Journal of Natural Sciences Research www.iiste.org
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.7, No.12, 2017

Effect of Three Medicinal Plants Extracts on the Growth of Some Yeasts

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Abstract
This study conducted to detect the effect of the ethanolic extracts for some medicinal plants on 16 yeast species isolated from the oral cavity of cancer patients, three plants which are *Lavandula angustifolia*, *Salvia officinalis* and *Syzygium aromaticum* were used to study their inhibition bioactivity and compare their effect with three antifungal drugs (Fluconazole, Ketoconazole and Nystatin), the results showed that Ethanolic extract of *L. angustifolia* exhibited antifungal bioactivity against all yeast species and revealed inhibition zones ranged from 16-36 mm. with highest effect on *C. parapsilosis* whereas the lowest effect was on species *H. uvarum*, while the ethanolic extract of *S. officinalis* and *S. aromaticum* showed inhibition zones 16-27 mm. and 17-31 mm. respectively, the results showed that the plants extracts having much more effect on the yeasts growth from the antifungal drugs.
Keywords: yeasts, medicinal plants, Ethanolic extracts.

1. Introduction

Candida species are a major resident commensal on skin, mucosal surfaces, gastrointestinal tract and the genitourinary tract (Underhill and Iliev, 2014). These species are possible to cause candidal infections in both patients and healthy individuals by the aid of many predisposing factors. *Candida* genus includes about 200 known species, however about 15 of these species are isolated from patients as a causative for infections. The incidence of infection resulting by *Candida* species has increased substantially as a cause of increased number of immunocompromised individuals, widespread use of broad-spectrum antibiotics, overuse of immunosuppressive drugs in cancer and organ transplant patients, and increased use of invasive procedures and devices (such as drains and catheters) (Mousavi *et al*., 2012; Kaur *et al*., 2014; Yapar, 2014).

Susceptibility of *Candida* to antifungal drugs are various between different species. For instance, some of them are resistant to Azole group and other to Amphotericin B drug and so on (Al-mamari *et al*., 2014). Furthermore, the increased use of antifungal agents also contributes in the development of resistance to these agents. Antifungal resistance is related with high mortality rates, more expensive and increased their side effects with more resistant versus antifungal agents. So, there is need to find new therapeutic alternatives acts against fungi with high-efficiency, low side effects and cheap price. For these reasons, it has been the trend to the potential plant extracts to detection of natural effective compounds as antifungal agents, therefore, it was the choice for the best solution to these problems by using of alternative drugs from plants (Zhang *et al*., 2011; Martins *et al*., 2015).

So the aims of this study were extracting natural compounds from three medicinal plants and assess their antifungal bioactivity against some yeast species, and identifying of the most effectiveness compounds in plants extracts by GC-MS analysis.

2. Materials and methods

2.1. Yeast species

Sixteen yeasts species were used to study the effect of the medicinal plants on it, all of these yeasts were isolated previously from the oral cavity of cancer patients (Aldossary *et al*., 2016)

2.2. Collection, Identification and preparation of Plant Samples

Samples of *Lavandula angustifolia*, *Salvia officinalis* and *Syzygium aromaticum* were purchased from local market in Basrah Province, Iraq. The taxonomic identification of the plants was done by Botanist Prof. Dr. Saher Abdulabbas, Department of Biology, College of Science, University of Basrah. After removal of the foreign materials, the samples were washed with distilled water, dried at room temperature and crushed by electric mill to make soft powder, then, powder for each sample was preserved individually in a sterile opaque and airtight bottle in the refrigerator at 4℃ until use.

2.3. Preparation of Ethanolic Plant Extracts

The method of extraction was adopted from Harborne (1984) which modified by Almansour (1995).Two hundred grams of powdered plant was put into a thimble paper and placed in the column of Soxhlet extractor. Three hundred milliliters of ethanol 80% was used as a solvent. Extraction process has lasted about 8 hours, after

that, the extract was evaporated on a rotary evaporator at 45℃ under reduced pressure by vacuum pump to get rid of excess ethanol, then the extract of each plant was weighed and stored in sterile dark bottle at 4℃ till use. Before detection of antifungal bioactivity, we prepared the plant extracts by dissolving 500 mg of each dried extract in1000 ml of distilled water to get the desired concentration (0.5mg/ml) and then sterilized by passing through 0.22µm filter papers (Sosa *et al*., 2016).

2.4. Gas Chromatography-Mass Spectrum Analysis (GC-MS)

This technique was used in our study to identify the bioactive compounds existing in the plant extracts. GC-MS technique was carried out at GC-MS Lab. College of Agriculture, University of Basrah by using GC Shimadzu QP 2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS). It was equipped with fused silica capillary column (DB5MS) (Length: 30m, Diameter: 0.32 mm, Film thickness: 0.25 µm, composed of 95% methyl poly-siloxane and 5% phenyl) and Helium gas (99.999%) was used as a carrier gas.

Injection was conducted in split mode, and the column temperature was programmed at 50℃ for 1 minute with an increase at a rate of 5℃/minute until reach to final temperature 280℃, then 1µl of sample was injected into the capillary column with fixing the injector and detector temperature at 280℃. Separation process of compounds was conducted as described in (Table 1).

Table 1: Analytical circumstances for both GC and MS.

No.	Gas Chromatography	Mass Spectrometer
1	Column Oven Temp.: 40.0℃	Ion Source Temp.: 200.00℃
2	Injection Temp.: 280.00℃	Interface Temp.: 280.00℃
3	Injection Mode:Split	Solvent Cut Time: 3.00 min.
4	Flow Control Mode: Linear Velocity	Start Time: 3.00 min.
5	Pressure: 96.1 kPa	End Time: 35.00-37.50 min.
6	Total Flow: 56.1 ml/min.	ACQ Mode:Scan
7	Colum Flow: 1.71 ml/min.	Event Time: 0.50 sec.
8	Linear Velocity: 47.2 cm/sec.	Scan Speed: 1250
9	Purge Flow: 3.0 ml/min.	Start m/z: 50.00
10	Split Ratio: 30.0	End m/z: 600.00

As a result of this technique for each sample, the percentage of each component was appeared as a peak. The identification of the peaks was done by comparison of their IR (Retention Indices) with those reported in NIST mass spectral library.

2.4. Determination of Ethanolic Plant Extracts Bioactivity

A loopful from pure colony culture of each yeast species were suspended in normal saline and adjusted to a turbidity of tube No. 1 in McFarland standards (equivalent to $3x10^6$ fungal cells per ml). 25 µl of the yeast suspension was added on the SDA plates and spread through glass L-shape spreader. The plates were dried for 15 minutes at room temperature and then wells of 6 mm diameter were made using cork borer. 100 µl of each plant extract was added to each well and incubated at 37˚C for 48 hours. The results were read by measurement the inhibition zone diameter manually (Owotade*et al*., 2016).

2.5. Preparation of Antifungal Drugs Concentrations

Three antifungal medications (Fluconazole, Ketoconazole and Nystatin) which purchased from local pharmacies were used in present study to comparison their activity with the plant extracts bioactivity. 500 mg of each drug dissolved in 1 liter of distilled water to get the desired concentration 0.5mg/ml.

2.6. Determination of Antifungal Drugs Activity

25 µl of the yeast suspension, which prepared previously, was added on the SDA plates and spread through glass L-shape spreader. The plates were dried for 15 minutes at room temperature and then wells of 6 mm diameter were made using cork borer. 100 µl of each antifungal drug was added to each well and incubated at 37˚C for 48 hours. The results were read by measurement the inhibition zone diameter manually (Saxena*et al*., 1995).

3. Results

3.1. GC-MS Analysis

The analysis of each ethanolic plant extract was performed by gas chromatography which coupled with mass spectrometry, for accurate detection of fatty acids. The antifungal compounds were identified by comparison of retention times and computer corresponding of the mass spectra with that of the National Institute of Standards and Technology (NIST 08) library.

3.2. L. angustifolia

GC-MS for ethanolic extract of *L. angustifolia* showed the presence of seventy peaks (Figure 1).The compounds matching to the peaks begin from 2(3H)-Furanone, 5-ethenyldihydro-5-methyl-, then end with Triacontane,1-bromo-. The major five peaks belong to compounds that were found in considerable quantities (46.04%) (Table 2).

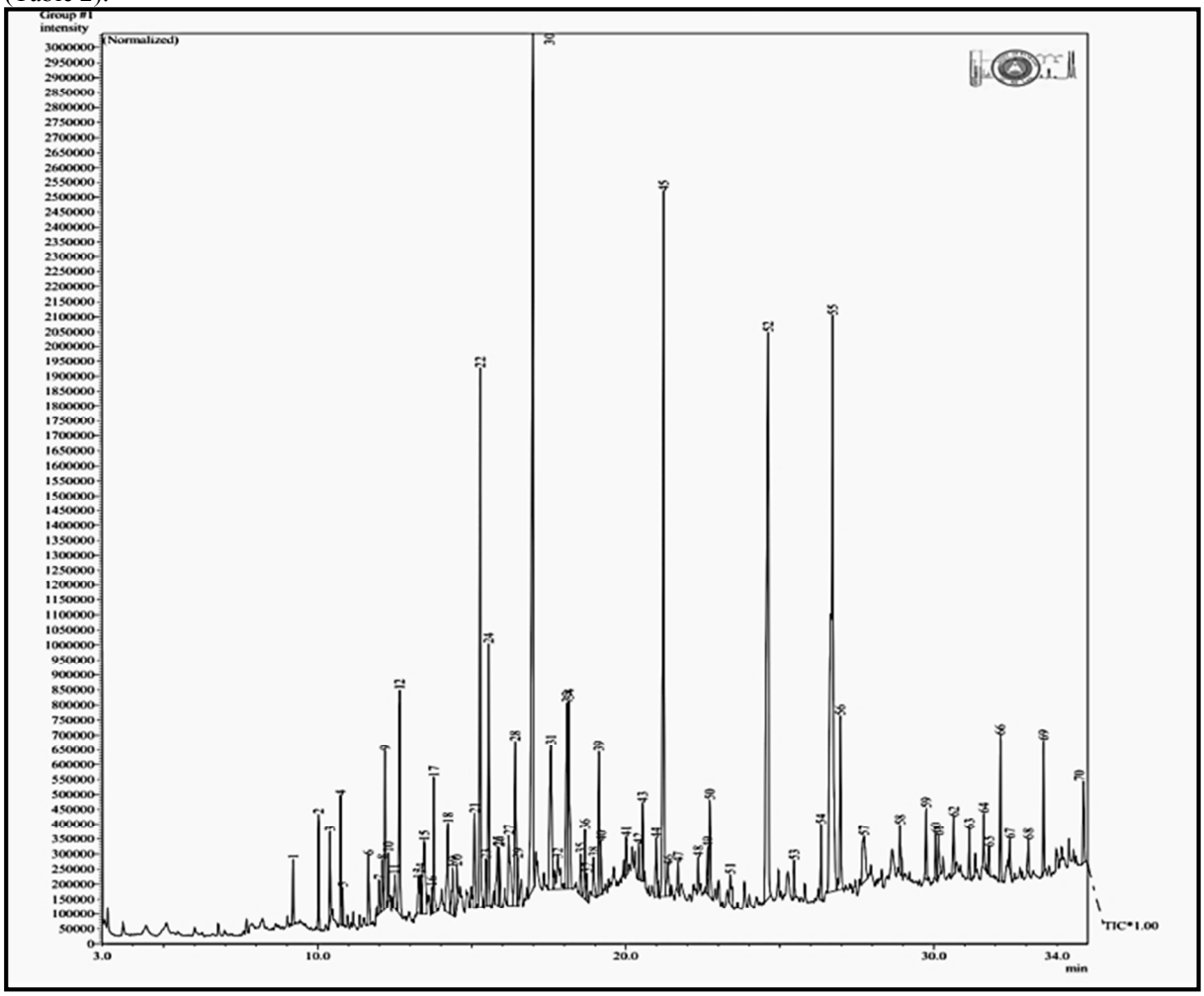

Figure 1: GC-MS chromatogram peaks of *L. angustifol*

Table 2: Compounds determined in ethanolic extract of *L. angustifolia*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	9.197	532460	0.57	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-
2	10.027	933527	1.00	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol
3	10.398	667665	0.72	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol
4	10.744	1039251	1.12	1,6-Octadien-3-ol, 3,7-dimethyl-
5	10.808	264694	0.28	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
6	11.646	705043	0.76	3-n-Butylthiolane
7	11.995	329966	0.35	Acetic acid, 3-methyl-6-oxo-hex-2-enyl ester
8	12.095	462335	0.50	4-Hexen-1-ol, 5-methyl-2-(1-methylethenyl)-, (R)-
9	12.183	1357510	1.46	Bicyclo[2.2.1]heptan-2-ol, 1,7,7-trimethyl-, (1S-endo)-
10	12.295	365774	0.39	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-
11	12.498	358820	0.39	2-Cyclohexen-1-one, 4-(1-methylethyl)-
12	12.658	2310430	2.48	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
13	13.267	502717	0.54	Benzofuran, 2,3-dihydro-
14	13.346	314557	0.34	Phenol, 3-(1-methylethyl)-
15	13.458	1402871	1.51	2-Furancarboxaldehyde, 5-(hydroxymethyl)-
16	13.700	205805	0.22	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
17	13.762	1198989	1.29	1,6-Octadien-3-ol, 3,7-dimethyl-, 2-aminobenzoate
18	14.221	1296368	1.39	1,7-Octadiene-3,6-diol, 2,6-dimethyl-
19	14.384	572215	0.62	(.+/-.)-Lavandulol, acetate
20	14.517	353135	0.38	Benzenemethanol, 4-(1-methylethyl)-
21	15.090	948971	1.02	5,5,6-Trimethylhept-3-en-2-one
22	15.271	4855317	5.22	3,7-Octadiene-2,6-diol, 2,6-dimethyl-
23	15.454	519007	0.56	Cyclohexanol, 1-methyl-4-(1-methylethenyl)-
24	15.546	1948532	2.10	3-Allyl-6-methoxyphenol
25	15.836	881909	0.95	1,7-Octadiene-3,6-diol, 2,6-dimethyl-
26	15.900	476492	0.51	trans-p-Mentha-2,8-dienol
27	16.203	1260651	1.36	9-Undecenal, 2,10-dimethyl-
28	16.408	1984410	2.13	Duryl-.beta.-phenylpropionate
29	16.492	357705	0.38	1,7-Octadiene-3,6-diol, 2,6-dimethyl-
30	16.983	10750008	11.56	2H-1-Benzopyran-2-one
31	17.574	2633802	2.83	.alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol
32	17.783	941187	1.01	trans-p-Mentha-2,8-dienol
33	18.082	2158389	2.32	Linalool oxide trans
34	18.148	2121969	2.28	Linalool oxide trans
35	18.534	341057	0.37	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-
36	18.673	646609	0.70	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-
37	18.742	223853	0.24	2-Cyclohexen-1-one, 3-(hydroxymethyl)-6-(1-methylethyl)-
38	18.949	451091	0.49	2H-Pyran-3-ol, 6-ethenyltetrahydro-2,2,6-trimethyl-
39	19.128	1285190	1.38	Caryophyllene oxide
40	19.192	433600	0.47	Terpinyl formate
41	20.010	316955	0.34	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (
42	20.420	284150	0.31	Aromadendrene oxide-(2)
43	20.545	579215	0.62	12-Heptadecyn-1-ol
44	20.985	721253	0.78	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)-
45	21.230	7642774	8.22	2H-1-Benzopyran-2-one, 7-methoxy-
46	21.375	460177	0.49	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol
47	21.696	213737	0.23	Tetradecanoic acid
48	22.348	289657	0.31	Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)-
49	22.657	404745	0.44	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
50	22.726	954171	1.03	2-Pentadecanone, 6,10,14-trimethyl-
51	23.391	461699	0.50	Triethylamine
52	24.618	8885418	9.56	l-(+)-Ascorbic acid 2,6-dihexadecanoate
53	25.463	327316	0.35	Eicosanoic acid
54	26.335	656348	0.71	Phytol
55	26.718	10676724	11.48	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
56	26.964	1646219	1.77	Octadecanoic acid
57	27.735	1074135	1.16	Benzyl .beta.-d-glucoside
58	28.903	448821	0.48	Urea, octadecyl-
59	29.748	577348	0.62	Tetratetracontane
60	30.059	340218	0.37	Tetracosane
61	30.157	392252	0.42	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester
62	30.644	521053	0.56	Tetracosane, 9-octyl-
63	31.146	426776	0.46	Octanoic acid, 1-ethenyl-1,5-dimethyl-4-hexenyl ester
64	31.624	506904	0.55	Hexatriacontane
65	31.790	243782	0.26	trans-p-Mentha-2,8-dienol
66	32.161	1215905	1.31	Hexacosane, 9-octyl-
67	32.467	224387	0.24	1,1,3,3-Tetramethyl-1,3-bis{[5-methyl-2-(1-methylethenyl)hex-4-en-1-yl]oxy}disiloxa
68	33.061	283504	0.30	Tetratetracontane
69	33.557	1080627	1.16	13,17,21-Trimethylheptatriacontane
70	34.857	730446	0.79	Triacontane, 1-bromo-
		92980597	100.00	

3.3. S. officinalis

GC-MS analysis for ethanolic extract of *S. officinalis* revealed chromatogram with thirty peaks (Figure 2). The compounds matching to the peaks begin from 3-Cyclohexene-1-methanol, alpha.,. alpha., 4-trimethyl-, (S)- then end with 6a, 14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-. The major four peaks belong to compounds that were found in considerable quantities (63.57%) (Table 3).

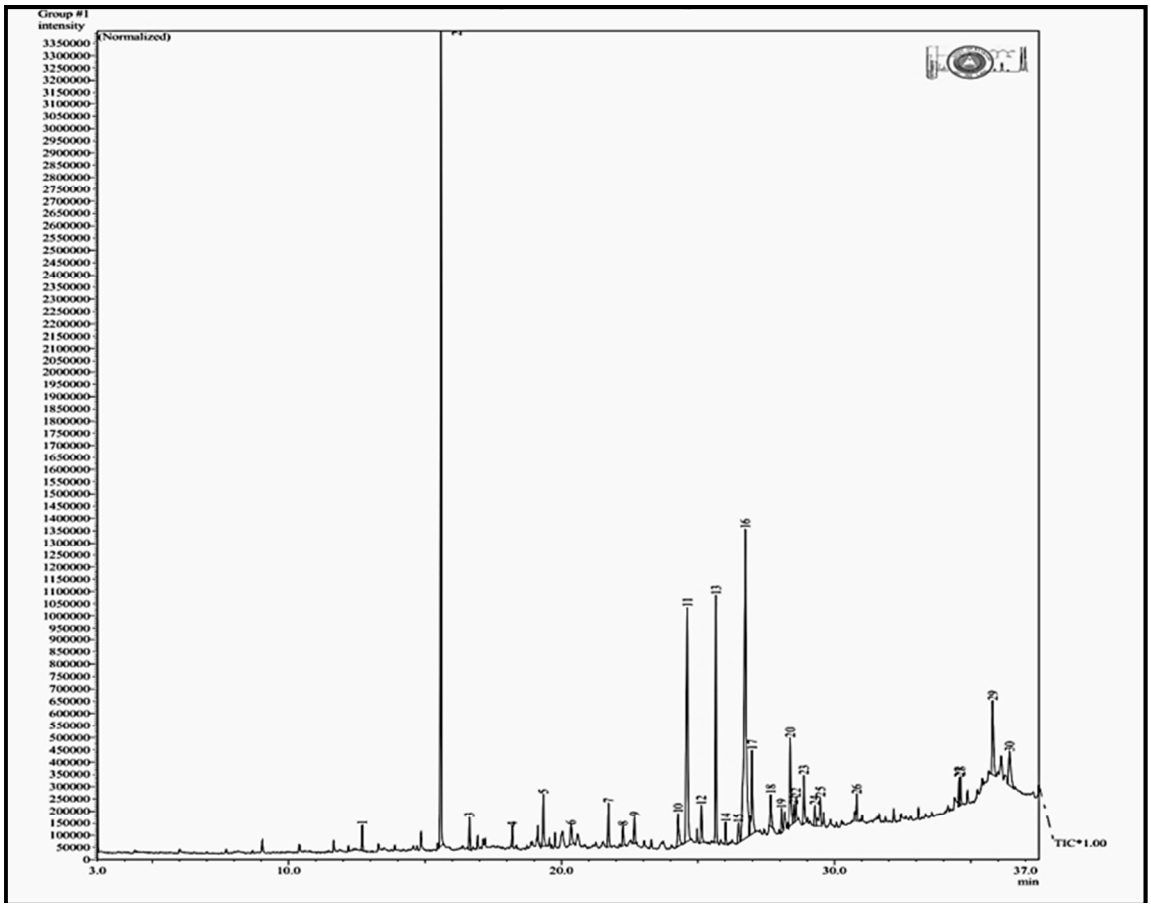

Figure 2: Chromatogram peaks of *S. officinalis*

Table 3: Compounds determined in ethanolic extract of *S. officinalis*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	12.696	248773	0.74	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)-
2	15.585	8167529	24.27	3-Allyl-6-methoxyphenol
3	16.628	312018	0.93	Caryophyllene
4	18.195	192939	0.57	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
5	19.331	605610	1.80	(-)-Globulol
6	20.351	354532	1.05	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)-
7	21.720	529685	1.57	Tetradecanoic acid
8	22.253	244061	0.73	trans-2-Hexadecenoic acid
9	22.675	290421	0.86	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
10	24.273	588538	1.75	cis-9-Hexadecenoic acid
11	24.610	3687061	10.96	l-(+)-Ascorbic acid 2,6-dihexadecanoate
12	25.126	480064	1.43	trans-2-Hexadecenoic acid
13	25.657	2694546	8.01	1-Naphthalenepropanol, .alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methyl
14	26.013	241145	0.72	Humulane-1,6-dien-3-ol
15	26.485	325239	0.97	Phytol
16	26.734	6840298	20.33	6-Octadecenoic acid, (Z)-
17	26.983	1290675	3.84	Octadecanoic acid
18	27.668	412765	1.23	4,6-Bis(1,1'-dimethylethyl)-2',5'-dimethoxy-1,1'-biphenyl-2-ol
19	28.064	301289	0.90	4,5,6,7-Tetrahydroxy-1,8,8,9-tetramethyl-8,9-dihydrophenaleno[1,2-b]furan-3-one
20	28.383	1058430	3.15	Estra-1,3,5(10)-trien-16-one, 3-[(trimethylsilyl)oxy]-
21	28.510	222181	0.66	Ferruginol
22	28.589	251967	0.75	Estra-1,3,5(10)-trien-16-one, 3-[(trimethylsilyl)oxy]-
23	28.877	676089	2.01	2(1H)-Phenanthrenone, 6-(acetyloxy)-3,4,4a,9,10,10a-hexahydro-1,1,4a-trimethyl-7-(
24	29.283	283117	0.84	Estra-1,3,5(10),9(11)-tetraen-17-one, 3-[(trimethylsilyl)oxy]-
25	29.486	503027	1.49	Dibenz[d,f]cycloheptanone, 2,3,9-trimethoxy-
26	30.813	276202	0.82	9(1H)-Phenanthrenone, 2,3,4,4a,10,10a-hexahydro-6-hydroxy-1,1,4a-trimethyl-7-(1-m
27	34.567	315165	0.94	.alpha.-Tocopherol-.beta.-D-mannoside
28	34.623	344078	1.02	4H-1-Benzopyran-4-one, 5-hydroxy-6,7-dimethoxy-2-(4-methoxyphenyl)-
29	35.802	1164824	3.46	.gamma.-Sitosterol
30	36.416	749274	2.23	6a,14a-Methanopicene, perhydro-1,2,4a,6b,9,9,12a-heptamethyl-10-hydroxy-
		33651542	100.00	

3.4. S. aromaticum

GC-MS analysis for ethanolic extract of *S. aromaticum* showed the presence of thirty peaks (Figure 3).The compounds matching to the peaks begin from Phenol, 4-(2-propenyl)-, acetate, then end with gama.-Sitosterol. The major four peaks belong to compounds that were found in considerable quantities (81.49%) (Table 4).

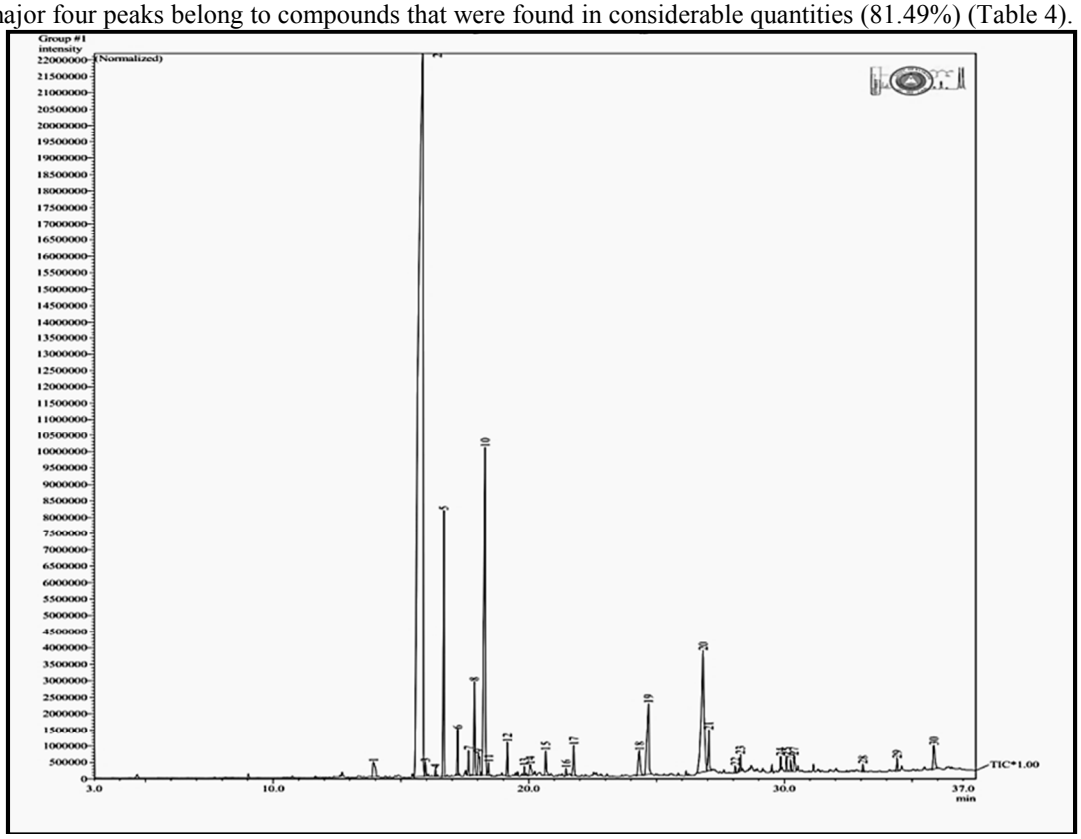

Figure 3: GC-MS Chromatogram of *S. aromaticum*

Table 4: Compounds determined in ethanolic extract of *S. aromaticum*

Peak Report TIC

Peak#	R.Time	Area	Area%	Name
1	13.924	3052918	0.70	Phenol, 4-(2-propenyl)-, acetate
2	15.854	276154341	63.54	3-Allyl-6-methoxyphenol
3	15.960	1134875	0.26	Copaene
4	16.351	544794	0.13	Vanillin
5	16.675	20672975	4.76	Caryophyllene
6	17.220	3257975	0.75	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
7	17.647	1894661	0.44	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
8	17.873	7226800	1.66	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-
9	18.031	2767692	0.64	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-
10	18.291	45440325	10.46	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
11	18.425	992827	0.23	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
12	19.161	2495792	0.57	Caryophyllene oxide
13	19.825	651436	0.15	Cubenol
14	20.060	1600324	0.37	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-
15	20.664	2379651	0.55	2',3',4' Trimethoxyacetophenone
16	21.464	551643	0.13	Benzene, 1,1'-(1,2-cyclobutanediyl)bis-, trans-
17	21.765	3202778	0.74	Tetradecanoic acid
18	24.330	4119627	0.95	cis-9-Hexadecenoic acid
19	24.686	11843644	2.73	l-(+)-Ascorbic acid 2,6-dihexadecanoate
20	26.823	28704851	6.60	6-Octadecenoic acid, (Z)-
21	27.043	4034955	0.93	Octadecanoic acid
22	28.078	551694	0.13	Cyclohexanecarboxylic acid, undec-10-enyl ester
23	28.283	1339915	0.31	Glycidol stearate
24	29.857	906588	0.21	Cyclohexanecarboxylic acid, undec-10-enyl ester
25	30.085	1150513	0.26	Heneicosanoic acid
26	30.251	846420	0.19	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-
27	30.381	1822692	0.42	1-Propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-
28	33.078	531017	0.12	Hexatriacontane
29	34.410	977712	0.22	Hentriacontane
30	35.847	3763009	0.87	.gamma.-Sitosterol
		434614444	100.00	

3.5. Bioactivity of Ethanolic Plant Extracts Against Yeast Species

The plant extracts were shown to be effective against all yeasts isolated during this study and the screening of

their bioactivity was detected by presence of inhibition zone around the colony using concentration of 0.5mg/ml for *L. angustifolia*, *S. officinals*, and *S. aromaticum*. Ethanolic extract of *L. angustifolia* exhibited antifungal bioactivity against all yeast species which isolated in this work. This activity revealed inhibition zones ranged from 16-36 mm. with highest effect on *C. parapsilosis* whereas the lowest effect was on species *H. uvarum*.

The ethanolic extract of *S. officinalis* showed antifungal bioactivity against all yeast species with inhibition zones ranged from 16-27 mm. The highest effect was on the *S. bayanus* x *S. cerevisiae* while the lowest effect was on *H. uvarum*. Antifungal effect for *S. aromaticum* extract showed bioactivity against all yeast species with inhibition zones ranged from 17-31 mm. The highest effect of this extract was on *K. marxianus*, while the lowest was on *H. uvarum* (Table 5) ,(Figures 4 to 19).

3.6. Evaluation of Antifungal Activity Against Yeast Species

The antifungal concentration for each agent was 0.5 mg/ml, and the wells without addition of drugs served as a control.The result of the susceptibility to antifungal drugs revealed variation in efficacy against yeasts. *Candida* species showed various degree of susceptibility to antifungal drugs, for instance, *C. albicans* showed high sensitivity to all three antifungal drugs followed by *K. marxianus*, while *H. uvarum* showed significant resistance to all antifungal agents used in this study. Antifungal agents showed different effects against yeast isolates, and nystatin drug was more effective than the other two agents, with the rate of diameter of the inhibitory zone 12.93 mm. (Figures 4 to 19)

Table 5: Sensitivity of tested yeast isolates to plant extracts and antifungal drugs

Yeast species	Inhibition zones of plant extracts			Inhibition zones of antifungal drugs		
	L. angustifolia (mm)	*S. officinalis* (mm)	*S. aromaticum* (mm)	Fluconazole (mm)	Ketoconazole (mm)	Nystatin (mm)
C. albicans	25	25	29	40	28	18
C. dubliniensis	33	24	27	12	17	14
C.glabrata	27	26	28	0	0	14
C. krusei	23	21	20	18	13	16
C.parapsilosis	36	22	28	23	17	13
C. prunicola	30	25	30	0	12	15
C. tropicalis	18	18	21	0	0	11
H. uvarum	16	16	17	0	0	0
K. exigua	20	21	22	0	6	14
K. marxianus	25	22	31	34	23	17
M. capitatus	21	19	23	0	0	11
*Magnusiomyces*sp.	23	20	19	0	12	8
P. kudriavzevii	19	23	20	9	7	8
P. manshurica	19	18	26	0	8	13
S. bayanus X *S. cerevisiae*	26	27	30	0	0	18
S. cerevisiae	21	20	27	0	0	17

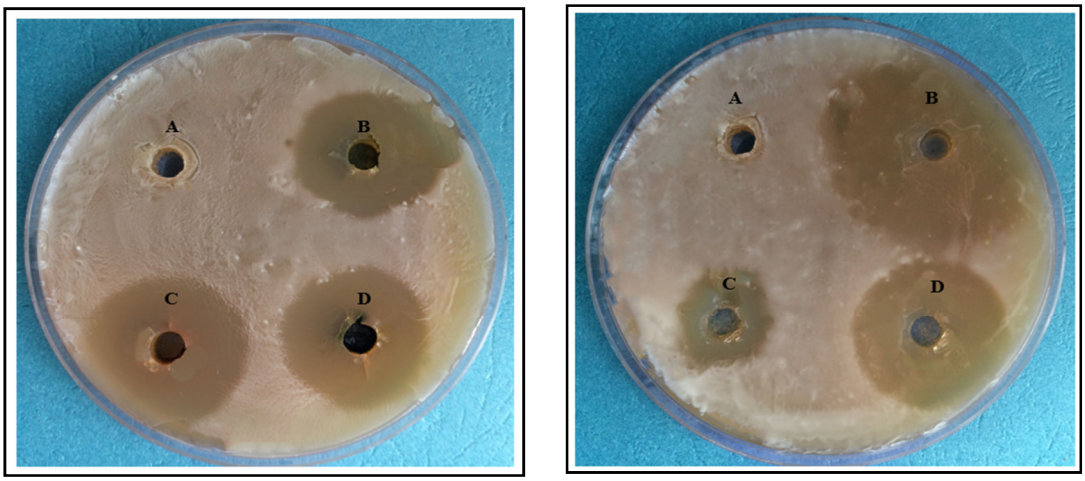

Figure 4: *C. albicans* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole,C- Nystatin, D- Ketoconazole).

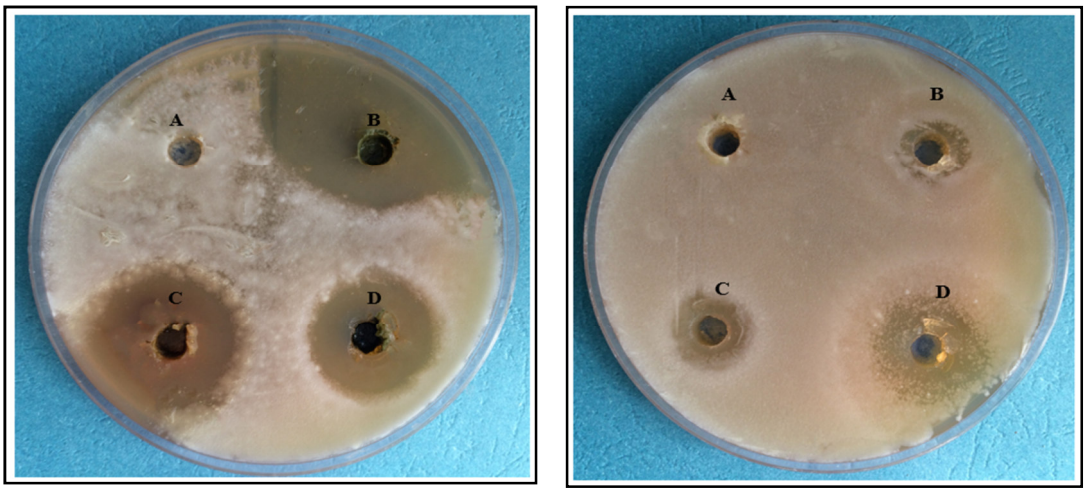

Figure 5: *C. dubliniensis* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

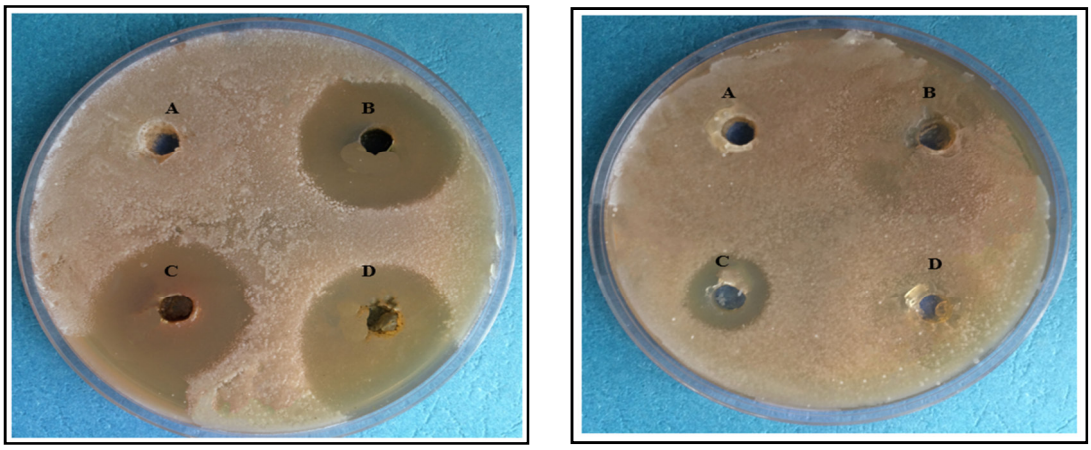

Figure 6: *C. glabrata* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

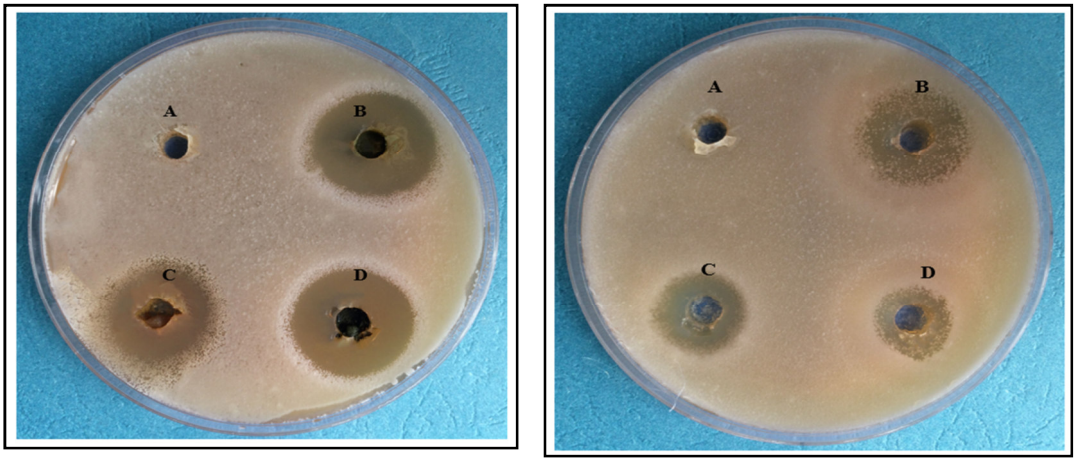

Figure 7: *C. krusei* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

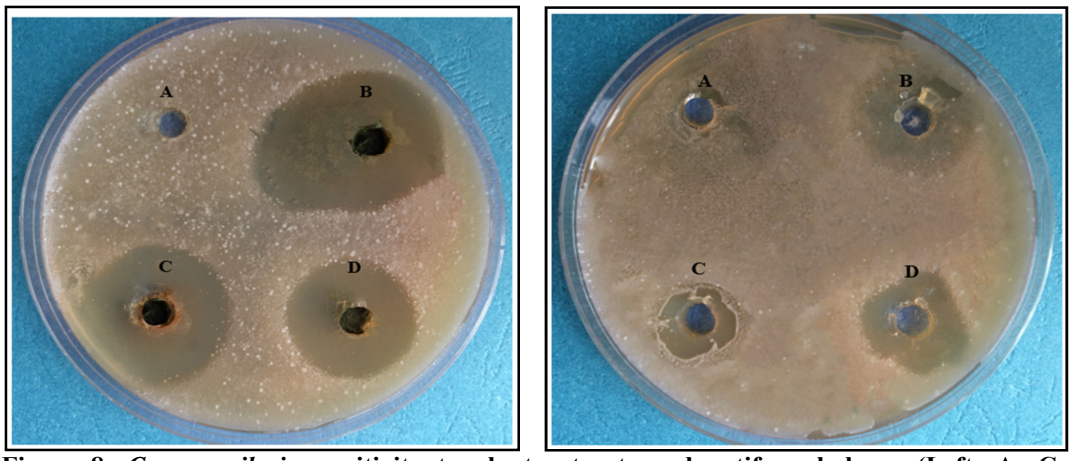

Figure 8: *C. parapsilosis* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

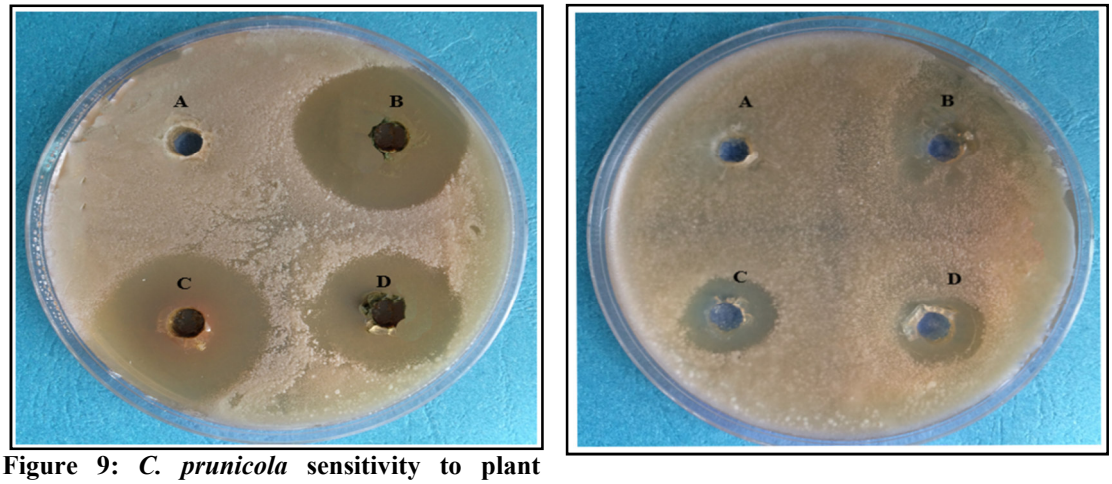

Figure 9: *C. prunicola* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

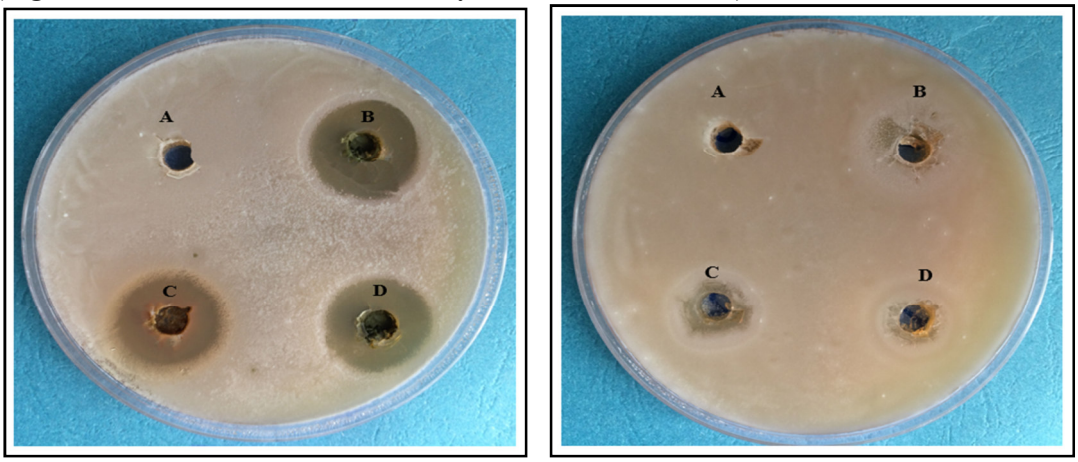

Figure 10: *C. tropicalis* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

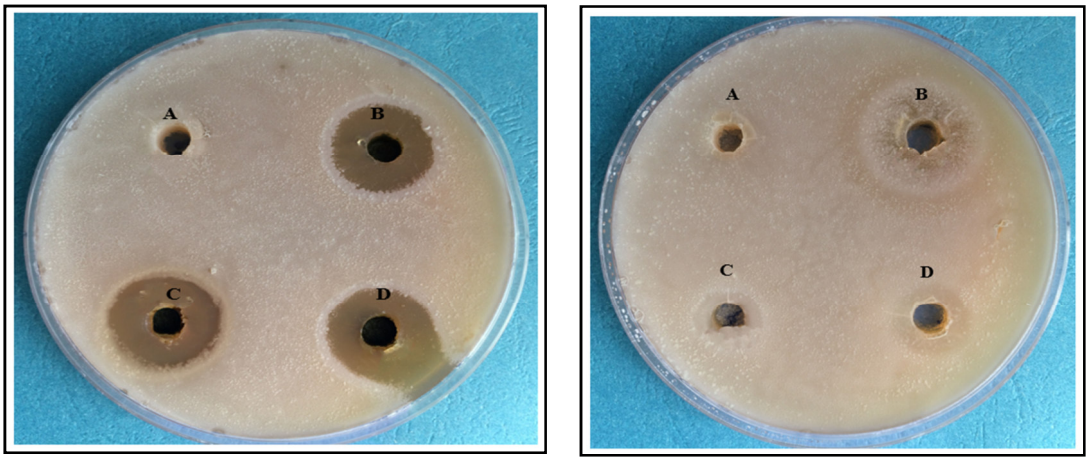

Figure 11: *H. uvarum* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

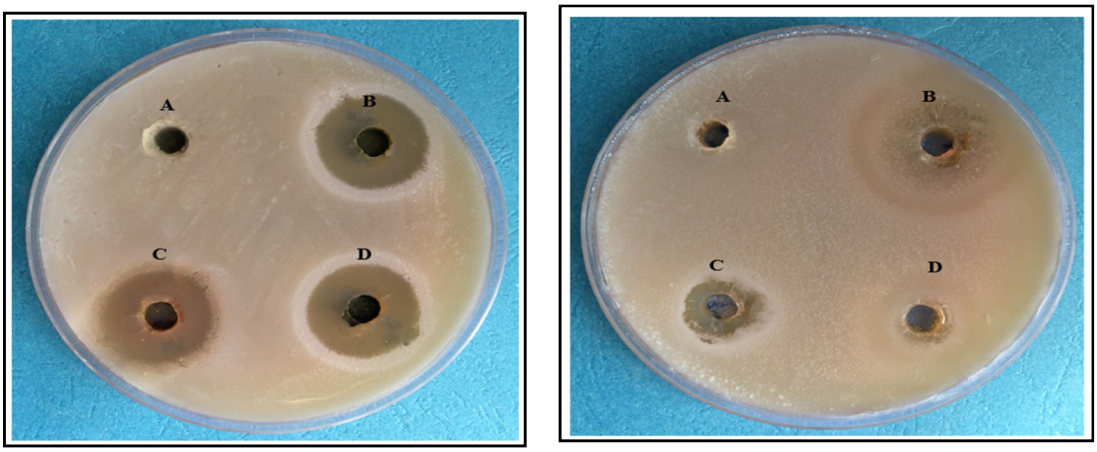

Figure 12: *K. exigua* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

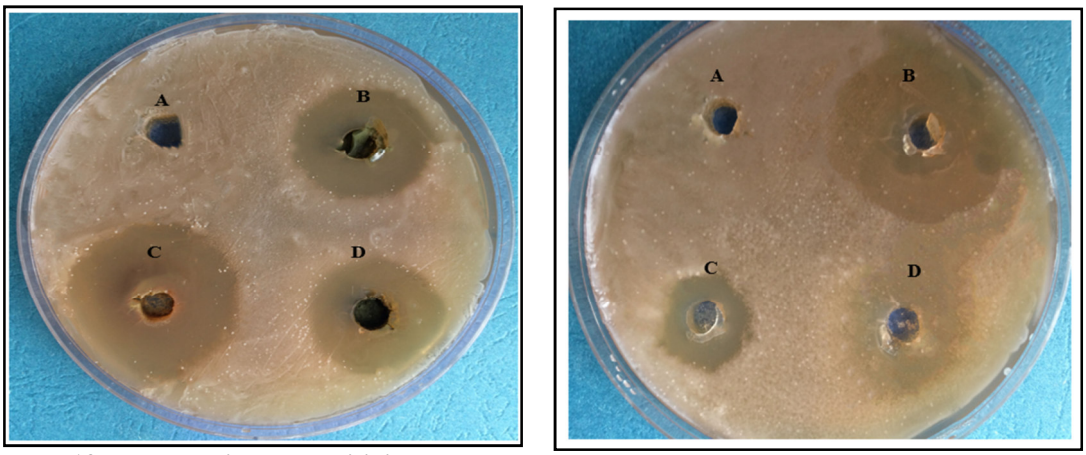

Figure 13: *K. marxianus* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

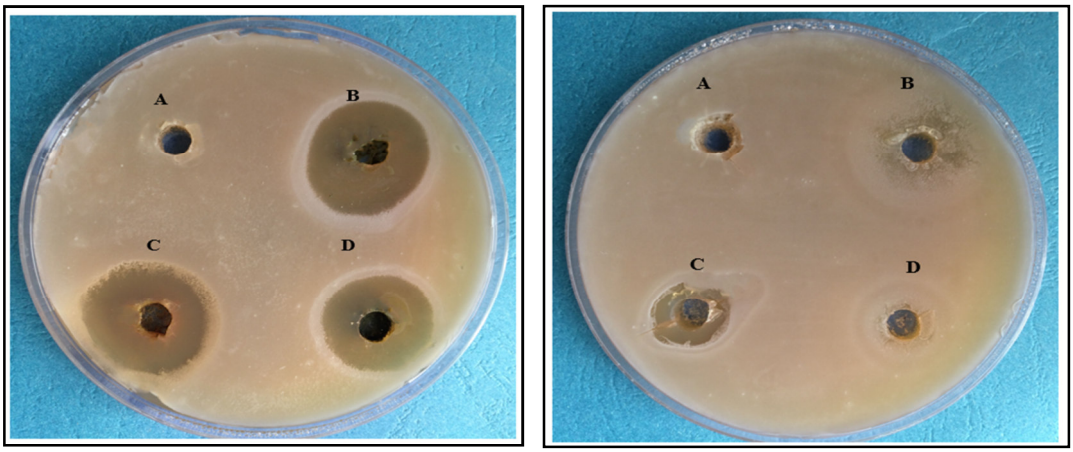

Figure 14: *M. capitatus* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

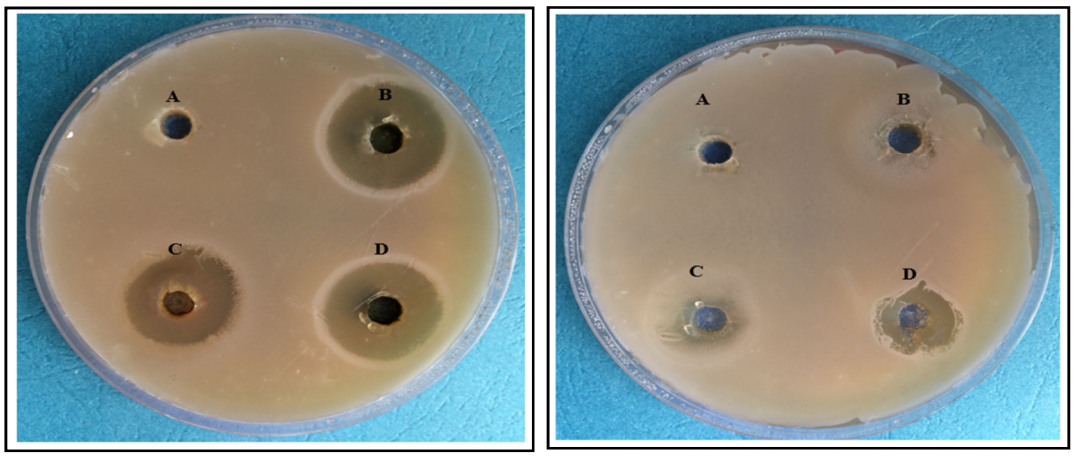

Figure 15: *Magnusiomyces* sp. sensitivity to plant extracts and antifungal drugs (Left: A -Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

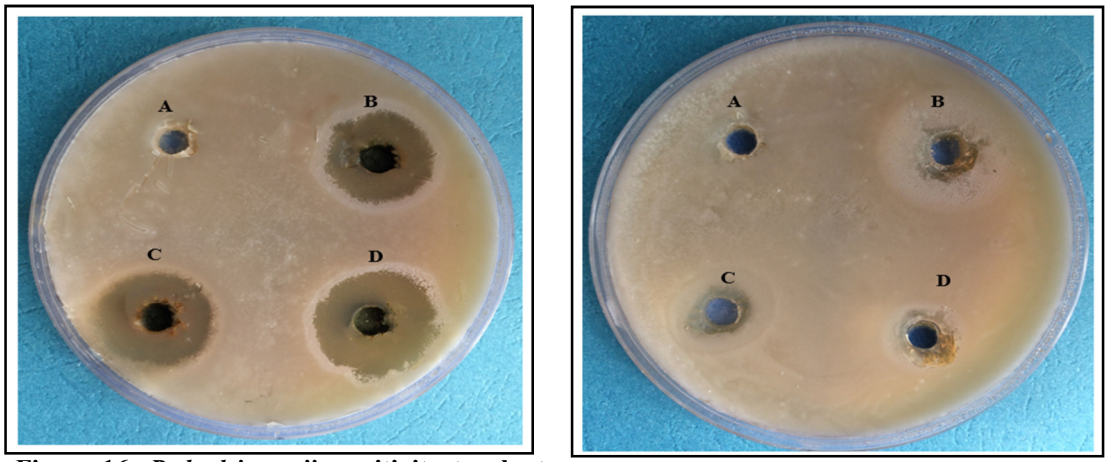

Figure 16: *P. kudriavzevii* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

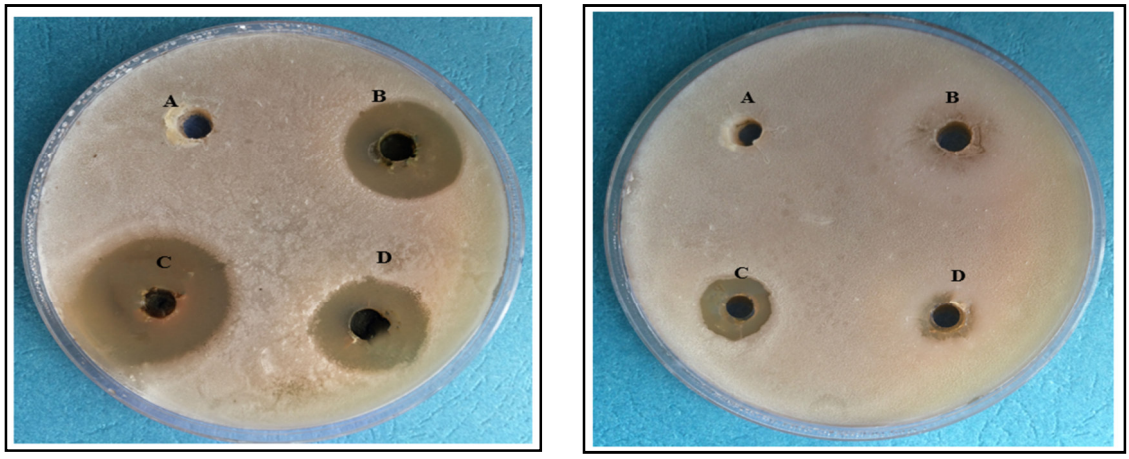

Figure 17: *P. manshurica* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

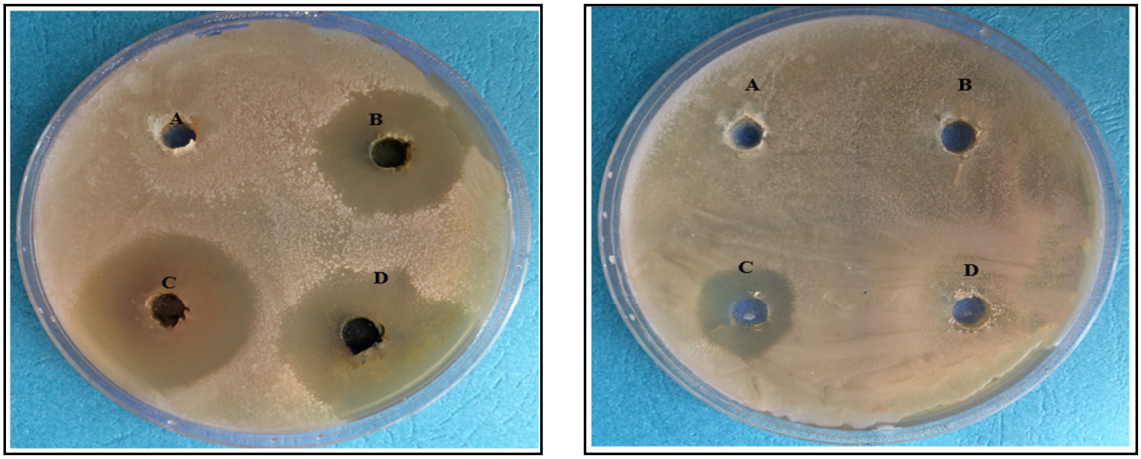

Figure 18: *S. bayanus* x *S. cerevisiae* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

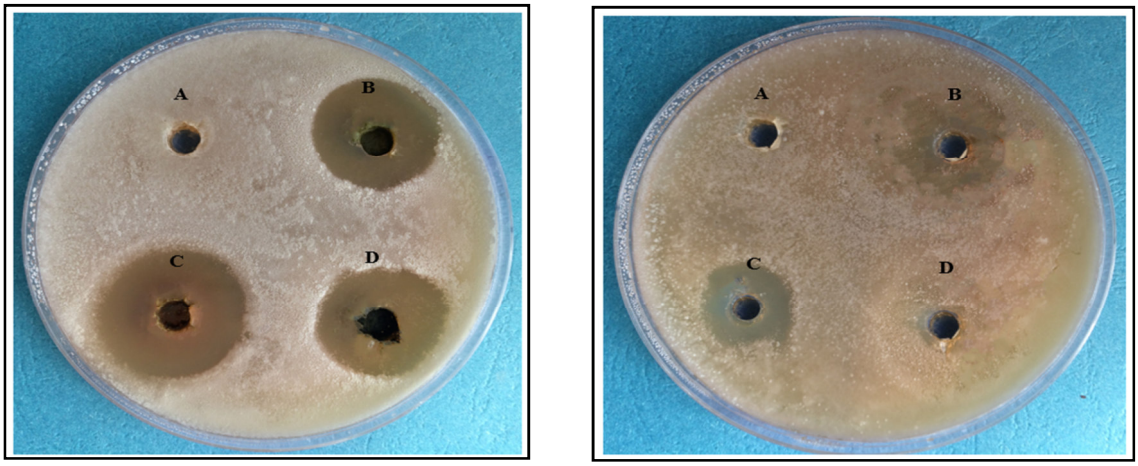

Figure 19: *S. cerevisiae* sensitivity to plant extracts and antifungal drugs (Left: A- Control, B- *L. angustifolia*, C- *S. aromaticum*, D- *S. officinalis*) (Right: A- Control, B- Fluconazole, C- Nystatin, D- Ketoconazole).

4. Discussion

In present study, it has been shown that ethanolic extract of *L. angustifolia* has antifungal activity. Some studies revealed weak inhibitory effect of lavender, whereas other studies reported significant positive effect (Behmanesh *et al*., 2015). Mahboubi *et al*. (2008) reported moderate activity of lavender against fungi.

GC-MS analysis for *L. angustifolia* revealed 5 major compounds were formed 46.04% of 70 different components of plant extract. The highest concentration compound 2H-1-Benzopyran-2-one (coumarin) has been reported for many medical applications such as anticancer, anti-allergic, anticoagulant, anti-inflammatory, antimicrobial and antifungal (Asif, 2015). The second major compound 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (Linolenic acid), also has antimicrobial activity (Rahman *et al*., 2014), therefore presence of these two compounds with others provide antifungal activity to extract of *L. angustifolia*.

L. angustifolia is considered a rich source of essential oils which used in aromatherapy, because it possessed different medical applications and lack the toxic influences. The rate of inhibitory zones of *L. angustifolia* on the yeast isolates was 23.87mm, and the highest positive activity emerged against *C. parapsilosis*, *C. dubliniensis* and *C. prunicola* respectively, while the lowest ffect was on *H. uvarum. L. angustifolia* has a moderate activity against *Candida*s species, so our findings agreed with previous studies of Mahboubi *et al*. (2008), Prusinowska and Smigielski (2013) and Behmanesh *et al*. (2015).

The rate of inhibitory zones of *S. officinalis* extract on the yeast isolated in this study was 21.68 mm, and the sensitivity of the yeast species to this crude extract is clearly dissimilar, because some species can more susceptible to the effect of plant extract than others (Nacsa-Farkas *et al*., 2014).

GC-MS analysis of *S. officinalis* extract showed 4 major compounds which represented the highest peaks in chromatogram. The major components are: 3-Allyl-6-methoxyphenol (24.27%), 1-(+)-Ascorbic acid 2,6-dihexadecanoate (10.96%), 1-phthalenepropanol, alpha.-ethenyldecahydro-.alpha.,5,5,8a-tetramethyl-2-methyl (8.01%), and 6-Octadecenoic acid, (Z)- (20.33%).

The essential oils of *S. officinalis* contain large amounts of monoterpenes which act on germ tube, pesudohyphae and biofilm formation by interaction with lipid components in the cell membrane and then prevent the filamentous form and adhesion of the *Candida* on the epithelial cells (Sookto *et al*., 2013).

The highest bioactivity of *S. officinalis* extract was on the *K. marxianus*, while the lowest impact was on the *H. uvarum*. The present result was agreement with previous works of Al-Wahab (2011), Jasim and Abd Al-Khaliq (2011), Karthikeyan *et al*. (2014) and Rathod *et al*. (2015). who indicated to effects of *S. officinalis* extract as the antifungal agent.

The aromatic plants are known to possess antifungal activity, therefore that were used as alternative agents in traditional medicine (Ahmad *et al*., 2013). Our findings showed that the rate of inhibitory effect of *S. aromaticum* against yeast isolates was 24.87mm which is higher than influences of *L. angustifolia* and *S. officinalis*.

Analysis of ethanolic extract for *S. aromaticum* showed thirty different compounds including five major peaks which represented 88.09% from the total components.

3-Allyl-6-methoxyphenol is a highest presence in the ethanolic extract of *S. aromaticum* and it is one of eugenol derivatives (m-eugenol) with molecular formula $C_{10}H_{12}O_2$. Eugenol is a phenolic compound, it has bioactivity against fungi by induce the production of H_2O_2 and elevation level of free Ca^{2+} in the cytoplasm of cells, which increased permeability changes, leading to damages of plasma membrane and plasma proteins (Kong *et al*., 2014; Cristina *et al*., 2015).

According to our results, eugenol an essential component in *S. aromaticum*, that agreement with the findings of many studies which demonstrated the same results (Cristina *et al*., 2015), and about the activity of eugenol against *Candida* species, the results of present study are corroborated with the previous studies of Anuj and Sanjay (2010), Kirui *et al*. (2014) and Wankhede (2015) who observed the high activity of eugenol as antifungal agent.

In present study, the highest rate of inhibitory zones for 3 tested antifungal drugs was revealed by nystatin (12.93 mm) and the large effect of this agent was on *C. albicans* and *S. bayanus* x *S. cerevisiae*, while the species *H. uvarum* showed zero susceptibility to these drugs. The mechanism action of nystatin is based on binding to sterol in the plasma membrane which lead to alteration their permeability and then the yeast cells lose the essential elements for survival (Nenoff *et al*., 2016).

The effect of ketoconazole agent revealed rate of inhibitory zones (8.93 mm) It interferes with cell membrane permeability by decreasing ergosterol synthesis via inhibition of fungal cytochrome P450 (14 α-demethylase) required for conversion of lanosterol to ergosterol (Lewis, 2011). In present work, the high effect of ketoconazole was on the *C. albicans* followed by *k. marxianus*, whereas the isolates of *C. glabrata*, *C. tropicalis*, *H. uvarum*, *M. capitatus*, *S. bayanus* x *S. cerevisiae* and *S. cerevisiae* were showed strong resistance to this drug.

Nine of yeast species resisted the effect of fluconazole completely, while the higher influence was on the *C. albicans*. The rate of inhibitory zones was 8.5mm which represented the lowest effect among three tested

drugs in this study. The action of fluconazole is on the ergosterol synthesis, thus inhibiting yeast growth. Many studies indicating that fluconazole had less activity against some of fungi because it is belonged to the triazole group which have the advantages of being metabolised more slowly and exerting less influence and perhaps the components of the medium SDA can interfere with the test (Pakshir *et al*., 2009; Muller *et al*., 2013).

The different yeast species vary in their susceptibility to the antifungal agents, and the absolute resistance to these drugs observed in several species, whereas all the yeasts were sensitive to the effect of the plant extracts. The results of the present study showed that extracts have the highest antifungal efficacy when compared with antifungal drugs, therefore antifungal susceptibility testing has become essential for effective patient management and resistance surveillance. The current study showed a significant difference between the efficacy of plant extracts and antifungal drugs ($p \leq 0.05$). The findings of this work were similar to many previous studies (Badiee *et al*., 2012; Behmanesh *et al*., 2015) who reported a broad spectrum of plant extracts bioactivity against a variety of pathogenic yeasts with decreased susceptibility of antifungal medications.

5. Conclusion

In the present study we demonstrate that all plant extracts were shown to be effective against all yeasts isolates , also the result of the susceptibility to antifungal drugs revealed variation in efficacy against yeasts. *Candida* species showed various degree of susceptibility to antifungal drugs, for instance, *C. albicans* showed high sensitivity to all three antifungal drugs followed by *K. marxianus*, while *H. uvarum* showed significant resistance to all antifungal agents used in this study, the G.C. analysis appeared that the ethanolic extracts having different types of antifungal compounds. This study revealed that ethanolic extracts having a good antifungal activity against all yeasts species.

6. Acknowledgment

The authors represent deep thanks for everyone help us during this study.

7.References

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