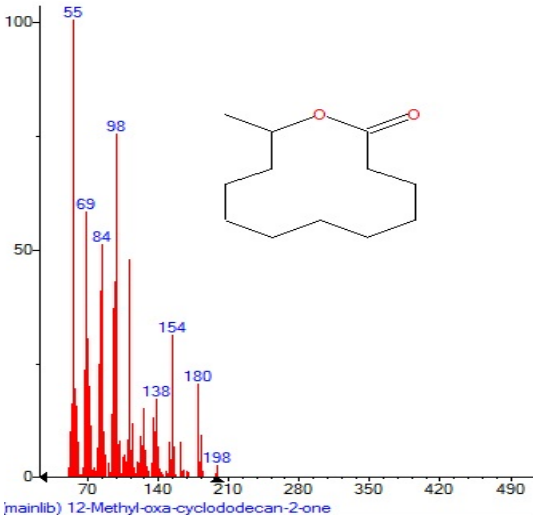


Figure 35: Mass spectrum of 12-Methyl-oxa-cyclododecan-2-one with Retention Time (RT)= 12.224.

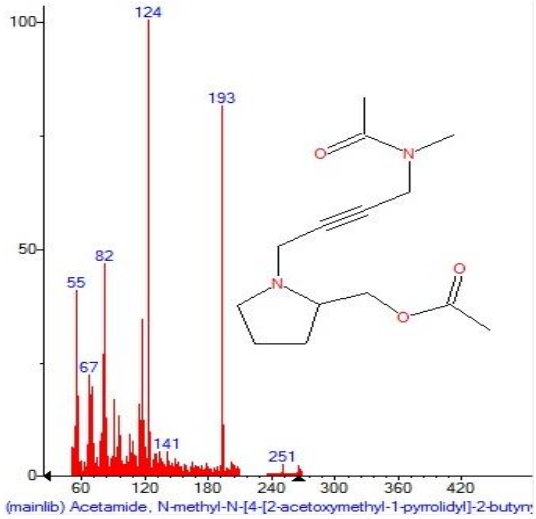


Figure 36: Mass spectrum of Acetamide , N-methyl-N-[4-[2acetoxymethyl-1-pyrrolidyl]-2-butyn with Retention Time (RT)= 13.100.

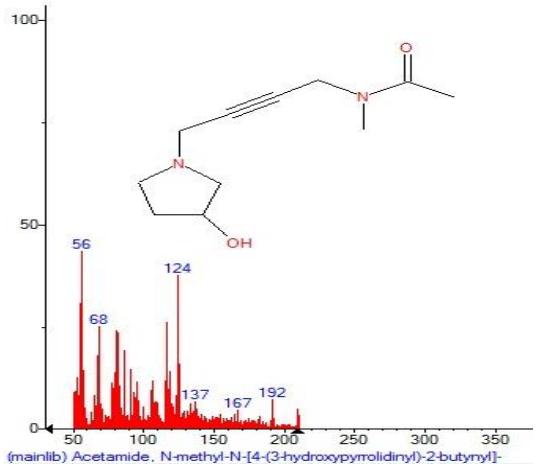


Figure 37: Mass spectrum of Acetamide , N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2butynyl]- with Retention Time (RT)= 13.598.

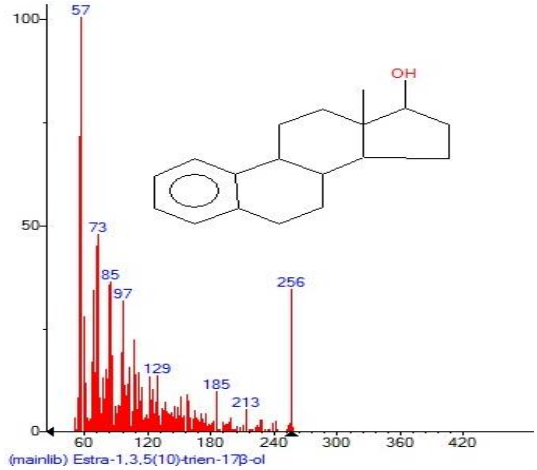


Figure 38: Mass spectrum of Estra-1,3,5(10)-triene-17β-ol with Retention Time (RT)= 13.678.

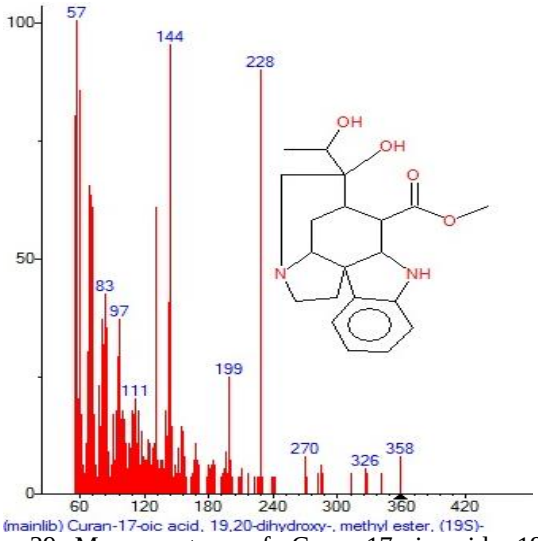


Figure 39: Mass spectrum of Curan-17-oic acid , 19,20-dihydroxy-,methyl ester,(19S)- with Retention Time (RT)= 14.336.

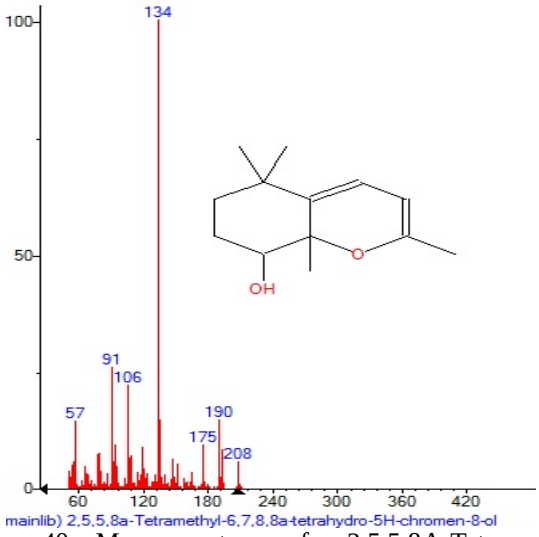


Figure 40: Mass spectrum of 2,5,5,8A-Tetramethyl-6,7,8,8atetrahydro-5H-chromen-8-ol with Retention Time (RT)= 15.160.

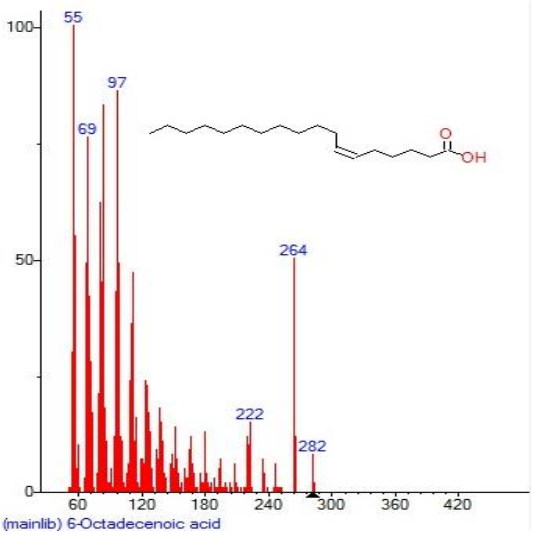


Figure 41: Mass spectrum of 6-Octadecenoic acid with Retention Time (RT)= 15.343.

*lathyrus, Foeniculum vulgare, Quercusinfectoria, Citrulluscolocynthis, Coriandrumsativum, Origanum vulgare, Urticadioica, Equisetum arvense, Artemisia annua, Punicagranatum*and*Cinnamomumzeylanicum*) were effective against *Candida albicans* Table 3. *Neriumolender*(Alkaloids) was very highly antifungal activity (7.67±0.21) mm against *Candida albicans*. *Candida albicans* 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.

CONCLUSION

In conclusion, this study provides new scientific information about *C. albicans*, based on its secondary metabolites, antibacterial potential and chemical. The antibacterial activity of *C. albicans* 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 *C. albicans* extract to inhibit several pathogenic microbes.

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