DOI Number: 10.5958/0976-5506.2018.00234.6

Analysis of Methanolic extract of Secondary Metabolites Released by *Candida glabratus* using GC-MS and Evaluation of Its Antimicrobial Activity

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ABSTRACT

The objectives of this study were analysis of the secondary metabolite products and evaluation antibacterial and antifungal activity. Bioactives are chemical compounds often referred to as secondary metabolites. Thirty nine bioactive compounds were identified in the methanolic extract of *Candida glabratus*. The identification of bioactive chemical compounds is based on the peak area, retention time molecular weight and molecular formula. *Coriandrum sativum* was very highly antifungal activity (6.80±0.25)mm. The results of anti-fungal and anti-bacterial activity produced by *Candida glabratus* showed that the volatile compounds were highly effective to suppress the growth of *Penicillium expansum* (5.95±0.20) and *Escherichia coli* (5.900±0.22).

Keywords: *Candida glabratus, GC-MS, Antifungal and Antibacterial, Secondary metabolites.*

INTRODUCTION

There are two widely cited potential virulence factors that contribute to the pathogenicity of *C. glabrata*. The first is a series of adhesins coded by the EPA (epithelial adhesin) genes [1-6]. These genes, located in the subtelomeric region, can respond to environmental cues that allow them to be expressed en masse so the organism can adhere to biotic and abiotic surfaces in microbial mats. This is also the suspected mechanism by which *C. glabrata* forms microbial "biofilms" on urinary catheters, and less commonly in-dwelling IV catheters. It also causes problems with dental devices, such as dentures. A major phenotype and potential virulence factor that *C. glabrata* possesses is low-level intrinsic resistance to the azole drugs[7-15], which are the most commonly prescribed antifungal (antimycotic) medications. It is still highly vulnerable to polyene drugs such as amphotericin B and nystatin, along with variable vulnerability to flucytosine and caspofungin. However intravenous amphotericin B is a drug of last resort, causing among other side effects, chronic renal failure[16-28]. Amphotericin B vaginal suppositories are used as an effective form of treatment in combination with boric acid capsules as they are not absorbed into the blood stream[29-33]. The aims of this study were screening of the metabolite products and determination antibacterial and antifungal activity.

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MATERIAL AND METHOD

Gas chromatography – Mass Spectrum analysis

Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The database consists of more than 62,000 patterns of known compounds[34-39]. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library.

Growth conditions of *Candida glabratus* and determination of metabolites

Candida glabratus was isolated from dried fruit and the pure colonies were selected, isolated and maintained

in potato dextrose agar slants. Spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25ºC in a shaker for sixteen days at 150 rpm[40-43]. 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. 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.

Determination of antibacterial and antifungal activity

The test pathogens (*Bacillus subtilis*, *Pseudomonas eurogenosa, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes*, *Staphylococcus aureus*, 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. After the incubation the diameter of inhibition zones around the discs was measured. *Candida glabratus* was suspended in potato dextrose broth and diluted to approximately 105 colony forming unit (CFU) per ml. They were "flood inoculated onto the surface of Potato dextrose agar and then dried. Standard agar well diffusion method was followed. The plates were incubated for 48 h at room temperature. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms[44,45]. Methanol was used as solvent control. Fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Table 1. Major phytochemical compounds identified in methanolic extract of *Candida glabratus*.

Phytochemical compound	RT (min)	Molecular Weight	Phytochemical compound	RT (min)	Molecular Weight
Methanesulfonic acid , 6-oxobicyclo[2.2.1]hept-2-yl	3.465	218.06128	5-Oxazolidinone,3-benzoyl-2-(1,1-dimethylethyl)-4-	5.753	365.166079
Cyclobut[c]inden-2-ol , decahydro-2-methyl-	3.493	180.151415	6-Acetyl-β-d-mannose	5.868	222.073953
3,3'-Methylenebis(1,5,8,11-tetraoxacyclotridecane)	3.522	392.241018	Muramic acid	5.954	251.100502
N-[2-[[2-Pyridylmethyl]amino]ethyl] aziridine	3.613	177.126597	Cyclohexanone , 4-ethoxy-	6.017	142.09938
Benzeneacetaldehyde	3.699	120.0575147	1-Nitro-2-acetamido-1,2-dideoxy-d-mannitol	6.360	252.095751
3-Benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylonitril	3.928	261.023533	8-Methylenecyclooctene-3,4-diol	6.692	154.09938
Glycerin	4.191	92.047344	1-Methyl-4-[nitromethyl]-4-piperidinol	7.270	174.100442
Propane , 2-fluoro-2-methyl-	4.231	76.0688286	Glucopyranuronamide , 1-(4-amino-2-oxo-1(2H)-pyri	7.350	443.17646
2-Bromotetradecanoic aid	7.579	306.119442	Cytidine , 5-methyl-	13.312	257.101171

Cont... **Table 1. Major phytochemical compounds identified in methanolic extract of *Candida glabratus*.**

Tertbutyloxyformamide , N-methyl-N-[4-(1-pyrrolidinyl	8.345	252.183778	Pyrrolo[1,2-a]pyrazine-1,4-dione , hexahydro-3-(2-me	14.285	210.136827
12-Hydroxy-14-methyl-oxa-cyclotetradec-6-en-2-on	8.511	240.1725445	n-Hexadecanoic acid	14.628	256.24023
1-Methyl-4-[nitromethyl]-4-piperidinol	9.084	174.100442	γ-Thionodecalactone	14.949	186.107836
3-Trifluoroacetoxypentadecane	9.312	324.227615	3-Oxa-16-demethoxycarbonyl-16-(2-methyl-sulphiny	15.263	410.166414
2H-Oxecin-2-one , 3,4,7,8,9,10-hexahydro-4-hydrox	9.524	184.109944	1-Propyl-3,6-diazahomoadamantan-9-ol	15.240	210.173213
9-Thiabicyclo[3.3.1]non-7-en-2-ol	10.686	156.060886	12,15-Octadecadiynoic acid , methyl ester	15.990	290.22458
Nitrosothymol	10.926	179.094628	Octadecanoic acid	16.516	284.27153
d-Mannose	11.716	180.063388	Pyridazine-3-carboxylic acid , 5-cyano-4-methyl-6-ox	16.591	255.064391

Table 2. Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of medicinal plants to Candida glabratus.

Plant	Inhibition (mm)	Plant	Inhibition (mm)
Ricinus communis	3.02±0.18	Cordia myxa	3.04±0.19
Datura stramonium	3.51±0.22	Malva parviflora	3.60±0.23
Linum usitatissimum	5.08±0.21	Mentha pulegium	5.19±0.21
Diplotaxis cespitosa	6.05±0.24	Daucus carota	6.00±0.23
Cassia angustifolia	5.69±0.25	Vitex agnus-castus	5.71±0.25
Euphorbia lathyrus	5.94±0.23	Cressa cretica	5.96±0.26
Rosmarinus oficinalis	5.68±0.25	Citrus sinensis	5.81±0.21
Citrullus colocynthis	3.90±0.16	Ruta graveolens	3.90±0.18
Althaea rosea	4.99±0.21	Thymus vulgaris	5.88±0.24
Coriandrum sativum	6.80±0.25	Passiflora caerulea	6.09±0.24
Origanum vulgare	5.71±0.23	Glycine max	5.73±0.23
Urtica dioica	4.14±0.24	Brassica oleracea	4.08±0.21
Foeniculum vulgare	3.19±0.19	Olea europaea	3.00±0.19
Ocimum basilicum	4.98±0.25	Calendula officinalis	4.93±0.24
Achillea millefolia	5.38±0.26	Taraxacum officinale	3.19±0.19
Medicago sativa	3.09±0.19	Borago officinalis	3.63±0.21
Celosia argentea	3.35±0.22	Sambucus nigra	3.07±0.24
Apium graveolens	5.08±0.24	C. morifolium	6.08±0.21
Brassica rapa	6.00±0.21	Equisetum arvense	5.81±0.23
Cichorium endivia	5.71±0.25	Portulaca oleracea	5.90±0.25
Anethum graveolens	5.88±0.22	Malva neglecta	5.49±0.22
Plantago major	5.39±0.24	L. angustifolia	3.10±0.18
Linum usitatissimum	3.84±0.18	Althaea Officinalis	6.01±0.21
A. esculentus	6.07±0.22	Melissa officinalis	6.51±0.27
Malva sylvestris	6.39±0.24	Control	0.00

RESULTS AND DISCUSSION

Identification of biochemical compounds

Analysis of compounds was carried out in methanolic extract of Salvadora persica, shown in Table 1. Clinical pathogens selected for antibacterial activity namely, Bacillus subtilis, Pseudomonas eurogenosa, Staphylococcus epidermidis, Escherichia coli, Proteus mirabilis, Streptococcus pyogenes, Staphylococcus aureus, and Klebsiella pneumonia maximum zone formation against Proteus mirabilis (6.19±0.20) mm. Methanolic extraction of Candida glabratus showed notable antifungal activities against M. canis, Aspergillus flavus, Aspergillus fumigatus, Candida albicans, Saccharomyces cerevisiae, Penicillium expansum, Trichoderma viride, and Aspergillus terreus. Penicillium expansum was very highly active against Candida glabratus (5.95±0.20). In agar well diffusion method the selected medicinal plants were effective against Candida albicans Table 2. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 µl of the samples solutions (Ricinus communis (Alkaloids), Datura stramonium(Alkaloids), Linum usitatissimum (Crude), Anastatica hierochuntica (Crude), Cassia angustifolia (Crude), Euphorbia lathyrus (Crude), Rosmarinus oficinalis (Crude), Citrullus colocynthis (Crude), Althaea rosea (Crude), Coriandrum sativum (Crude), Origanum vulgare (Crude), Urtica dioica (Crude), Foeniculum vulgare (Crude), and Ocimum basilicum (Crude), Achillea millefolia, Medicago sativa, Celosia argentea, Apium graveolens, Brassica rapa, Cichorium endivia, Anethum graveolens, Plantago major, Linum usitatissimum, A. esculentus, Malva sylvestris, Cordia myxa, Malva parviflora, Daucus carota, Vitex agnus-castus, Cressa cretica, Citrus sinensis, Ruta graveolens, Thymus vulgaris, Passiflora caerulea, Glycine max, Brassica oleracea, Olea europaea, Taraxacum officinale, Borago officinalis, Sambucus nigra, C. morifolium, Equisetum arvense, Portulaca oleracea, Portulaca oleracea, Malva neglecta, L. angustifolia, Althaea Officinalis, and Melissa officinalis) were delivered into the wells. Coriandrum sativum was very highly antifungal activity (6.80±0.25) mm.

CONCLUSION

Candida glabratus produce many important secondary metabolites with high biological activities. Based on the significance of employing bioactive compounds in pharmacy to produce drugs for the treatment of many diseases, the purification of compounds produced by Candida glabratus can be useful.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: In this research, all experimental protocols were approved under the Department of Biology, College of Science for women, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

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