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Research Article

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Assessment of antioxidant activity in *Vaucheria sessilis* extracts and their efficacy against isolated *Candida* spp. from diabetic foot ulcers

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Abstract

This study was carried out in order to assess the biological efficacy of separated extracts of V. sessilis as an Antioxidant and antifungal against isolated candida spp. from diabetic foot ulcers. Several tests were used to identify the presence of active chemical compounds in V. sessilis and we note that the V. sessilis is rich in active substances. showed phytochemical results were found for the extracted ethanol 70% contain carbohydrates, flavonoids, phenols, alkaloids, glycosides, triterpenoid and saponin, and negative detection for Tannin, Steroid, and Amino acid. The antioxidant activity of V. sessilis extracts was assessed by decreasing the radical scavenging activity of (1,1-diphenyl-2-picrylhydrazyl (DPPH). Results exhibited that all extracts have antioxidant activity with different concentrations, showed the superiority of the polyphenol 86.02 % inhibition ratio at (1000 μ g/ ml), flavonoid 84.05 % at (1000 µg/), and hexane extract 68.5 % at (1000 µg / ml) compared to ascorbic acid, which recorded 81.17% at a concentration of 1000 µg/ml. Whereas, the hexane extract recorded the lowest inhibition rate at (50 µg/ml). Efficacy showed of separated extracts from V. sessilis as antifungal activity using well diffusion plate method. The polyphenol extract (VP) was the highest activity against all isolated candida spp. from foot ulcers of diabetic patients, while the other extracts showed varying activity against different candida spp.at P. value < 0.01. The results that a high Zone rate of inhibition/mm² was (26.7 \pm 0.6) against C. albicanis, while no inhibition/mm² (9.8 \pm 0.8) for hexane extract against the same candida. HPLC analysis indicated the presence of 7 compounds in separated polyphenol extract of V.sessilis (Chlorogenic acid, Ferulic acid, Gallic acid, Rutin, Qurcetine, Kaempferol and Apigenin) with specific retention times. The detected compounds possess antioxidant and antimicrobial activities. These results suggested that separated extracts from V. sessilis have high antioxidant and antifungal activities. In the future, V. sessilis extracts could become one of the important alternatives in infection treatment, even if in a limited way due to the spread of *candida spp*. that are resistant to antifungals.

Keywords: Antioxidant Activity, HPLC, V. sessilis, Active chemical compounds, Antifungal Activity

Introduction

Vaucheria is commonly known as "water felt"; the sparsely branched or unbranched coenocytic filaments often form felt like mats, belong to Vaucheriales (Xanthophyceae) (Potter et al. 1997; Naveen et al.,2020). Vaucheria frequently occurs in freshwater, marine, and terrestrial habitats forming greenish mats, often appearing as velvety, felt-like coatings on various substrata. It has a cosmopolitan distribution with over 79 accepted species worldwide, although there are no records in South America, Africa, Central Asia, and Antarctica (Guiry and Guiry 2014). Taxonomy of the genus is based on a morphological species concept, where species are characterized by morphology, especially the shape, size, and arrangement of antheridia and oogonia (Muralidhar et al., 2014). In the case study the use of *V. sessilis*, because it is readily available, renewable, low cost, and environmentally friendly (Sagar and Rastogi, 2019). Oxidative stress is among the main causative factors in the induction of many chronic and degenerative diseases, such as cancer, diabetes, Alzheimer's disease, stroke, viral infections, neurodegenerative processes, infarction, brain edema, and aging (Incent et al., 2017). Antioxidants are ions, radicals, or compounds that, when present, can delay or prevent the oxidation of other molecules (Dibacto et al., 2021). Antioxidants protect the body by combating free radicals generated by oxidative stress and maintaining a balance between oxidants and antioxidants, which is triggered by stray oxygen atoms. Antioxidants are either hydrogen donors or free radical sensors (Parcheta et al., 2021). When hydrogen atoms and radicals combine, they form a stable molecule (Khadim and Al-Fartusie, 2021). They protect us by either directly reducing free radical production or preventing their spread (Aziz et al., 2019). Antioxidants can be found in vegetables, fruits, cereals, most medicinal herbs, nuts, some meats, poultry, and fish (Nemzer et al., 2019; Jideani et al., 2021). Antioxidants are found naturally in foods and improve human health without causing harm, as opposed to synthetic antioxidants (Loizzo and Silva, 2021). It is the most effective method of protecting the body from free radical damage in an emergency. They are self-defense systems that protect the cell from oxidative stress and play an important role in its function (Aziz et al., 2019).

The most important previous studies on the genus Vaucheria show its chemical compounds and their importance as antioxidants and antimicrobials according to the results of a spectrophotometer analysis of the total phenolic compounds present in a methanol extract of V. geminata, results were given as gallic acid equivalent (Vuran et al., 2012). In the study El-Tablawy et al. (2020), HPLC chromatography was used to identify roughly seven varieties of each phenol and flavonoid chemical in the four algae (Vaucheria geminate, Pleurochloris pyrenoidosa, Botrydiopsis eriensis, and Scenedesmus obliquus). Gallic, chlorogenic, caffeic, and ferulic were the most often found phenols, while rutin, quercetin, apigenin, and quercitrin were the most prevalent flavonoids among all the investigated algae. All the studied algae were noted for having significant antioxidant properties. As the Pérez-Gutiérrez study (2009). A sesquiterpene lactone 10β-Hydroxy-11βH-guai-12,6α-olide has been isolated from hexane extract of V. sessilis and the compound showed inhibits growth of Bacillus subtilis, B. cereus, E. coli, Staphylococcu aureus and S. epidermidis in vitro with at a minimun inhibitory concentration (MIC) ranging from 25 to 50 µg/mL, while It had no activity at 100 µg/mL against other bacterial species Vibrio cholerae, Salmonella typhi and Kiebsiella pneumoniae.

Materials and methods

Algae collection

Vaucheria sessilis are collected from Euphrates River in Nasiriya city/ Iraq in March 2020 using sterilized plastic containers, then labeled and transported to the laboratories of

the Department of Biology - College of Science at the University of Thi–Qar. They were classified, cleaned, washed with distilled water, and dried at room temperature (25 °C) for two weeks. It is collected, crushed, and then preserved in dark glass containers until use.

Candida spp. collection

A total of 126 swabs were collected from diabetic patients suffering from foot ulcers at the Diabetes Center/Thi-Qar during the period from September to November 2020, The patients' ages ranged from 23 to 74 years of sexes, and the swabs were placed in sterile tubes with transport media. They were then taken to the laboratory immediately for inoculation on Sabauroud dextrose agar.

Extraction of phytochemicals from V. sessilis

Preparation of ethanol extract, 20 g of *V. sessilis* were powdered and soaked in solution (200 ml of 70% ethanol and distilled water). The process of secondary metabolites extraction from *V.sessilis* by ultrasonic for (48 hr. 45°C). The next day, all the extracts were filtered using titron cloth three times (Sani et al., 2013, Al-Kunani, 2015; Ibrahim et al., 2015). Qualitative phytochemical tests were performed as per standard methods (Harborne, 1998). Prepare hexane extract following the method (Bobby et al., 2012), in which 20 g of *V.sessilis* were placed in a thimble and then placed in a Soxhlet extractor using 200 ml of hexane for 24 h.

Extraction and fractionation of different active constituent

Isolation of Polyphenols from V. sessilis by ultrasoundassisted extraction (UAE)

The extraction of phenolic compounds from *V. sessilis* by ultrasound was carried out using 500 mL ethanol (70%) (a water–ethanol mixture (30:70 ml) with 50g of *V. sessilis*, at a temperature of 45 °C and the extraction time (48 hours) (Alternimi et al., 2015).

Isolation of flavonoids from V. sessilis

Powder of *V. sessilis* (50gm) was treated with 250 ml of (80%) methanol by stirring at room temperature for 24 hours (Benavente-Valdés et al., 2020).

Percentages of V. sessilis extracts

Extraction Percentages were calculated from the equation as:

Weight of extracts (g)

Extraction percentage (%) =

X 100

Powder Weight of algae (g)

powder weight of algae (g)=20g

Estimation of antioxidant activity for *V. sessilis* extracts by non-enzymatic method Free radical scavenging assay (DPPH), It is a fast method to show the radical scavenging activity

Iml DPPH solution (A freshly formulated (0.004 w/v) by dissolving 0.004 gm in 100ml methanol: DMSO (75:25), was mixed with 1 ml at various concentrations (50,100, 250, 500, 1000 μ g/ml) of the (hexane extract, Polyphenols and flavonoids from *V. sessilis*), solvent was methanol: DMSO (75:25). Then the samples were kept in drak at r.t 25°C for 30 min. then The absorbance was measured at a wavelength of 517 nm with a UV-Vis Spectrophotometer, using ascorbic acid at the same concentrations as a standard solution. The following formula was used to measure radical scavenging behavior, which was expressed as the percentage of free radicals inhibited by the sample (Xiao et al., 2020).

Investigation of studied extracts activity as antifungal

Investigation of studied extracts activity of *V. sessilis* as antifungal using well diffusion plate method, that the candida suspension was spread on the culture medium (Sabauroud dextrose agar), then the suspension was spread by means of a sterile cotton swab on the plates and left for two minutes to dry. studied extracts were dissolved with the organic solvent DMSO to obtain concentration 100 μ g/ml. Then added to each hole 50 μ l of the studied extracts Using a micropipette, and incubated a at a temperature of 35 °C, for 24 hours (Khalif and Al-Waheeb, 2020).

High Performance Liquid Chromatography (HPLC) Analysis for detection of Active Compounds

An HPLC analysis was performed for the detection and estimation of High Performance Liquid Chromatography (HPLC) Analysis of active compounds in Separated polyphenol from *V. sessilis.* Analysis was carried out by HPLC. Reversed-phase HPLC processing was used to quantify individual phenolic compounds, using a SYKAMN HPLC chromatographic device fitted with a UV detector, Chemstation, and a Zorbax Eclipse Plus-C18-OSD. In the Ministry of Science and Technology's laboratories in Baghdad –Iraq

HPLC Conditions

• Mobile phase: solution A (0.5 ml trifluoro acetic acid in one liter of water) + solution B (0.5 ml trifluoroacetic acid in one liter of acetonitrile) Solution A: solution B (20:80 %)

- Column type C-18
- Column temperature: 22 C°
- Flow rate: 1.0 ml/min
- Injection volume

Results and discussion

Fig. 1 indicates the results of percentages of V. sessilis extracts. It is noted that the ethanolic 70% extract has the highest percentage of extraction from other general extracts because ethanol is a good solvent for the extraction of alkaloid, saponins, tannins, phenols, glycosides, steroids, flavonoids and carbohydrates, and some medium polar compounds (Fayaz et al., 2017).

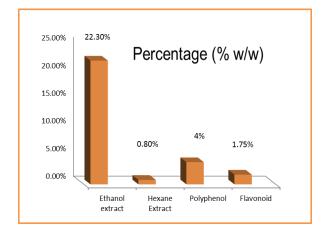


Fig. 1 Percentages of V. sessilis extracts

The variance in the percentages of extracting the active substances in different solvents is due to several reasons, including type of algae used, the solubility intensity of each specific structure in the solvents used and the polarity of the solvent. Solvent extraction is one of the basic methods for separating active ingredients that may be affected by various factors such as sample quantity and quality, time of collection, solvent, solvent to substance ratio. All of these factors are responsible for the overall amount extracted (Chisté et al., 2014).

Several tests were used to identify the presence of active chemical compounds in *S V. sessilis* and phytochemical results were found for the extract ethanol 70% contain carbohydrates, flavonoids, phenols, alkaloid, glycosides, triterpenoidand saponin, and negative detection for Tannin, Steroid and Amino acid.

Through the results obtained from the phytochemical study, we note that the *V. sessilis* is rich in active substances, and this is expected because previous studies found many active compounds present in the form of chemical hosts in the *V. sessilis* such as tannin, flavonoid, alkaloid, saponin, and phenolic compound (Gaya et al., 2016).

The DPPH assay is a commonly used test to determine the antioxidant function of plant extracts. It is a more stable and well-known nitrogen-centered free radical dependent on the reduction of accepting hydrogen or electron donor. A stable free radical with a dark purple color is used in this research, which changes color to yellow as antioxidant compounds are returned by giving it an electron or a hydrogen root (Hu et al., 2011) to become a stable molecule as follows:

 $(DPPH\bullet) + HO-R-OH \rightarrow (DPPH): H + HO-R-O\bullet$

Antioxidants cause a decrease in optical absorbance of 517 nm, which determines the DPPH radical's return strength.

The DPPH assay is fast test for determining the antioxidant activity of *V. sessilis* extracts. the effects of DPPH radical scavenging operation appear to improve with an increased concentration ratio of extracts, also the study results shown in the Table 1. and Fig. 2, compared to standard ascorbic acid, showed the superiority of the polyphenol 86.02 % inhibition ratio at a concentration of $1000 \mu g/ml$, flavonoid isolated 84.05% at a concentration of $1000 \mu g/ml$, and hexane extract 68.5% at a concentration of $1000 \mu g/ml$ Compared to ascorbic acid, which recorded 81.17% at a concentration of 1000 $\mu g/ml$.

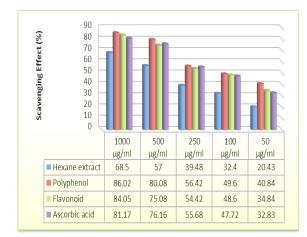


Fig. 2 DPPH radical scavenging activity of separated V. *sessilis* extracts with five different concentrations when compared to the standard (ascorbic acid).

Extract type	Scavenging Effect (%)				
	50 µg/ml	100 µg/ml	250 μg/m	500 μg/m	1000 µg/m
Hexane extract (VH)	20.43	32.4	39.48	57	68.5
Polyphenol (VP)	40.84	49.6	56.42	80.08	86.02
Flavonoid (VF)	34.84	48.6	54.42	75.08	84.05
Ascorbic acid (SCA)	32.83	47.72	55.68	76.16	81.17

Table 1. Antioxadant activity of separated extracts of V. sessilis by DPPH method

As we note the hexane extract and the isolated groups have high inhibition results compared to ascorbic acid, and this indicates that V. sessilis plant has a high activity as an antioxidant. also the current results showed were apparent outperforms the other extracts. The that the polyphenol presence of hydroxyl groups in some compounds, such as phenolic compounds or flavonoids, may influence their ability to scavenge free radicals (Bhakya et al., 2016). Because of the relationship between the concentrations of the compounds, the high contents of total phenolic and total flavonoid compounds can lead to essential antioxidants. This finding suggests that the extracts have a part of the oxidative action, and this role has a synergistic effect on the antioxidant effect of the DPPH radical. The potency of antioxidants and the phenolic removed, indicating that these substances are mainly responsible for It acts as an antioxidant and has a polar appearance so it is an effective antioxidant (Ubando-Rivera et al., 2005). This influence may also be traced back to the flavonoids present in this extract, as several studies have shown a connection between flavonoids' chemical structure and their displacement effect (Seyoum et al., 2006).

Antioxidant phytoconstituents can neutralize (DPPH) radicals either by transferring hydrogen atoms or by transferring an electron (Taia, 2006).

Studied extracts activity of V. sessilis as antibacterial

The polyphenol extract (VP) exhibited the highest activity against all isolated *Candida spp*. from foot ulcers of diabetic patients, while the other extracts showed varying activity against different *Candida* species at P. value < 0.01 as demonstrated in Table 2. and Fig. 3. According to results of the present study, *V. sessilis* polyphenol extract (vp) of had highly activity against *Candida* spp.

The high efficacy of the polyphenol extract (VP) separated from *V. sessilis* was indicated by their containment of effective compounds, as in Fig. 4 and Table 3. HPLC analysis indicated the presence of polyphenol compounds possess antioxidant and antimicrobial activities, and this is consistent with the findings of researchers (Chibane et al., 2019; El-Tablawi et al., 2020).

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Candida spp.	Inhibition Zone/ mm2 Mean + SD			
	VH	VP	VF	
C. tropicalis	19.7±1.5	25.3 ±0.5	14.2 ± 0.8	
C. albicanis	9.8±0.8	26.7 ± 0.6	12.7 ± 1.2	
C. glabrata	15.2 ±1.0	24.2 ± 0.8	15.3 ± 0.6	
C. krusei	15.2 ± 0.3	24.7 ± 0.6	13.8 ± 1.0	
P. Value	< 0.01			

Table 2. Activity of separated extracts of V. sessilis against isolated Candida spp. from foot ulcers of diabetic patients

Hexane extract (VH), Polyphenol (VP), Flavonoid (VF)

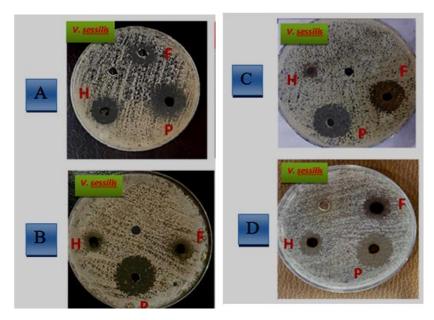


Fig. 3 Activity of separated extracts of V. sessilis against isolated Candida spp. (A= C. tropicalis, B= C. albicanis, C= C. glabrata, D= C. krusei)

Table 3. Retention	time of sample separated	polyphenol compounds of	V. sessilis extract
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No.	Retention Time	Area	Formula	Compound
	[min]	[%]		Name
1	2.50	10.25	C16H18O9	Chlorogenic acid
2	4.30	15.55	C10H10O4	Ferulic acid
3	5.99	15.89	C7H6O5	Gallic acid
4	8.21	12.65	C27H30O16	Rutin
5	9.92	15.39	C15H10O7	Qurcetine
6	10.30	13.47	C15H10O6	Kaempferol
7	12.09	14.80	C15H10O5	Apigenin
Total		100.0		

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No .	Compound	Retention Time	Area	Formula	Chemical Structure
	Name	[min]	[%]		
1	Chlorogenic acid	2.54	100.00	C ₁₆ H ₁₈ O ₉	HO. CO ₂ H HO. OH
2	Ferulic acid	4.32	100.00	C ₁₀ H ₁₀ O ₄	ОН ОСН3
3	Gallic acid	5.94	100.00	C7H6O5	но но соон
5	Rutin	8.20	100.00	C27H30O16	
6	Qurcetine	9.92	100.00	C ₁₅ H ₁₀ O ₇	
7	Kaempferol	10.36	100.00	C15H10O6	НО ОН ОН ОН
8	Apigenin	12.19	100.00	C ₁₅ H ₁₀ O ₅	HO OH OH OH OH OH OH

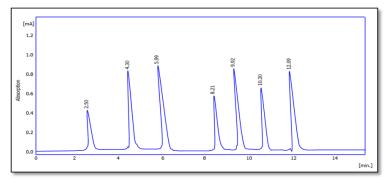


Fig. 4 HPLC study of sample separated polyphenol compounds of V. sessilis extract

Conclusion

Results of the present study showed the *V. sessilis* extracts have the presence of most of the phytochemicals (carbohydrates, alkaloid, flavonoids, tannins, polyphenols, glycosides, steroids, and saponins).

All hexane extract and two isolated groups of *V. sessilis.* possess an antioxidant activity by DPPH that increases with increasing the concentration of the extract as compared with the antioxidants (ascorbic acid) and was highest Polyphenol (VP) activity Whereas, the hexane extract recorded the lowest activity, and these Polyphenol extract (VP) may also exhibit strong antifungal action in addition to their well-known antioxidant activity.

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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