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Research Article



The antifungal effects of *Chlorella vulgaris* methanol extract on *Candida* spp. isolated from skin infections

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Abstract

This study was carried out in order to assess the biological efficacy of methanol extract of *Chlorella vulgaris* as antifungal against isolated *Candida* spp. from skin infections. Several tests were performed to determine the existence of active chemical compounds in *C.vulgaris*, and we discovered that it is rich in active chemicals. The methanol 70% extract was discovered to contain carbohydrates, glycosides, phenols, flavonoids, triterpenoid, amino acid, tannin, alkaloid, and saponin, with no Anthocyanin and steroid detected. GC-MS analysis indicated the presence of (50) compounds in 1 ml methanol extract of *C. vulgaris* (organic compounds high percentage ex. n-Hexadecanoic acid, 2,4-Di-tert-butylphenol, cis-Vaccenic acid, Hexanoic acid, 4-oxo-6-(1-piperidyl)Stigmasta-3,5-diene and Octadecanoic acid) with specific retention times. The detected compounds possess antioxidant and antimicrobial activities. The efficiency of *C. vulgaris* extract as an antifungal was demonstrated utilizing the well diffusion plate method. Methanol extract showed significant efficacy against all isolated candida spp. from skin infections (P-value < 0.01). The results showed a high zone rate of inhibition/mm² (36.2 ±0.5) against *C. albicans* and less inhibition/mm² (9.5 ±1.6) against *C. krusei*.

Keywords: Antifungal Effects, *Chlorella vulgaris*, *Candida* spp.

Introduction

Chlorella vulgaris is a type of microalgae that falls under the Chlorococcales order within the Oocytaceae family. Its green coloration is a result of the chloroplasts that are present in it. Its size ranges from 1 to 10 microns, and its form is spherical. Because these microalgae include a substantial amount of intracellular proteins, carbohydrates, lipids, vitamin C, β-carotenes, and B vitamins (B1, B2, B6, and B12) in addition to chlorophyll, they are frequently used in the formulation of dietary supplements, cosmetics, and medicinal treatments (Alberto et al., 2022). These aquatic algae are cultivated because of the high protein, fatty acids content and biologically active substances, the freshwater algae of the genera *Chlorella* sp. were called super foods because they have a positive effect on every system in the body, have antioxidant properties and strengthen the immune system,

due to the small size and high multiplication rates (Al-Hammadi et al., 2024; Ibrahim et al., 2020).

Chlorella has the potential to be used as food, fuel, and feed. It is known to produce a wide range of secondary metabolites with different biological actions, including antifungal, antiviral, and antibacterial activities (Silva et al., 2024). These metabolites can be produced both intracellularly and extracellularly. The use of dried algal biomass and biologically active compounds derived from algae as pharmaceuticals has drawn a lot of attention lately. The biologically active compounds extract from microalgae were the best sources of various, including sterols, polysaccharides, phenolic compounds, carotenoids, and fatty acids (Fu et al., 2017). Microalgal polysaccharides are powerful anti-inflammatory, immunomodulatory, hypocholesterolemic, hypolipidemic, and hypoglycemic substances (Olasehinde et al., 2017).

Materials and methods

1. Samples Collection: Algae collection

A sample of algae was collected from the Euphrates River and transported to the laboratories of the Biology Division - College of Science at Dhi Qar University. *C. vulgaris* is isolated, classified, then purified and grown on ch-10 medium.

Algae Culture

After the diagnosis, the algae were transferred to the liquid medium by a sterile pipette, and by the sterile loop in the case of solid medium or from both mediums to a number of 1000 ml glass jugs. Each beaker contains 100 ml of sterile culture medium. The jugs were closed with cotton and transferred to growth cabins with a temperature of 25 ± 3 °C, and light ranged between (130- 150 Micro-Einstein/M²/S and lighting system 8: 16 hours lighting / dark and the culture was shaken several times and continued planting until a good growth (Alrumman and Hesham, 2016).

Candida spp. collection

The Al-Imam Al-Hussein Teaching Hospital and Special Clinics in Thi-Qar province/Southern Iraq were the source of 100 swabs from patients with skin infections between August 2, 2023, and December 3, 2023. The patients' ages ranged from 11 to 70 years old for both sexes, and the swabs were placed in sterile tubes with transport media before being sent straight to the laboratory for inoculation on Sabauroud dextrose agar.

2. Extraction of phytochemicals from *C. vulgaris*

20 g of *C. vulgaris* were previously produced, powdered, and steeped in a solution (200 ml of 70% methanol and distilled water) to prepare the methanol extract. The extraction of secondary metabolites from *C. vulgaris* using an ultrasonic method (45°C for 48 hours). The following day, all of the extracts were filtered through Whatmann No. 15 filter paper. The filtrate was then concentrated under low pressure on a rotary evaporator at 50°C and dried at 25°C. The extract was then stored in glass tubes that had been sterilized until it was needed (Sani et al., 2013, Al-Kunani, 2015; Ibrahim et al., 2015; Waheeb et al., 2023). Standard procedures were followed for conducting qualitative phytochemical testing (Harborne, 1998).

3. Detection of Active Compounds of methanol extract by using Spectrometry Gas Chromatography-Mass

Active chemical compounds were isolated and diagnosed from methanol extract of micro algae under study in the Water-Environmental Research Center - Ministry of Science

and Technology Department of Environment according to method obtained by (Shobier and Abdel Ghani, 2016).

The following conditions were used in isolation and diagnosis by GC

- Instrument voltage was (70) volts
- Optima type column (5μs)
- Dimentions column (30mm-0.25μm)
- Column with thickness (0.23μm)
- The wave temperature was (100-180 °C at 15°C/min, 180-300 °C at 5 °C/min and 10 min hold at 300 °C)
- The degree of injector was (280 °C)
- Carrier gas flow rate was (3ml/min)
- Split ratio was (10)
- Quantity of injected solution is (1 μml)

4. Investigation of studied extract activity as antifungal

The methanol extract activity of *C. vulgaris* as an antifungal was investigated using the well diffusion plate method, which involved spreading the candida suspension on the culture medium (Sabauroud dextrose agar), then spreading the suspension on the plates with a sterile cotton swab and leaving for two minutes to dry. The extracts were diluted in DMSO to achieve various concentrations (25%, 50%, 75%, and 100% mg/dl). 50 μl of the extracts were then applied to each hole using a micropipette and incubated at 35°C for 24 hours (Khalif and Al-Waheeb, 2020).

Results and discussion

Table 1 showed the results of Qualitative disclosures for methanol extract for *C. vulgaris*. It is noted that the methanol 70% extract has contain carbohydrates, glycosides, phenols, flavonoids, triterpenoid, Amino acid, Tannin, alkaloid, and saponin, and negative detection for Anthocyanin and Steroid because alkaloids, saponins, tannins, phenols, glycosides, steroids, flavonoids, carbohydrates, and several medium-polar substances may all be extracted well with methanol. (Fayaz et al., 2017).

Through the results of the phytochemical study, we can see that *C. vulgaris* is abundant in active substances. This is to be expected, as previous research has shown that *C. vulgaris* contains a variety of active compounds in the form of chemical hosts, including tannin, carbohydrates, alkaloids, saponin, terpenoids, and phenolic compounds (Pradhan et al., 2021).

Organic compounds in the extracts from *C. vulgaris* algae were detected by using GC-Mass technology in laboratories of the Environmental Research Center/Ministry of Science and Technology, according to (Chen and Walker, 2011). The methanol extract of *C. vulgaris* was found to include fifty chemical components, each of which represents a certain

distance, according to the data. As demonstrated, the organic compounds high percentage which is the greatest believed to

have efficacy on the dermatophyte are those that are farthest apart from one another as shown in Fig. 1 and Table 2.

Table 1. Qualitative disclosures for methanol extract for *C. vulgaris*

Active compounds	Present/absent
Carbohydrates	+
Alkaloids	+
Glycosides	+
Phenols	+
Flavonoids	+
Triterpenoid	+
Saponin	+
Steroids	-
Amino acid	+
Tannin	+
Anthocyanin	-

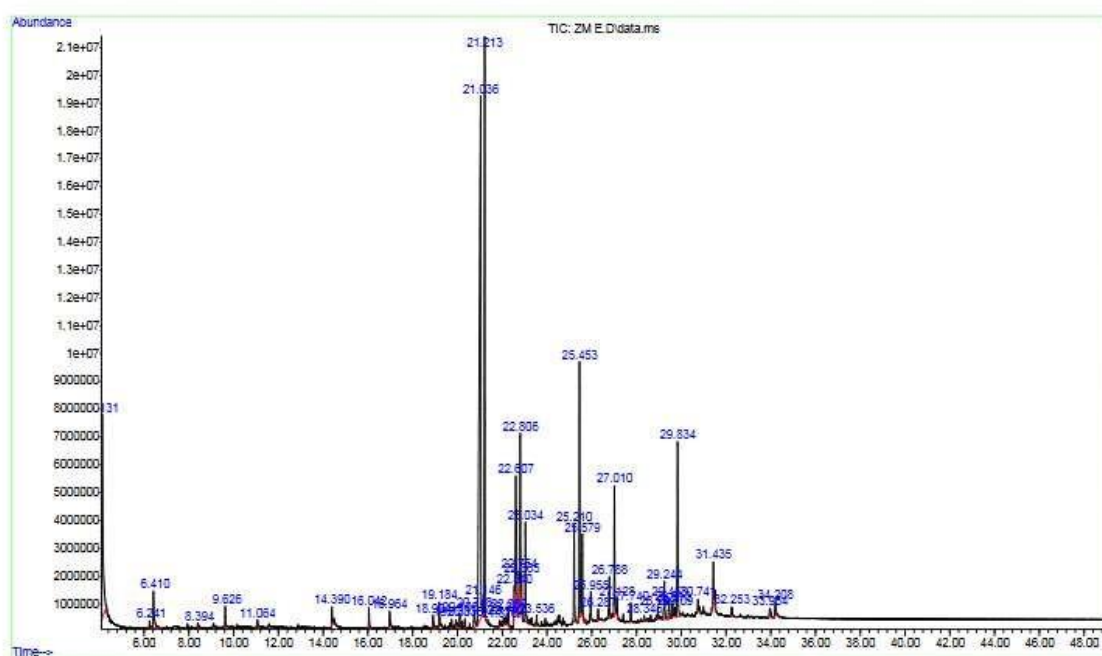


Fig. 1 GC-Mass spectrum of the chemical compounds from methanol extract of *C. vulgaris*

Table 2. Organic compounds in methanol extract of *C.vulgaris*

Peak	R. Time	Common Name	Peak Report TIC Area (%)
1	4.131	Dimethyl Sulfoxide	0.0345
2	6.241	Formamide, N-methoxy-	0.2419
3	6.41	Ethanol, 2,2-diethoxy-	1.6209

4	8.394	Formamide, N-methoxy-	0.2227
5	9.626	2-Furaldehyde diethyl acetal	0.5381
6	11.064	Hexane, 1,1-diethoxy-	0.211
7	14.39	Ethyl(dimethyl)ethoxysilane	0.4928
8	16.042	2,4-Di-tert-butylphenol	0.5177
9	16.964	Cetene	0.4534
10	18.904	Tetradecanoic acid	0.4639
11	19.184	9-Eicosene, (E)-	0.7398
12	19.738	2-Pentadecanone, 6,10,14-trimethyl-	0.2306
13	20.106	Carbonic acid, isobutyl undec-10-enyl ester	0.3347
14	20.335	2-Isopropyl-5-methylcyclohexyl methylphosphonofluoridate	0.206
15	20.748	6-Pentadecenoic acid, 13-methyl-, (6Z)-	0.8617
16	21.036	n-Hexadecanoic acid	31.1287
17	21.146	cis-Vaccenic acid	0.2732
18	21.213	2,4-Di-tert-butylphenol	12.0261
19	21.876	n-Hexadecanoic acid	0.1892
20	22.142	9,12-Octadecadienoic acid, methyl ester	0.1918
21	22.201	9-Octadecenoic acid (Z)-, methyl ester	0.2075
22	22.289	Tricosyl heptafluorobutyrate	0.4225
23	22.54	Linoelaidic acid	0.4231
24	22.607	cis-Vaccenic acid	7.1954
25	22.754	Linoleic acid ethyl ester	0.4121
26	22.806	Octadecanoic acid	5.7211
27	22.865	Ethyl Oleate	0.4933
28	23.034	Octadecanoic acid, ethyl ester	2.2182
29	23.536	Tetradecanedioic acid	0.3405
30	25.21	(3H,6H)Thieno[3,4-c]isoxazole, 6-(4-chlorophenyl)-3a,4-dihydro-	2.5434
31	25.453	Hexanoic acid, 4-oxo-6-(1-piperidyl)-	7.7387
32	25.579	Octadecanedioic acid	1.9441
33	25.955	Bis(2-ethylhexyl) phthalate	0.4789
34	26.287	Docosanoic acid, ethyl ester	0.2363
35	26.788	Hexadecanoic acid, propyl ester	1.1854
36	27.01	Cyclobutanone, 2-tetradecyl-	3.2941
37	27.128	Hexadecanedioic acid, dimethyl ester	0.3973
38	27.74	Ethyl tetracosanoate	0.4448
39	28.345	Anthracene, 9,10-dihydro-9,9,10-trimethyl-	0.1838
40	28.949	Benzene, 1-(1,1-dimethylethyl)-4-ethenyl-	0.2329

41	29.244	Cholest-5-en-3-ol (3.beta.)-, carbonochloridate	1.3359
42	29.458	Chola-5,22-dien-3-ol, (3.beta.,22Z)-	0.7698
43	29.613	6-Fluoroindole, TMS derivative	0.3486
44	29.709	1H-Indole, 1-methyl-2-phenyl-	0.2721
45	29.834	Stigmasta-3,5-diene	7.1915
46	30.741	Campesterol	0.3421
47	31.435	.gamma.-Sitosterol	1.2818
48	32.253	Stigmasta-3,5-dien-7-one	0.342
49	33.964	N-Methyl-1-adamantaneacetamide	0.4614
50	34.208	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, octadecyl ester	0.5628
			100%

The present study results were similar to Al-okayli et al. studied (2019), showed that the ethanol extract of *C. vulgaris* contain to active Organic compounds such as Hexadecanoic Acid, Phytol and Gamolenic Acid.

Studied methanol extract activity of *C. vulgaris* as antifungal:

The current study indicated the highly activity of extract with concentration 100% extracted from *C. vulgaris* has highly activity against *C. albicans* and the lowest activity showed against *C. krusei*. Also the antifungal activity showed increased with increase concentration of extract as shown in Table 3.

Table 3. Activity of *C. vulgaris* methanol extract against isolated *Candida* spp.

<i>Candida</i> spp.	<i>C. glabrata</i>	<i>C. krusei</i>	<i>C. famata</i>	<i>C. albicans</i>
	inhibition Zone/ mm ² Mean ±SD			
100%	21.5 ±1.0	18.25 ±1.5	26.61±0.8	36.2 ±0.5
75%	19.4 ±0.8	15.30 ±1.2	21.2 ±1.0	23.20 ±0.6
50%	14.62 ±0.6	12.22 ±1.0	15.5 ±1.2	19.9 ±0.8
25%	11.08 ±1.0	9.5 ±1.6	13.7 ±0.8	16.10 ±0.6
P.Value	< 0.01			

The high efficacy of methanol extract from *C. vulgaris* was indicated by their containment of active compounds, as in (Fig. 1 and Table 2) GC-Mass analysis indicated the occurrence of active compounds possess antioxidant and antimicrobial activities, and this is constant with the findings of researchers (Al-okayli et al., 2019; Pradhan et al., 2021).

Conclusion

The study demonstrates the potent antifungal properties of methanol extract from *Chlorella vulgaris* against *Candida* species isolated from skin infections. The extract was rich in bioactive compounds such as carbohydrates, glycosides, phenols, flavonoids, and triterpenoids, as confirmed by phytochemical tests and GC-MS analysis. Notably, the presence of compounds like n-Hexadecanoic acid and 2,4-Di-tert-butylphenol, known for their antioxidant and

antimicrobial activities, likely contributes to the extract's efficacy. The well diffusion plate method revealed significant antifungal activity, particularly against *C. albicans*, with inhibition increasing with higher extract concentrations. The study's findings suggest that *C. vulgaris* methanol extract could be a promising natural antifungal agent, offering an effective alternative for managing skin infections caused by *Candida* species.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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