

Analysis of Methanolic Extract of *Fusarium Chlamydosporum* Using GC-MS Technique and Evaluation of its Antimicrobial Activity

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ABSTRACT

Chromatography is the term used to describe a separation technique in which a mobile phase carrying a mixture is caused to move in contact with a selectively absorbent stationary phase. The objectives of this study were analysis of the secondary metabolite products and evaluation antimicrobial activity. Bioactives are chemical compounds often referred to as secondary metabolites. Eleven bioactive compounds were identified in the methanolic extract of *Fusarium chlamydosporum*. DL-Arabinose, D-Glucose, 6-O- α -D-galactopyranosyl, α -D-Glucopyranoside, O- α -D-glucopyranosyl, 5-Hydroxymethylfurfural, N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylidene), 1H-Purin-2-amine, 6-methoxy-N-methyl, 2-Methyl-9- β -D-ribofuranosylhypoxanthine, 1-Hexadecanesulfonic acid, 3,5-dichloro-2,6-dimeth, Methyl-6,7-benzoisoquinoline, Undeca-2,4,6,8,10-pentaenal, 11-(2-furyl)-oxime, and 2-Bromotetradecanoic acid. *Malva sylvestris* was very highly active 6.85 ± 0.25 mm. The results of anti-fungal and anti-bacterial activity produced by *Fusarium chlamydosporum* showed that the volatile compounds were highly effective to suppress the growth of *Aspergillus terreus* (6.09 ± 0.21) and *Staphylococcus aureus* (5.99 ± 0.19) mm.

Keywords: Anti-Microbial, *Fusarium chlamydosporum*, GC-MS, Secondary metabolites.

INTRODUCTION

Fusarium commonly infects barley if there is rain late in the season. It is of economic impact to the malting and brewing industries, as well as feed barley. *Fusarium* contamination in barley can result in head blight, and in extreme contaminations, the barley can appear pink¹⁻⁷. Most species are harmless saprobes, and are relatively abundant members of the soil microbial community. The genus includes a number of economically important plant pathogenic species. *Fusarium oxysporum* f.sp. cubense is a fungal plant pathogen that causes Panama disease

of banana (*Musa* spp.), also known as fusarium wilt of banana⁸⁻¹⁵. Panama disease affects a wide range of banana cultivars, which are propagated asexually from offshoots and therefore have very little genetic diversity. In humans with normal immune systems, fusarial infections may occur in the nails (onychomycosis) and in the cornea (keratomycosis or mycotic keratitis). Some consumers of *fusarium* products have shown food allergies similar in nature to peanut and other food allergies. People with known sensitivities to molds should exercise caution when consuming such products. *Fusarium* species possess several virulence factors, including the ability to produce mycotoxins, including trichothecenes, which suppress humoral and cellular immunity and may also cause tissue breakdown¹⁶⁻²⁷. In addition, *Fusarium* species have the ability to adhere to prosthetic material and to produce proteases and collagenases¹⁷⁻³². The aims of this research were screening of secondary bioactive chemical products and determination of its antibacterial and antifungal activities.

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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 compounds³³⁻³⁹. The spectrum of the extract was matched with the spectrum of the known components stored in the NIST library⁴⁰⁻⁴³.

Analysis of methanolic extract of *Fusarium chlamydosporum* and evaluation of its antimicrobial activity: *Fusarium chlamydosporum* spores were grown in a liquid culture of potato dextrose broth (PDB) and incubated at 25°C 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 culture⁴⁴⁻⁴⁷. The test pathogens

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 diameter⁴⁸⁻⁵⁰. The plates were incubated at 37°C for 24 hr and examined. After the incubation the diameter of inhibition zones around the discs was measured. 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 agent⁵¹⁻⁵³. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation.

Statistical Analysis: Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests.

Table 1: Major phytochemical compounds identified in methanolic extract of *Fusarium chlamydosporum*

S. No.	Phytochemical compound	RT (min)	Molecular Weight	Exact Mass
1.	DL-Arabinose	3.230	150.052823	150
2.	D-Glucose , 6-O- α -D-galactopyranosyl-	3.642	342.11621	342
3.	α -D-Glucopyranoside , O- α -D-glucopyranosyl-(1.fw	4.929	504.169035	504
4.	5-Hydroxymethylfurfural	6.440	126.031694	126
5.	N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylidene)	7.756	411.100124	411
6.	1H-Purin-2-amine , 6-methoxy-N-methyl-	10.949	179.080709	179
7.	2-Methyl-9- β -d-ribofuranosylhypoxanthine	11.527	282.09642	282
8.	1-Hexadecanesulfonic acid , 3,5,-dichloro-2,6-dimeth	14.897	479.20277	479
9.	3-Methyl-6,7-benzisoquinoline	14.931	193.089149	193
10.	Undeca -2,4,6,8,10-pentaenal , 11-(2-furyl)-,oxime	16.637	241.110279	241
11.	2-Bromotetradecanoic acid	17.392	306.119442	306

Table 2: Zone of inhibition (mm) of test different bioactive compounds and standard antibiotics of medicinal plants to *Fusarium chlamydosporum*

S. No.	Plant	Inhibition (mm)
1.	<i>Datura stramonium</i>	3.50 \pm 0.20
2.	<i>Diplotaxis cespitosa</i>	6.10 \pm 0.23
3.	<i>Cassia angustifolia</i>	5.62 \pm 0.23
4.	<i>Rosmarinus officinalis</i>	5.45 \pm 0.22
5.	<i>Citrullus colocynthis</i>	3.89 \pm 0.16
6.	<i>Althaea rosea</i>	5.85 \pm 0.21
7.	<i>Coriandrum sativum</i>	6.77 \pm 0.25
8.	<i>Origanum vulgare</i>	5.59 \pm 0.24
9.	<i>Urtica dioica</i>	4.08 \pm 0.21
10.	<i>Foeniculum vulgare</i>	3.09 \pm 0.18
11.	<i>Medicago sativa</i>	3.09 \pm 0.18
12.	<i>Celosia argentea</i>	3.56 \pm 0.20
13.	<i>Apium graveolens</i>	5.08 \pm 0.21
14.	<i>Brassica rapa</i>	6.08 \pm 0.23
15.	<i>Cichorium endivia</i>	5.73 \pm 0.23

Contd...

16.	<i>Anethum graveolens</i>	5.99 \pm 0.23
17.	<i>Malva sylvestris</i>	6.85 \pm 0.25
18.	<i>Malva parviflora</i>	3.51 \pm 0.19
19.	<i>Daucus carota</i>	6.11 \pm 0.25
20.	<i>Vitex agnus-castus</i>	5.73 \pm 0.24
21.	<i>Citrus sinensis</i>	5.69 \pm 0.14
22.	<i>Ruta graveolens</i>	3.90 \pm 0.18
23.	<i>Thymus vulgaris</i>	5.64 \pm 0.20
24.	<i>Passiflora caerulea</i>	6.76 \pm 0.25
25.	<i>Glycine max</i>	5.78 \pm 0.23
26.	<i>Brassica oleracea</i>	4.08 \pm 0.21
27.	<i>Olea europaea</i>	3.03 \pm 0.18
28.	<i>Borago officinalis</i>	3.37 \pm 0.20
29.	<i>Sambucus nigra</i>	5.06 \pm 0.22
30.	<i>C. morifolium</i>	6.05 \pm 0.23
31.	<i>Equisetum arvense</i>	5.66 \pm 0.23
32.	<i>Portulaca oleracea</i>	5.99 \pm 0.24
33.	<i>Malva neglecta</i>	5.48 \pm 0.22
34.	Control	0.00

RESULTS AND DISCUSSION

Analysis of compounds was carried out in methanolic extract of *Fusarium chlamydosporum*, shown in **Table 1**. Chromatogram GC-MS analysis of the methanol extract of *Fusarium chlamydosporum* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. All peaks were determined to be DL-Arabinose, D-Glucose, 6-O- α -D-galactopyranosyl, α -D-Glucopyranoside, O- α -D-glucopyranosyl, 5-Hydroxymethylfurfural, N-(4,6-Dimethyl-2-pyrimidinyl)-4-(4-nitrobenzylidene), 1H-Purin-2-amine, 6-methoxy-N-methyl, 2-Methyl-9- β -d-ribofuranosylhypoxanthine, 1-Hexadecanesulfonic acid, 3,5-dichloro-2,6-dimeth, Methyl-6,7-benzoisoquinoline, Undeca -2,4,6,8,10-pentaenal, 11-(2-furyl)-oxime, and 2-Bromotetradecanoic acid. Clinical microorganism 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 *Staphylococcus aureus* (5.99 ± 0.19) mm. Methanolic extraction of *Fusarium chlamydosporum* showed notable antifungal activities against *Aspergillus niger*, *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans*, *Saccharomyces cerevisiae*. *Fusarium chlamydosporum* was very highly active against *Aspergillus terreus* (6.09 ± 0.21). In comparison to the antibiotics used in this study, the plants extracts were far more active against the test bacterial strains. Five-millimeter diameter wells were cut from the agar using a sterile cork-borer, and 25 μ l of the samples solutions *Datura stramonium* (Alkaloids), *Anastatica hierochuntica* (Crude), *Cassia angustifolia* (Crude), *Rosmarinus officinalis* (Crude), *Citrullus colocynthis* (Crude), *Althaea rosea* (Crude), *Coriandrum sativum* (Crude), *Origanum vulgare* (Crude), *Urtica dioica* (Crude), *Foeniculum vulgare* (Crude), *Medicago sativa*, *Celosia argentea*, *Apium graveolens*, *Brassica rapa*, *Cichorium endivia*, *Anethum graveolens*, *Malva sylvestris*, *Malva parviflora*, *Daucus carota*, *Vitex agnus-castus*, *Citrus sinensis*, *Ruta graveolens*, *Thymus vulgaris*, *Passiflora caerulea*, *Glycine max*, *Brassica oleracea*, *Olea europaea*, *Borago officinalis*, *Sambucus nigra*, *C. morifolium*, *Equisetum arvense*, *Portulaca oleracea*, *Portulaca oleracea*, and *Malva neglecta* were delivered into the wells. The plates were incubated for 48 h at room temperature. In agar well diffusion method

the selected medicinal plants were effective against *Fusarium chlamydosporum* **Table 2**. *Malva sylvestris* was very highly antifungal activity 6.85 ± 0.25 mm.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Department of Biology, College of Science, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

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