

**ANTIMICROBIAL ACTIVITIES OF SOME YEAST STRAINS
AND GC/MS ANALYSIS OF *Rhodotorula mucilaginosa*
AUMC13565 BIOACTIVE METABOLITES**

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Received: 16/8/20 **Accepted:** 13/9/2018 **Available Online:** 6/12/2018

Primary screening of the antibacterial activity of yeast methanolic extracts against six pathogenic bacterial strains were examined by disc diffusion methods. Antimicrobial activities of the highest 10 molecular identified yeast strains methanolic extracts were confirmed against pathogenic (bacteria, yeast, dermatophyte and filamentous fungi) by using disc diffusion method and wells diffusion method were tested. The major metabolites in methanolic extract of the highest antimicrobial active *Rhodotorula mucilaginosa* AUMC13565 strain were determined by GC/MS analysis. The GC/MS analysis showed 41 active metabolites are classified based on their structure into 13 alcohols, 7 aldehyds, 6 fatty acids, 5 azoles derivatives and 10 other metabolites. All the available information about the detected metabolites were reported and included GC/MS %, Retention Time, CID number, molecular formula, molecular weight g/mol, uses and bioactivity. The most detected metabolites had many bioactivities included seven metabolites had anticancer activity followed by 5 from “anti-bacteria, antifungal and flavoring agents”, 3 anti-inflammatory, 2 antioxidant, one metabolite from each “antidiuretic, estrogen receptor, insecticide, herbicide, plant growth regulator, antineoplastic diseases, antischizophrenia, immune system disorders nematotoxic, antimalaria, anti-leukemia and immunosuppressant agents according to the references in Pub Chem. citation.

Keywords: Yeast antimicrobial bioactivity; *Rhodotorula mucilaginosa*, GC/MS analysis.

1.INTRODUCTION

Since antibiotics discovered numerous peoples suffering from the inflammation. So, the world needs a new natural and safe antimicrobial agent's source without side effect on the human health. Bacterial resistance to antimicrobial agents is the most problems facing the treatments of the bacterial diseases [1].

Consequently, the discovery of new antibacterial agents is important for human. Various investigations reported that numerous yeast genera have the ability to produce natural antimicrobial agents and act as food

preservatives for prolonging the shelf life of food with improve the safety of food efficacy [2-8].

Saccharomyces cerevisiae strains are able to produce impart desirable flavor, aroma and antimicrobial activity metabolites includes different levels of isobutanol, isoamyl alcohol, acetaldehyde and acetic acid [9]. Alcohols act as flavoring agents, antifreeze, antiseptics, fuels, preservative, solvents, antioxidant and antimicrobial [10].

Fungal alcohols, aldehyds, phenols & flavonoids and organic acids have been reported as antibacterial activity [11].

Hydroxyl groups in alcohols or phenols is quit reactive and easily forms hydrogen bonds with active sites of target enzymes. Alcohols and aldehyds are very effective metabolites against bacteria and fungi [12-13]. Azoles are largest and most antifungal metabolites isolated from yeast. They inhibit the fungal cell membrane synthesis by inhibiting 14- α -methylation of lanosterol in ergosterol biosynthetic pathway. Azoles are classified into many derivatives such as fluconazole active against pathogenic yeast *Candida* and *Cryptococcus*, azoles derivatives "itraconazole, posaconazole and voriconazole" are active against filamentous fungi [9].

This article has been designed for screening the antibacterial activities of yeast methanolic extracts isolated from different sources against six bacterial strains. The highest antibacterial extract(s) were confirmed against pathogenic bacteria, yeast and fungi. The major metabolites of the highest antimicrobial active strain (*R. mucilaginosa* AUMC13565) were also determined by GC/MS analysis.

2. MATERIALS AND METHODS

2.1 Source of yeast strains:

Yeast strains (Table 1) were isolated from different sources and identified by molecular technique in Korean lap and deposited in Assiut University Mycological Center (AUMC) [1].

2.2 Preparation of yeast inoculums and cultivation the yeast samples:

The medium contains yeast extract, maltextract, peptone, and glucose (YMEPG) was prepared and used for preparation of yeast inoculum. Medium was adjusted to pH 3.7 and autoclaved at 121°C for 20 min. A loop full of yeast inoculum was taken from a pure culture of the yeast isolate and inoculated into 50 ml of sterilized medium then incubated for 72 hours at 28°C on a shaker at 100 rpm [14].

Each broth culture was centrifuged for 15 min at 5000rpm. The cell biomass was dried and weighted and homogenized with 40 ml methanol in a high-speed blender at 16.000 rpm. The homogenized mixture was kept in a shaker overnight. Then the mixture was filtered through Whitman filter paper No.1, and the residue of the extract was dried and stored in a dark glass vial for further work [15].

2.3 Antibacterial activity:

The antibacterial spectrum of the methanolic extracts of tested yeast was examined against six AUMC B bacterial strains included 3 from Gram -ve (*Escherichia coli* 53, *Pseudomonas aeruginosa* 73, and *Serratia marcescens* 55) and Gram +ve (*Bacillus cereus* 52, *Staphylococcus aureus* 54 and *Micrococcus luteus* 112). Nutrient Agar (NA) medium was prepared and sterilized at 121°C for 20min, then pour into sterilized Petri dishes. Using micropipette 200µm from bacterial spore suspension were spread on the surface of the medium. Each extract was dissolved into 500µm methanol and then the disc of filter paper was impregnated in methanolic extract and completely air dried. Each filter paper disc was saturated with methanolic extract and applied on the surface of the bacterial culture. The inhibition zone was recorded after 24 hours [12,16].

2.4 Antimicrobial activities by wells diffusion methods with DMSO as a solvent:

The antimicrobial activity of the selected methanolic extracts of the highest antibacterial active yeast strains were applied against pathogenic bacteria, yeast, dermatophytic and filamentous fungal strains were provided from AUMC using methanol & disc diffusion method and DMSO & Welles cavity method for determined their microbial activity against pathogenic bacteria and fungi.

The antifungal activity of the methanolic extract of the highest bioactive yeast strains were applied against six pathogenic fungal strains which provided by the AUMC No. included four filamentous fungi (*Aspergillus flavus* 1276, *Fusarium oxysporium* 215, *Geotrichum candidum* 226 and *Scopulariopsis brevicaulis* 16), one dermatophytic strain (*Trichophyton rubrum* 1804) and one yeast strain (*Candida albicans* 1299). Each fungal strain was grown for 4 days in Universal tubes containing 20 ml of Sabouraud's dextrose broth [17]. The bioassay was done in 10 cm sterile Petri plates in which microbial suspension (1ml/plate) and 15 ml appropriate agar medium were poured. Nutrient agar and Sabouraud's dextrose agar was respectively used for bacteria

and fungi [14]. Yeast extract were dissolved in dimethyl sulfoxide (DMSO) at 2%w/v (=1 mg/ml) were pipetted in the 3 cavities per dish (50 µl/cavity), the incubated the bacterial cultures for 48 hours and four days for fungal cultures at 28°C. Results were recorded as the diameter (in mm) of the inhibition zone around cavities [18].

2.5 GC/MS analysis:

GC/MS analysis of the *R. mucilaginosa* AUMC13565 which has the most highest antimicrobial activity for determining its active antimicrobial metabolites profile [10]. The analysis was performed using Apparatus: GC-MS (7890A-5975B) by injecting into a DB-Column. The GC/MS conditions included, oven program: 40°C for 2 min; then 10 °C/min to 150 °C for 3 min; then 10 °C/min to 220°C for 6 min; then 15 °C/min to 280 °C for 28 min; run time 61 min and 2 min (Post Run) 260 °C. Flow program: 0.5 mL/min for 10.9 min and 1 mL/min per min to 1 mL/min for 30 min. Analysis of the extraction was performed using Agilent GC/MS, (Agilent Technologies, Palo Alto, CA, USA) at the analytical Chemistry Unit, ACAL, Chemistry Department, Faculty of Science, Assiut University, Assiut, Egypt.

3. RESULTS&DISCUSSION

3.1 Screening of the yeast antibacterial activity:

Table 1 clearing the antibacterial activity of yeast strains methanolic extract were tested using disc diffusion method. The activity of each extract was determined by measuring the inhibition zone. Based on the diameter of the inhibition zone the antibacterial activity of the tested methanolic extracts was classified into high, moderate and low. All the tested yeast strains have antibacterial bioactivity against Gram-negative bacterial strains strongly compared to its effect on Gram+ve bacterial strains. The high antibacterial activates (showed inhibition zone ≥ 20 mm). *Pseudomonas aeruginosa* B73 has highly sensitive effect against tested yeast extracts with inhibition zone diameter range between 21-46 mm. The most resistance bacterial strain against yeast extract was *M. luteu* B112 not affected by yeast extracts with undetected inhibition zone.

A total tested molecular identified yeast strains were selected for confirmation their highest antibacterial activity, these yeast strains are related to five genera included *R. mucilaginosa*, *D. rugosa*, *S. cerevisiae*, *D. hansenii* and *P. laurentii* were 5, 3, 2, 1 and 1 strains, respectively (Table 1).

The highest antibacterial effect against *P. aeruginosa* B73 was recorded by *R. mucilaginosa* MH298828 (AUMC13564) with 46 mm inhibition zone. The extract of *D. rugosa* MH333102 (AUMC13568) was the highest antibacterial against *S. marcesens* B55 record 47mm inhibition zone. The highest antibacterial effect against *E. coli* B53 was recorded by an *D. rugosa* MH341116 (AUMC13567) reached to 28mm inhibition zone. The extract of *R. mucilaginosa* MH298828 (AUMC13564) recorded as highest antibacterial yeast against *B. cereus* B52 (30mm inhibition zone).

Rhodotorula mucilaginosa MH298828 (13565) and *D. rugosa* MH341116 (13566) were the most active yeasts against all the tested bacteria, followed by *D. rugosa* MH333102 (13568), *R. mucilaginosa* MH298827 (13567) and *D. rugosa* MH333095 (13571) as recorded in Table (1).

Pseudomonas aeruginosa B73 was the most affected and sensitive bacterium by all the tested yeast methanolic extracts, followed by *S. marcesens* 55, *B. cereus* 52, *M. luteus* 112, *St. aureus* 54 and *E. coli* 53 (Table 1).

The extract of *D. rugosa* MH333102 (AUMC13568) had a highest activity against *M. luteus* B112as well as *R. mucilaginosa* MH298828 (AUMC13564). The extract of *R. mucilaginosa* MH298828 (AUMC13564) recorded as highest antibacterial yeast against *S. aureus* B54 (27mm inhibition zone) (Table 1).

The results of used wells methods cleared that the *R. mucilaginosa* MH298828 (13565) was the most active yeast strain against all tested bacteria.

The *D. rugosa* MH333102 (13568) strain was active against *Trichophyton rubrum* 1804 dermatophytic tested fungus.

The *R. mucilaginosa* MH298828 (13565) strain methanolic extract was used for GC/MS to make the chemical profile of the antibacterial metabolites.

Table 1.Antibacterial activities of 12 selected yeast strains methanolic extracts against six pathogenic bacterial strains by using disc diffusion method determined by mm diameter of the inhibition zone and flavonoids contents

No. of the tested yeast strains in GBAN (AUMC)

Tested pathogenic Gram +ve & Gram -ve bacterial strains AUMC No.

| | Source of isolation | G-ve | | | | | | G+ve | | Categories |
|------------------------|--|--------------------|--------------------------|--------------------------|----------------------|-----------------------|-----------------------|----------|-------------|------------|
| | | <i>E. coli</i> B53 | <i>P. aeruginosa</i> B73 | <i>S. marcescens</i> B55 | <i>B. cereus</i> B52 | <i>M. luteus</i> B112 | <i>St. aureus</i> B54 | | | |
| 1. | <i>Rhodotorula mucilaginosa</i> MH298828 (13565) | Molasses | 22 H | 46 H | 32 H | 30 H | 30 H | 30 H | 27 H | 6H |
| 2. | <i>Diutina rugosa</i> MH341116 (13566) | Orange juice | 28 H | 35 H | 32 H | 26 H | 23 H | 25 H | 25 H | 6H |
| 3. | <i>D. rugosa</i> MH333102 (13568) | Dough | 15 M | 20 H | 47 H | 20 H | 30 H | 15 M | 15 M | 4H+2M |
| 4. | <i>R. mucilaginosa</i> MH298827 (13567) | Molasses | 23 H | 15 M | 29 H | 23 H | 20 H | 17 M | 17 M | 4H+2M |
| 5. | <i>D. rugosa</i> MH333095 (13571) | Guava | 22 H | 27 H | 23 H | 19 M | 17 M | 19 M | 19 M | 3H+3M |
| 6. | <i>Debaryomyces hansenii</i> KR264905 | Tomato | 19 M | 21 H | 16 M | 21 H | 17 M | 24 H | 24 H | 3H+3M |
| 7. | <i>Saccharomyces cerevisiae</i> KM504287 | Cane juice | 18 M | 27 H | 22 H | 19 M | 19 M | 23 H | 23 H | 3H+3M |
| 8. | <i>Papiliotrema laurentii</i> MH333092 (13569) | Carrot pickled | 18 M | 22 H | 26 H | 16 M | 15 M | 20 H | 20 H | 3H+2M+L |
| 9. | <i>R. mucilaginosa</i> MH333091 | Molasses | 12 L | 12 L | 15 M | 18 M | 21 H | 9 L | 9 L | H+2M+3L |
| 10. | <i>S. cerevisiae</i> GHM | Germany | 12 L | 21 H | 11 L | 13 L | 16 M | 8 L | 8 L | H+M+4L |
| 11. | <i>R. mucilaginosa</i> MH341115 (13570) | Carrot pickled | 10 L | 22 H | 12 L | 17 M | 8 L | 15 M | 15 M | H+2M+3L |
| 12. | <i>R. mucilaginosa</i> MH333100 (13564) | Molasses | 8 L | 25 H | 12 L | 7 L | 8 L | 9 L | 9 L | H+5L |
| Total recorded results | | | 4H+4M+4L | 10H+M+L | 7H+2M+3L | 5H+5M+2L | 5H+5M+2L | 5H+4M+3L | 36H+20M+16L | |

Antibacterial categories abbreviations according to the inhibition zone diameter determined by mm.

H: High activity ≥ 20 mm

M: Moderate activity=19.9 to 16mm

L: Low activity=15.9 to 0.1mm

Table 2. Metabolites recorded by GC/MS in methanolic extract of *Rhodotorula mucilaginosa* MH298828strain.

| IUPAC of the recorded by GC/MS library | % | RT | CID | MF | MW | Uses and bioactivity |
|--|------|------|----------|--|---------|--|
| 1. α -D-Mannofuranoside-isopropyl ^{alcohol} | 11.7 | 10.6 | 537903 | C ₉ H ₁₈ O ₆ | 222.237 | |
| 2. α -Furfuryl alcohol or 2-Furanmethanol ^{alcohol} | 6.9 | 7.0 | 3761 | C ₅ H ₆ O ₂ | 98.101 | Irritate skin, eyes and mucous membranes |
| 3. D(-)Mannitol; hexane-1,2,3,4,5,6-hexol; sorbitol, (D)-gulitol ^{alcohol} | 5.4 | 13.3 | 453 | C ₆ H ₁₄ O ₆ | 182.172 | Anti-dental bacteria , anti-inflammation, antidiuretic |
| 4. (4-Ethenyloxy)-1-butanol ^{alcohol} | 4.1 | 12.5 | | | | |
| 5. 1,2,3,4-butanetetrol or erythritol ^{alcohol} | 3.3 | 11.6 | 8998 | C ₄ H ₁₀ O ₄ | 122.12 | Anti-dental bacteria , |
| 6. 1-S-Nonyl-1-thio-d-mannitol ^{alcohol} | 1.9 | 17.1 | 537510 | C ₁₅ H ₃₂ O ₅ S | 324.476 | |
| 7. 2-Ethoxyethanol; cellosolve ^{alcohol} | 0.7 | 13.2 | 8076 | C ₄ H ₁₀ O ₄ | 90.122 | Anti-leukemia & solvent has many other uses |
| 8. 1,3-Diamino-2-propanol ^{alcohol} | 0.3 | 10.8 | 17882627 | C ₃ H ₁₀ N ₂ O | 90.126 | Antioxidants, cytoprotective, protease inhibitors |
| 9. l-Threitol ^{alcohol} | 3.3 | 11.9 | | | | |
| 10. 2- Methyl-1-propanol ^{alcohol} | 0.3 | 6.4 | | | | |
| 11. 1-Tetracosanol ^{alcohol} | 0.1 | 23.8 | 10472 | C ₂₄ H ₅₀ O | 354.663 | Anti-inflammatory, vitamin formulation |
| 12. Nonadecanol ^{alcohol} | 0.06 | 19.8 | 80281 | C ₁₉ H ₄₀ O | 284.528 | Antibacterial , immunosuppressant |
| 13. 10,6,2,14-tetramethyl-15-hexadecen-1-ol ^{alcohol} | 0.04 | 25.1 | | | | |
| 1. 5-Hydroxymethylfurfural ^{aldehyd} | 23.6 | 13.0 | 237332 | C ₆ H ₆ O ₃ | 126.111 | Flavoring agents, antibacterial |
| 2. 5-Hydroxymethyl-2-furane- carboxaldehyd ^{aldehyd} | 23.5 | 13.0 | | | | |
| 3. D3,2-Dihydroxypropanal ^{aldehyd} | 2.2 | 13.2 | 751 | C ₃ H ₆ O ₃ | 90.078 | Anticancer |
| 4. 1-Methylpentyl hydrosulfide ^{aldehyd} | 1.8 | 13.1 | 519310 | C ₆ H ₁₄ S | 118.243 | |
| 5. 2- Furaldehyde ^{aldehyd} | 1.3 | 6.6 | 7362 | C ₃ H ₄ O ₂ | 96.085 | Antimalarial parasite |
| 6. Methyl- (2-propenyl)-hydrazine-formaldehyde ^{aldehyd} | 1.2 | 14.0 | | | | |
| 7. 5-Methyl-2-furfural ^{aldehyd} | 0.3 | 8.7 | 12097 | C ₆ H ₆ O ₂ | 110.112 | Nematotoxi,c flavoring Agents |
| 1. Heptadecanoic acid or margaric acid ^{Fatty acid} | 5.4 | 13.3 | 10465 | C ₁₇ H ₃₄ O ₂ | 270.457 | Antineoplastic, anti-inflammatory, anticancer, antischizophrenia, immuneenhancer |
| 2. Hexadecanoic acid or palmitic acid ^{Fatty acid} | 0.8 | 23.4 | 175 | | | Anti-cancer |
| 3. 9-Octadecenoic acid or oleic acid ^{Fatty acid} | 0.6 | 25.9 | 445639 | C ₁₈ H ₃₄ O ₂ | 282.46 | Anticancer, plant growth regulator, flavoring agent |
| 4. Heneicosanoic acid ^{Fatty acid} | 0.2 | 8.6 | 16898 | C ₂₁ H ₄₂ O ₂ | 326.565 | Antioxidant |
| 5. 12,9-Octadecadienoic acid ^{Fatty acid} | 0.2 | 25.8 | 5282457 | C ₁₈ H ₃₂ O ₂ | 280.452 | |
| 6. Octadecanoic acid or stearic acid ^{Fatty acid} | 0.1 | 26.3 | 5281 | C ₁₈ H ₃₆ O ₂ | 284.484 | Flavoring agents and estrogen receptor |
| 1. 3-[3,5-bis(trifluoromethyl)phenyl]-5-(4-methyl-piperidyl)meth-1,2,4-oxadiazole ^{Azole} | 2.4 | 6.5 | | | | Antifungal |

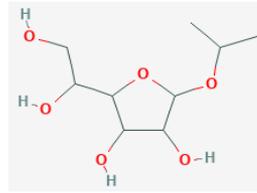
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| | | | | | | | | | |
|--|--------------------------|-----|------|--------|--|---------|--|--|--|
| 2. 2-t-butyl-4-(1-hydroxy-1-methylethyl)-3-methoxycarbonyl-5-me,2,3-dihydrooxazole | Azole | 2.8 | 7.1 | | | | | | Antifungal |
| 3. 2-fluoro-5-hydroxy-1-ribofuranosyl-Imidazole | Azole | 0.2 | 8.6 | | | | | | Antifungal |
| 4. 1,4,5- trimethylimidazole | Azole | 0.3 | 8.7 | | | | | | Antifungal |
| 5. 4-acetylpyrazole | Azole | 0.2 | 7.9 | 565593 | C ₅ H ₆ N ₂ O | 110.116 | | | Antifungal |
| 1. D1-Phenylephrine | Amino-phenols | 3.6 | 21.6 | 4782 | C ₉ H ₁₃ NO ₂ | 167.208 | | | Ant-Staphylococcus aureus bacteria |
| 2. 1,6:2,3-Dianhydro-4-O-acetyl-.β.-d-mannopyranose | | 2.8 | 7.1 | | | | | | |
| 3. 6-Deoxy-3-C-methyl-2-O-methyl-L-talose | | 1.4 | 14.9 | | | | | | |
| 4. 2-(Acetylamino)-2-deoxy-α-D-Galactopyranose | Amino Sugar | 0.9 | 10.1 | | | | | | |
| 5. β-D-Glucopyranose,1-thio-1-[hydroxy-5-(methylthio)pentanimidat | Thio-amid-Sugar | 0.2 | 11.6 | | | | | | |
| 6. 2-Deoxy-D-galactose | Deoxy Sugar | 0.4 | 12.3 | | | | | | |
| 7. d-Glycero-d-ido-heptose | HalogenatedKetonic Sugar | 0.3 | 6.4 | | | | | | |
| 8. Galacto-heptulose | Ketonic Sugar | 2.2 | 13.2 | 102926 | C ₇ H ₁₄ O ₇ | 210.182 | | | Anticancer |
| 9. D-manno-2-Heptulose | Ketonic Sugar | 2.0 | 10.9 | 12600 | C ₇ H ₁₄ O ₇ | 210.182 | | | Antibacterial endotoxin, antitumor necrosis |
| 10. 1-Nitro-.β.-d-arabinofuranose,tetraacetate | Nitrogenous Furanose | 0.7 | 13.2 | 536786 | C ₁₃ H ₁₇ NO ₁₁ | 363.275 | | | |

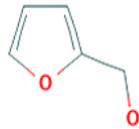
Fourty one active recorded metabolites are classified based on their structure into 13 alcohols, 7 aldehyds, 6 fatty acids, 5 azoles derivatives and 10 other metabolites. GC/MS %, Retention Time RT, CID Number, Molecular Formula, Molecular Weight g/mol, uses and bioactivity according to Pub Chem citation [19].

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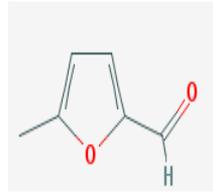
Figure1: Chemical structures of the antimicrobial metabolites recorded by GC/MS in methanolic extract of *Rhodotorula mucilaginosa* MH298828 strain according to Pub Chem citation [19].



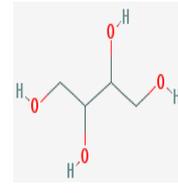
α -D-Mannofuranoside-isopropyl



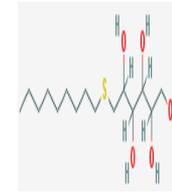
2-furanmethanol



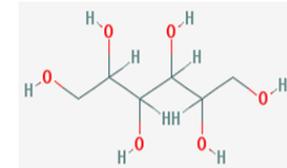
5-Methyl-2-furaldehyde



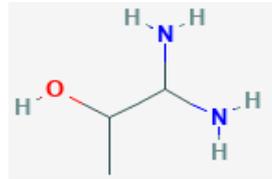
Erythritol



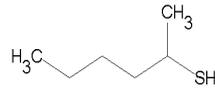
1-S-Nonyl-1-thio-D-mannitol



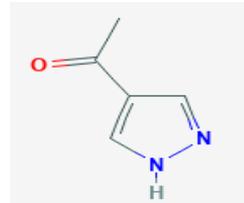
Sorbitol



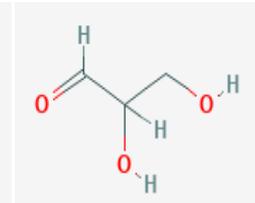
2-Ethoxyethanol; cellosolve



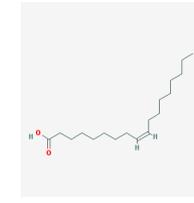
1-Methylpentylhydro sulfide



4-acetylpyrazole



2,3-Dihydroxy propanal



9-Octadecenoic acid or oleic acid



1-Tetracosanol



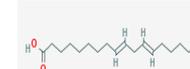
Nonadecanol



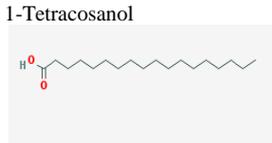
Margaric acid



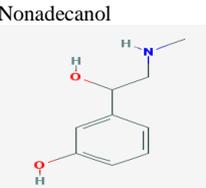
Heneicosanoic acid



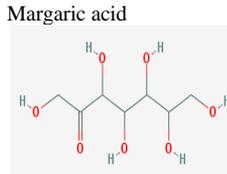
12,9-Octadecadienoic acid



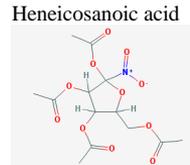
Stearic acid



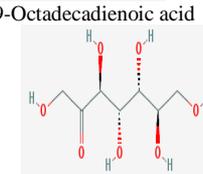
DI-Phenylephrine



Galacto-heptulose



1-Nitro- β -D-arabinofuranose, tetraacetate



D-manno-2-Heptulose

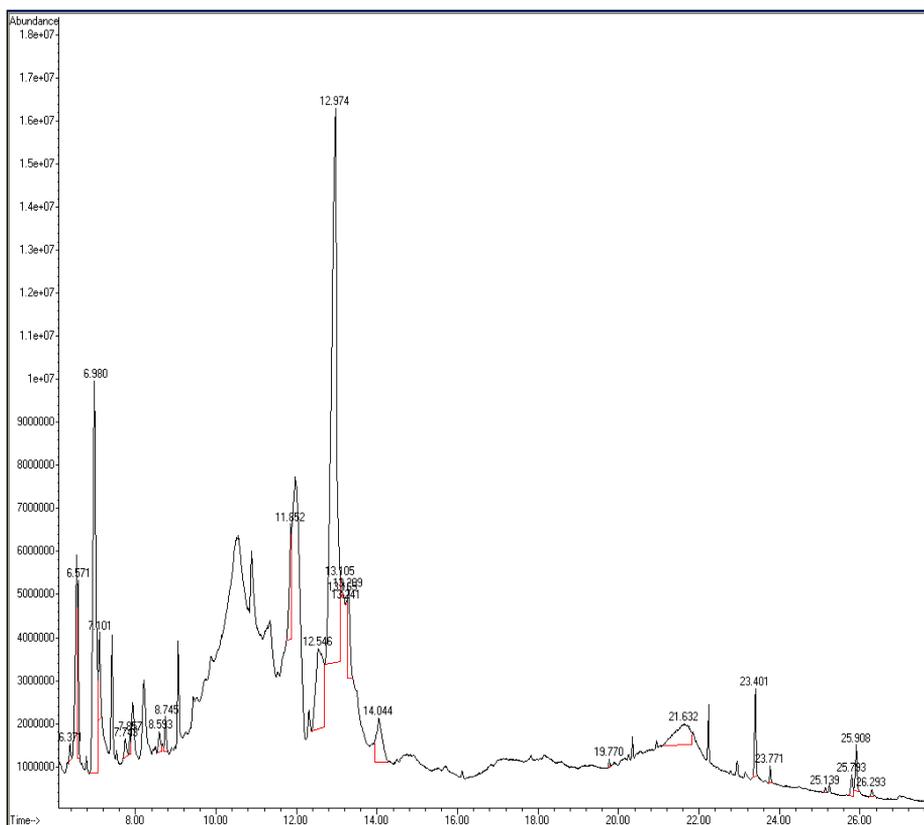


Figure 2: Antimicrobial metabolites detected by GC/MS in methanolic extracts of *Rhodotorula mucilaginosa* MH298828 strain

The chemical profile of the antimicrobial metabolites was detected by GC/MS of *R. mucilaginosa* AUMC13565 methanolic extracts was the highest antimicrobial activity analyzed and recorded 41 active metabolites are classified based on their structure into 13 alcohols, 7 aldehyds, 6 fatty acids, 5 azoles derivatives and 10 other metabolites. All the available information about the detected metabolites were reported and included GC/MS %, Retention Time, CID number, molecular formula, molecular weight g/mol, uses and bioactivity Table 2. The most detected metabolites had many bioactivities included seven metabolites had anticancer activity followed by 5 from “anti-bacteria, antifungal and flavoring agents”, 3 anti-inflammatory, 2 antioxidant, one metabolite from each “antidiuretic, estrogen receptor, insecticide, herbicide, plant growth regulator, antineoplastic diseases, antischizophrenia, immune system disorders nematotoxic, antimalaria, anti-leukemia and immunosuppressant agents according to the references in Pub Chem citation [19].

Eight detected metabolites were recorded as antibacterial agents citation and included mannitol; hexane-1,2,3,4,5,6-hexol; sorbitol/gulitol; 1,2,3,4-

butanetetrol or erythritol; nonadecanol; phenylephrine; galacto-heptulose; manno-2-heptulose. Also 5 azoles metabolites were recorded as antifungal agents according to Pub Chem citation [19] (Table 2 and Figure 1 & 2).

The antimicrobial bioactivity was increased by increasing the number of –OH group on the alcohols [20]. *Saccharomyces cerevisiae* strains produced antimicrobial metabolites includes isobutanol, isoamyl alcohol, acetaldehyde and acetic acid [21].

Also had antimicrobial activity of azoles against plant and human pathogens and dermatophytes included *Candida*, *Aspergillus flavus*, *A. tamarii*, *Cladosporium sphaerospermum*, *P. digitatum* and *P. italicum* were recorded [1].

Nevertheless, disc-diffusion assay offers many advantages over other methods: simplicity, low cost, the ability to test enormous numbers of microorganisms and antimicrobial agents, and the ease to interpret results provided. Moreover, several studies have demonstrated the great interest in patients who suffer from bacterial infection of an antibiotherapy based on the antibiogram of the causative agent [22,23].

About 5-20% of the yeast and filamentous fungi dry weight are sugar alcohols, polyols or polyhydric alcohol or polyalcohol $[H(HCHO)_{n+1}H]$. Fungal polyols act as defense metabolites against the unfavorable conditions and act as antifreeze, anti-water stress for restoring the turgor pressure, act as compatible solutes (glycerol, arabitol, erythritol, and mannitol). Psychrophilic fungi produced polyols and sugar (by mg/100mg dry wt) after 8 weeks at 15°C, *H. marvinii* psychrophilic fungus have "glycerol 0.35; erythritol 0.27; arabitol 0.21; mannitol 41.1 & trehalose 7.76". Also polyols found in fungal spores for serves and energy source [24].

Mushrooms antimicrobial active metabolites detected by GC/MS analysis included (alcohols, aldehyds, phenols & flavonoids and organic acids); anticancer (fatty acids "linoleic, linolenic, oleic, myristic, palmitic, stearic and vaccenic" and polysaccharides "glucan and PSK"); anticholesterols, anti-cardiovascular and enhancement the blood circulations (vaccenic acid and other fatty acids, pyran, glycoproteins and sterols); human health supporting (fatty acids, sterols and sugar alcohols); immune enhancer (fatty acids, glycoproteins, polysaccharides and sterols); hepato-protective (triterpenoids); and food flavoring or aroma metabolites (alcohols, aldehydes, amides, amines, carboxylic acid, esters, ketones, terpenoids, thiols and mercapto) [12].

Saccharomyces cerevisiae anti-bacterial volatile active metabolites recorded by GC/MS have highly effective to against *Proteus mirabilis*.

These antibacterial metabolite included thieno[2,3-c]furan-3-carbonitrile; 2-amino-4,6-dihydro-4,4,6,6-; Oxime; Methoxyphenyl-acetic acid-; N'-[3-(1-hydroxy-1-phenylethyl)phenyl]-hydrazide; 1-Aminononadecane; N-trifluoroacetyl; Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-,17-[O-(phenyl)me; Benzeneacetamide, α -ethyl-; 4-Benzyloxy-6-hydroxy methyl -tetrahydropyran-2,3,5-triol; 1,2-Ethenediol; 1-(2-phenyl-1,3,2-dioxaborolan-4-yl)-; 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,(3 β ,22E)-; Ethyliso-allocholate; (5 β)Pregnane-3,20 β -diol,14 α ,18 α -[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-Dimethyl-2- pyrimidinyl)-4-(4nitrobenzylideneamino)-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-Bromo tetradecanoic acid [25].

Yeasts have antimicrobial active metabolites against undesirable spoilage microbes during production of fermented foods and beverage, responsible for food, fruit and beverage losses, and these impacts negatively on the economy of the producing countries. The yeast produced extracellular metabolites which act as control agents and as preservatives. Antimicrobial active compounds produced from *Candida pyralidae* KU736785 against *Botrytis cinerea*, *Brettanomyces bruxellensis* and *C. guilliermondii* included proteins, glycoproteins and volatile organic compounds [26].

Aromatic aldehyds have antimicrobial activity against the following microbes included filamentous fungus "*Aspergillus niger*", yeast "*C. albicans* and *S. cerevisiae*" and bacteria "*E. coli*, *B. cereus*, *P. aeruginosa* and *St. epidermidis*" were tested by Disc Diffusion Methods. Salicyl aldehydes, had highly inhibitory zones up to 49 mm in diameter [27]. Antibacterial activity of six aliphatic unsaturated aldehyds included [2-hexenal, 2-eptenal, 2-octenal, 2-nonenal, 2-decenal and 2,4-decadienal were tested and recorded except hexenal, all aldehyds caused significant changes in membrane permeability and damaged the bacterial cells [28].

4. CONCLUSION

Tested yeasts had antibacterial activities with different levels. Gram -ve *Serratia* and *P. aeruginosa* bacterial strains are more sensitive than the gram +ve bacteria. Yeast may represent novel sources of antimicrobial metabolites and may allow the development of a pharmacologically, food preservatives in food industry for improving human health. Yeast has many advantages such as weather independent, producing simply by

fermentation with few nutritional and environmental requirements. Yeast easily grow on inexpensive substrates especially agriculture and agro-industrial residues, through few days, gives high yield with of the natural metabolites, good quality, easily extracted and easily separated from the growth media with high safety, stability, solubility in water and alcohol.

5. ACKNOWLEDGEMENTS

The authors thank Assiut University Mycological Centre (AUMC). Thanks to Research Finance Unit, Faculty of Science, Assiut University.

6. REFERENCES

- [1] Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Review. *International Journal of Antimicrobial Agents* 26(2005)343–356.
- [2] Jorgensen HJ, Ferraro MJ. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. *Clinical Infectious Diseases* 49(11)(2009)1749–1755. <https://doi.org/10.1086/647952>.
- [3] Lim SL, Tay ST. Diversity and killer activity of yeasts in Malaysian fermented food samples. *Tropical biomedicine* 28(2011)438-443. https://www.researchgate.net/publication/5175_9129
- [4] Caron F. Antimicrobial susceptibility testing: a four facts tool for the clinician, *Clinical Journal des Anti-Infectious* 14(2012)174-186.
- [5] Li Q, Huang J, Guo H, Guo X, Zhu Y, Dong K. Bactericidal activity against meticillin-resistant *Staphylococcus aureus* of a novel eukaryotic therapeutic recombinant antimicrobial peptide. *International Journal Antimicrobial Agents* 39(2012) 496-499.
- [6] Liu J, Sui Y, Wisniewski M, Droby S, Liu Y. Utilization of antagonistic yeasts to manage post-harvest fungal diseases of fruit. Review. *International Journal Food Microbiology* 167(2013) 153-160.
- [7] Carocho M, Morales P, Ferreira IC. Natural food additives: *Quo Vadis*. *Trends in Food Science Technology* 45(2015)284-295. <https://bibliotecadigital.ipb.pt>.
- [8] Sui Y, Wisniewski M, Droby S, Liu J. Responses of yeast bio-control agents to environmental stress. *Applied Environmental Microbiology* 81(2015) 2968-2975.
- [9] Mohammad A. Review on antimicrobial agents. *Organic Medicinal Chemistry* 1(5)(2017)1-7.
- [10] Eman MM. Chemical profile, agaritine content and selenium in *Agaricus bisporus*. *Brazilian Archives Biology Technology* 55(2012) 95-111.

- [11] Eman MM, Farghaly FA. Bioactive compounds of fresh and dried *Pleurotus ostreatus* mushroom. *International Journal Biotechnology For Wellness Industries* 3(2014)4-14.
- [12] El-Kady IA, El-Maraghy MSS, Eman Mostafa M. Antibacterial and anti-dermatophyte activities of some essential oils from spices. *Qatar University Science Journal* 13(1993)63-69.
- [13] Eman MM, Ali MM, Nassar SM Isolation of yeast from different food staffes affected by isolation (media and techniques) and molecular technique identification. (2018) *Studies in fungi* In Press.
- [14] Vimalkumar CS, Hosagaudar VB, Suja SR, Vilash V, Krishnakumar NM, Latha PG, Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of *Oleadioica* Roxb., infected with the rust fungus *Zaghouania oleae* (EJ Butler) *Cummins* and non-infected plants. *Journal of Pharmacognosy Photochemistry* 31(4)(2014) 69-72. www.phytojournal.com.
- [15] Bag GC, Devi PG, Bhaigyabati T. Assessment of total flavonoids content and antioxidant activity of methanolic rhizome extract of three *Hedychium* species of Manipur Valley. ISSN 0976-044X. *International Journal of Pharmaceutical Sciences Review Research* 30(1)(2015) 154-159.
- [16] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Pathology* 45(4)(1966)493-496.
- [17] Johanson TR, Caes CL. *Laboratory experiments in Microbiology*. 9th Edition, San Francisco, USA (2010) pp.470
- [18] Kwon-Chung KJ, Bennett JE. *Medical Mycology*. Lea & Febiger, Philadelphia (1992) PA 397-446.
- [19] Pub Chem citation. <https://pubchemdocs.ncbi.nlm.nih>.
- [20] Rauha JP, Remes S, Heinonen M, Hopia et al. Antimicrobial effects of Finnish plant extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology* 56(2000)3-12. www.elsevier.nl/locate/
- [21] Romano P, Fiore C, Paraggio M, Caruso M, Capece A. Function of yeast species and strains in wine flavor. *International Journal of Food Microbiology* 86(2003)169-180.
- [22] Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiology Reviews* 12(4) (1999) 564-582.
- [23] Kreger BE, Craven DE, McCabe WR. Gram-negative bacteria. IV. Re-evaluation of clinical features and treatment in 612 patients, *American Journal Medical* 68(1980) 344-355.

- [24] Lewis DH, Smith DC. Sugar alcohols(polyols) in fungi and green plants. Distribution physiology and metabolism. *New Phytologist* 66(1967)143-184.<https://nph.onlinelibrary.wiley.com/doi/>
- [25] AL-Jassani M, Mohammed GJ, Secondary Metabolites Analysis of *Saccharomyces cerevisiae* and evaluation of antibacterial activity. *International Journal of Pharmaceutical Clinical Research* 8(2016) 304-315.
- [26] Mewa-Ngongang M, Ntwampe SK, du Plessis HW, Mekuto L, Jolly NP. Bio-preservatives from yeasts with antimicrobial activity against common food, Agricultural produce and beverage spoilage organisms. Antimicrobial research: Novel bioknowledge and educational programs (A Méndez-Vilas Ed) (2017) 219-228
- [27] Pelttari E, Karhumäki E, Langshaw J, Peräkylä H, Elo H. Antimicrobial properties of substituted salicylaldehydes and related compounds. *Verlag der Zeitschrift für Naturforschung, Tübingen* 62 (2007) 487-497.
- [28] Trombetta D, Saija A, Bisignano G, Arena S, Caruso S, Mazzanti G, Uccella N, Castelli F. Study on the mechanisms of the antibacterial action of some plant b-unsaturated aldehyds. *Letters in Applied Microbiology* 35(2002)285–290.
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النشاط الحيوى المضاد للميكروبات لبعض سلالات الخمائر وباستخدام جهاز (ت أ ل ك) تحليل الاطياف اللونية والكتلة للمركبات الايضية الموجودة فى خميرة الرودوتوريولا ميليجانس ذات الرقم ١٣٥٦٥

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المسح الشامل الأولى لقدرة الخمائر على إنتاج المركبات ذات النشاط الحيوى المضاد للبكتريا بأستخدام طريقة الأنتشار للمادة الفعالة خلال الأقراص الورقية تم أختبار ثمانين مستخلص الكحول الميثيلى للخمائر لمعرفة نشاطها المضاد لسته سلالات من البكتريا الممرضة.

كما تم تأكيد النشاط المضاد للميكروبات لاثنى عشر سلالة من المستخلص الكحول الميثيلى للخمائر المعرفة بواسطة البصمة الحينية لمعرفة نشاطها المضاد للميكروبات الممرضة التى شملت (سته من سلالات البكتريا، وخميرة الكانديدا، وفطر من فطريات اصابة الجلد والفطريات الخيطية) حيث تم اختبارها بأستخدام (الدمسوا كمادة مذيبة واستخدام طريقة الحفر فى الاجار) ثم المقارنة بين النتائج عند (أستخدام الكحول كمذيب وطريقة الانتشار عبر الأقراص الورقية). حيث سجلت طريقة أستخدام الكحول كمذيب وطريقة الانتشار عبر الأقراص الورقية ادق واكفاً نتائج.

ومن النتائج السابقة تم اختيار اعلى وأكفاً سلالات الخمائر للتعرف على المواد الفعالة والمسببة للنشاط العالى المضاد للبكتريا والفطريات بأستخدام جهاز التحليل الكروماتوجرافى والمحلل الطيفى للكتلة تم تحليل المستخلص السابق ذكره حيث سجلت النتائج أن الرودوتوريولا ميليجانس ذات الرقم ١٣٥٦٥ تحتوى على واحد واربعون مركب نشط ومضاد للميكروبات وهذه المركبات شملت ثلاثة عشر نوعا من الكحوليات وسبع الديهيدات وستة أحماض دهنية وخمس مركبات ازولية وعشر من المركبات الاخرة. وكذلك تم تجميع كل المعلومات المتاحة عن المركبات المسجلة السابقة مثل نسبة وجودها فى المستخلص، زمن الجريان داخل جهاز التحليل، التركيب الكيمايى والرمز الكيمايى، الوزن الجزيئى ونشاطها الحيوى.