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Chemical composition and antioxidants of *Polystichum setiferum* (Dryopteridaceae)

Karzan O. Qader[1], Sahar A.A. Al-Saadi[2*] & Dhia F. A. Al-Fekaiki[3*]

[1] Department of Biology, College of Science, University of Sulaimani, Iraq.
[2] Department of Biology, College of Science, University of Basrah, Iraq
[3] Department of Nutrition Sciences, College of Agriculture, University of Basrah, Iraq.

[*]**Corresponding author email: K.O.Q.:** karzanqader65@gmail.com, **S.A.A.A.:** saharmalik2010@gmail.com, **D.F.A.:** dhia.alfekaiki@uobasrah.edu.iq

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Abstract: Antioxidant activity and chemical compounds of the alcoholic extract of *Polystichum setiferum* were studied in 2023. GC analysis indicated that *P. setiferum* had 45 chemical compounds, discovered the following primary constituents: n-Hexadecanoic acid (8.53%), 9, 12, 15-octadecatrienoic acid (7.42%), methyl mannofuranoside (6.82), oxirane (butoxymethyl)-(6.02%), cis-13-octadecenoic acid (5.61%), gamma-sitosterol (2.66%), and phytol (1.29%). The activity of the leaf that scavenges free radicals was measured by adding varying amounts of extract to 2,2-Diphenyl-1-Picrylhydrazyl. Six levels of *P. setiferum* leaf extracts (0, 25, 50, 100, 150, 200) µg.ml^{-1} were used to measure the inhibitory activity. The results showed that the radical scavenging action at 200 mg ml^{-1} concentrations was as powerful as at low concentrations. Determined the total antioxidant power of the test specimens by using equivalents of ascorbic acid per gram of leaf extract. The ascorbic acid was ($y = 0.3171x + 44.758$, $R^2 = 0.8883$). The outcomes of *P. setiferum* were ($y = 0.3061x + 46.716$, $R^2 = 0.8788$). Based on their high IC50 values, the species with the widest concentrations had the best antioxidant activity. The best performance was seen in ascorbic acid (IC50 = 5.90 µgml^{-1}), which was significantly higher than *P. setiferum* (3.73 µgml^{-1}).

Keyword: Antioxidant, Ferns, GC-MS analysis, Hexadecanoic acid. *Polystichum setiferum*.

Introduction

Around 12,000 different types of pteridophytes, including ferns exist worldwide (Fons *et al*., 2010; Yeou, 2015). Experts have not paid enough attention to the phytochemical analysis and biological activities of ferns, despite their numerous medicinal uses and ability to treat ailments (Bahadori *et al.,* 2015). Some kinds of ferns reproduce entirely by spores; they have neither seed nor flowers. Most ferns referred to as fiddleheads have distinctive leaf morphology while they are young. When the fiddleheads ripen, they will gradually transform into beautifully split fronds. Ferns are abundant in naturally occurring antioxidants, and the flavonoid, phenolic, alkaloid, and terpene families make up most of their bioactive constituents (Yeou, 2015). Researchers have conducted several studies on ferns to identify alkaloids, phenolics, glycosides, terpenes, and amino acids (Soeder, 1985; Widén *et al*., 2001; Fons *et al*., 2010), as well as volatile organic

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compounds (Imbiscuso *et al*., 2009). The main essential oils were caryophyllene, α-terpineol, nerolidol, linalool, and pinenes with a significant quantity of derivatives of the carotenoid type among all ferns (Fons *et al*., 2010).

The fern *Polystichum setiferum*, which belongs to the Dryopteridaceae family, is found in the Atlantic, Europe, Africa and sub-Mediterranean regions (Tlałka *et al.,* 2021). The fronds of big evergreen *P. setiferum* are dark green and preference grows in shade and moisture regions (Froissard *et al*., 2011). Among the 31 essential components of *P. setiferum* are a-pinene, and 3-hexenoic acid. According to Saleem *et al.* (2021), the primary components of *P. lonchitis* L. were α-galactopyranoside and n-hexadecanoic acid. Additionally, glycoside and flavonoids such as quercitrin were extracted from seven different kinds of ferns (Gyeltshen *et al.*, 2022). Twenty chemicals were found in *P. lonchitis*, these chemicals were made up of fructose, phytol, 9,12-octadecadienoic acid, L-proline, n-hexadecenoic acid. Additionally, caryophyllene oxide and succinic acid (Sher *et al.,* 2023).

Some species of *Polystichum* are used as food, and others have been used with several medicinal implications, including treating Meniere's syndrome and as anthelmintic, schistosomicidal, antihemorrhagic, cholagogue, anti-rheumatism, and regenerating agents (Dalli *et al*., 2007). *P. lonchitis* methanolic extracts have been found by Sher *et al*., (2023) to possess analgesic, antioxidant, antipyretic, and antispasmodic properties.

The goal of the current research was to chemically analyze *P. setiferum* leaves extracts using GC-MS spectroscopy and find out how effective they were as antioxidants in the lab.

Materials & Methods

Plant collection

Leaves *P. setiferum* were collected in 2024 from Basrah, Iraq. Then the plant was identified using information from Iraq's flora. The air-drying of sampled leaves followed.

Plant extraction

Using the Harborne (1984) method with some modifications, by mixing 250 ml of 70% ethyl alcohol with 25 g of plant powder. The mixture was butting on a magnetic stirrer for 24 hours prior to the filtering out of the ethanol extract. Concentrating the extract was done using a rotary evaporator and fully drying it at 30 degrees Celsius. The extracts were placed in tubes and stored frozen until needed.

DPPH test for measuring antioxidant activity

Evaluated the antioxidant activity of *P. setiferum* leaves using the DPPH test, with minor modifications (Hatano *et al.,* 1988; Abd-Alrasoul *et al*., 2022). *P. setiferum* extract was made at different dilutions with methanol (0, 10, 25, 100, 150, 200) µg ml^{-1} by using three replicates. The methanol (100 ml) was used to dissolve 0.004 mg of DPPH. A spectrophotometer was to measure 517 nm absorbance against control after 30 minutes of room temperature incubation. After 30 minutes of room temperature incubation, using a spectrophotometer, the 517 nm absorbance was determined in comparison to the control. The extract's 50% inhibitory concentration was discovered using the calibration curve, which also indicated the antiradical activity as the IC50 of DPPH and the antioxidant actions % determined by formula:

Activity of antioxidant % = (A control– A sample) ÷A control) x 100

Determine of total contents of *P. setiferum*.

The ultra-gas chromatograph Shimadzu GC-QP 2010 performed a GC-MS scan. Temperature adjustments for the GC oven were configured to operate at 50°C to 260°C at five °C/minute. The use of helium. The rate of column flow was 1 mL min^{-1}, and the rate of purge flow was 3 $mL.min^{-1}$, all at 280°C using the split injection temperature mode. The MS scans were conducted at two different temperatures: 200°C and 280°C at the interface. set the scan speed to 1666 N2, starting at 50 $m.z^{-1}$ and ending at 800 $m.z^{-1}$. Discovered *P. setiferum* chemical compounds via comparison their spectrum for those of recognized chemicals kept within the library at NIST (2005).

Statistical analysis

The statistical software SPSS v17 was used to produce all the results from the analyses of variance (ANOVA) that were conducted on the data. The means were compared with a probability of 0.05.

Results & Discussion

The chemical makeup of the leaves of *P. setiferum*.

The GC-MS analysis of *P. setiferum* extract showed 45 different chemical components (Fig 1; Table 1). The major components were found to be: n-Hexadecanoic acid (8.53%), Hexamethyl -7, 12– di (3', 5', 5' -trimethyl hexyl) octadecane (8.33%), Octadecatrienoic acid (7.42%), Methyl mannofuranoside (6.82%), Oxirane, (butoxymethyl)-(6.02%), cis-13-Octadecenoic acid (5.61%), gamma. -Sitosterol (2.66%), Phytol (1.29%). The current results agree with some species of *Polystichum.* The main constituents of compounds in *P. tripteron* leaves were phytol, hexadecanoic acid, 9,12,15-octadecatrienoic acid, stigmasterol, and 9,12-octadecadienoic acid (Yu & Wen-yi, 2008). Saleem *et al.* (2020) who reported that *P. braunii* 9,12,15-Octadecatrienoic acid, methyl- ester (10.644%) and 10-Octadecenoic acid - methyl ester (44.385), Furthermore, the results align with the findings of Sher *et al*., (2023), who found that the concentrations of n-hexadecenoic acid, alpha-linoleic acid, and phytol were 0.621. By separating the bioactive components, we can produce novel medications with fewer adverse effects. The results clearly show that ferns are beneficial against a variety of diseases, offering them the potential to be a cure.

A phytol compound is a significant substance with cytotoxic, antibacterial, and antioxidant characteristics documented (Wei *et al*., 2011). There are several main phytochemicals in plants. Hexadecenoic acid methyl ester is one of them because of their bactericidal activity (Preethi *et al*., 2010), the methyl form of α-d-galactopyranoside can help with diabetes and is an antioxidant (Kumar *et al*., 2015), n-hexadecanoic acid is an antioxidant and an anti-inflammatory (Aparna *et al*., 2012; Al-Shammary *et al*., 2013; Amran *et al*., 2024) 9,12-octadecadienoic acid, methyl ester, can help with inflammation, and it can fight cancer (Yu & Wen-yi, 2008). Several studies have shown that 7-dehydrodiosgenin can kill bacteria, fungi, cells, microbes, and free radicals (Karabay-Yavasoglu *et al.,* 2007; Sher *et al*., 2023). According to Saleem *et al*., (2021) n-hexadecenoic acid possesses antioxidants, analgesic, and anti-inflammatory properties, along with antipyretic and antispasmodic properties.

Table (1): *P. setiferum* leaf compounds discovered by GC-MS analysis.

	Compounds	Area %	Retention time
1	Ethanedioic acid, dimethyl ester	1.09	3.845
2	Pentadecafluorooctanoic acid, tridecyl ester	0.42	5.903
3	1-Hexadecanaminium, N, N, N-trimethyl-, octadecenoate	0.57	5.970
4	Platinum, tetrakis(triethylphosphine)-, (t-4)	0.56	6.047
5	hexadecanoic acid, 1,6-hexanediyl ester	0.67	8.232
6	2,4-Hexadiyne-1,6-diol	2.60	10.431
7	4-Vinylphenol	1.54	10.618
8	2-Pentene, 2,3,4-trimethyl-	0.80	12.567
9	2- Oct, 1-en-3- yloxy- 6- [(3, 4, 5- trihydroxyoxan -2-yl)	0.27	12.762
10	Nonahexacontanoic acid, propyl ester	7.97	13.733
11	Octadecane, 2,2, 4,15,17,17- hexamethyl -7, 12- bis (3,5,5-	8.33	14.008
12	Oxirane, (butoxymethyl)-	6.02	14.069
13	Spiro (4.5) Isopropyl decan-7-one, 1,8-dimethyl-8,9-epoxy	1.11	15.858
14	Imidazole, 2-fluoro-5-hydroxy-1-ribofuranosyl-	1.07	15.938
15	Diethyl Phthalate	3.24	16.044
16	Hexadecane, 1,1-bis(dodecyloxy)-	4.66	16.116
17	. alpha. -Methyl mannofuranoside	6.82	16.351
18	Tetradecanoic acid	0.29	18.448
19	Imidazole, 2-fluoro-1-tribenzoylribofuranosyl-	1.26	19.464
20	Neophytadiene	1.17	19.598
21	Hexadecanoic acid, methyl ester	1.55	20.401
22	n-Hexadecanoic acid	8.53	20.739
23	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	1.85	22.170
24	10-Octadecenoic acid, methyl ester	0.39	22.227
25	Phytol	1.29	22.411
26	9,12,15-Octadecatrienoic acid (alpha- Linoleic acid)	7.42	22.509
27	cis-13-Octadecenoic acid	5.61	22.557
28	cis-5-Dodecenoic acid	4.49	22.604
29	Octadecanoic acid	4.31	22.821
30	Rhodium, [1,2-bis (. eta.2-ethenyl)	0.83	22.904
31	1, E-11, Z-13-Octadecatriene	0.50	23.311
32	cis-5,8,11-Eicosatrienoic acid, methyl ester	0.49	23.724
33	trans-1,4-Cyclohexanedimethanol, bis(heptafluorobutyrate)	0.25	24.288
34	Cyclohexanone, 4-hydroxy-	0.21	24.737
35	Hexadecanoic acid, 2 -hydroxy-1, (hydroxymethyl) - ethyl	1.82	25.851
36	Methyl 11-oxo-9-undecenoate	0.98	26.275
37	Tristearin	0.30	26.507
38	Rhodium, bis (eta.2- ethenyl) - 4- ethenylcyclohexane] di-. mu.	1.13	27.348
39	1-Hexadecanaminium, N, N, N-trimethyl-, octadecanoate	0.26	27.901
40	5-Cholestene-3-ol, 24-methyl-	0.88	28.064
41	Octaethylene glycol monododecyl ether	0.20	28.158
42	. alpha. -Tocospiro A	1.26	28.910
43	. alpha. -Tocospiro B	1.64	29.128
44	14-Methylcholest-8-en-3-one	0.73	29.317
45	. gamma. -Sitosterol	2.66	29.839
		100.00	

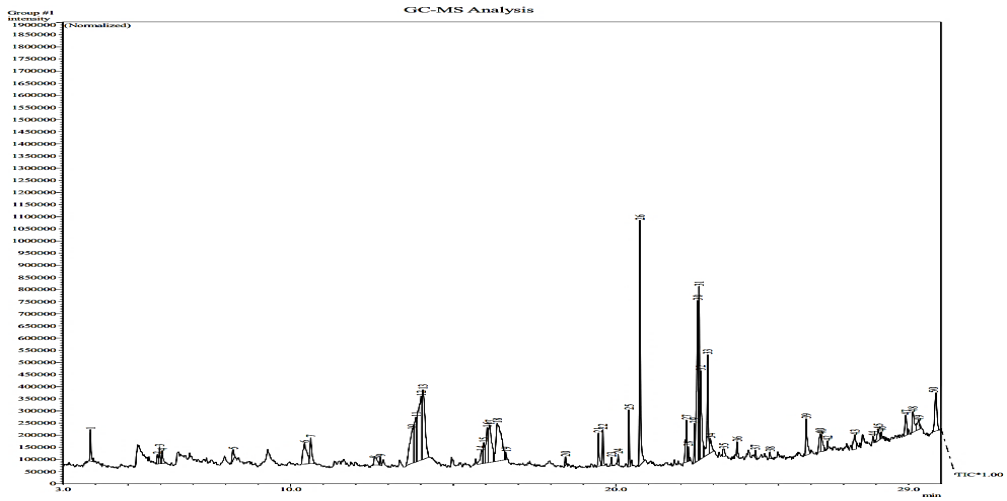

Fig. (1): Chromatogram of *P. setiferum* leaves.

***P. setiferum* antioxidant testing utilizing DPPH radical scavenging:**

By adding varying quantities of DPPH, the alcoholic extract of *P. setiferum* leaves were determined. It was shown that the process of scavenging radicals at a 200 µg.ml^{-1} concentration was just as strong as it was at lower concentrations (Fig 2). At 200 µg ml^{-1}, *P. setiferum* was shown to have an inhibitory percentage of 98%, whereas normal ascorbic acid had a percentage of 99% (Fig 2).

The test samples' overall antioxidant capacity was calculated using ascorbic acid equivalents of leaf extract. The results showed that ascorbic acid was ($y = 0.3171x + 44.758$, $R^2 = 0.8883$), while *P. setiferum* was ($y = 0.3061x + 46.716$, $R^2 = 0.8788$) (Fig 3 & 4). Our findings showed that as the concentration of the *P. setiferum* extract grew, so did its ability to scavenge DPPH radicals. This could be because of the confirmed discovery that certain chemical molecules have antioxidant properties (Vuong *et al*., 2013; Sultan *et al*., 2019; Tahir & Hasan, 2021). Moreover, free radicals can damage cellular membranes, changing their permeability and allowing the escape of intracellular materials, they can also damage cellular organelles, including mitochondria, which are vital for the production and survival of cellular energy (Sravani & Sunitha, 2023).

IC 50 essay:

The antioxidant capacity of *P. setiferum* at various concentrations was shown using their IC50 values, where all data were compared to the IC50 of traditional ascorbic acid (Fig 3).

A plant's antioxidant property increases with a lower IC50. The neutralization of this radical was observed at 200 µg ml^{-1}. *P. setiferum* (3.73 µg ml^{-1}) had the best ability to get rid of DPPH radicals, while ascorbic acid (5.90 µg ml^{-1}) was the second-best choice based on other studies. Not much research has been done on testing the antioxidant activity of *Polystichum* species. The study by Valizadeh *et al*., (2016) found that *P. aculeatum* is a useful antioxidant, having an IC50 of 0.45 ± 0.02 µg ml^{-1}. Saleem *et al.*, (2020) reported that *P. braunii*'s IC_{50} was greater than 1000 µg ml^{-1}. In the DPPH test, the aerial portions of *P. squarrosum* were very good at getting rid of free radicals (IC50, 6.03 µg ml$^{-3)}$ (Khulbe *et al*., 2022). *P. lonchitis* ethanol extract has an antioxidant activity of 1.5 mg ml^{-1} (Sher *et al.,* 2023). According to Carranza *et al.* (2019), while *C. ramosii* extract

has an IC50 = 0.306 mg ml^{-1}. *C. ramosii* contains 17.86% phytol and n-hexadecanoic acid 29.03% were the constituents of the extracts. The presence of the different chemical components in plants causes free radical scavenging (Qader *et al*., 2018; Saleem *et al.,* 2020; Alsaad & Mohammed, 2021; Althuluth *et al*.,2024) these may have been the primary bioactive compounds. So, these extracts protect the cell from oxidative stress by scavenging radicals; as a result, a lower IC50 result in a higher level of antioxidant features in plants (Al-Saadi *et al.,* 2017; Al-Harbi, 2019; Ait-yahia *et al.,* 2018; Abd-Alrasoul *et al*., 2022).

Reduced IC50 values for the ethanolic extracts of *P. setiferum* suggested that they exhibited significant antioxidant activity. These findings highlight the potential of these ferns in the development of novel therapies for a variety of illnesses linked to oxidative stress (Sravani & Sunitha, 2023).

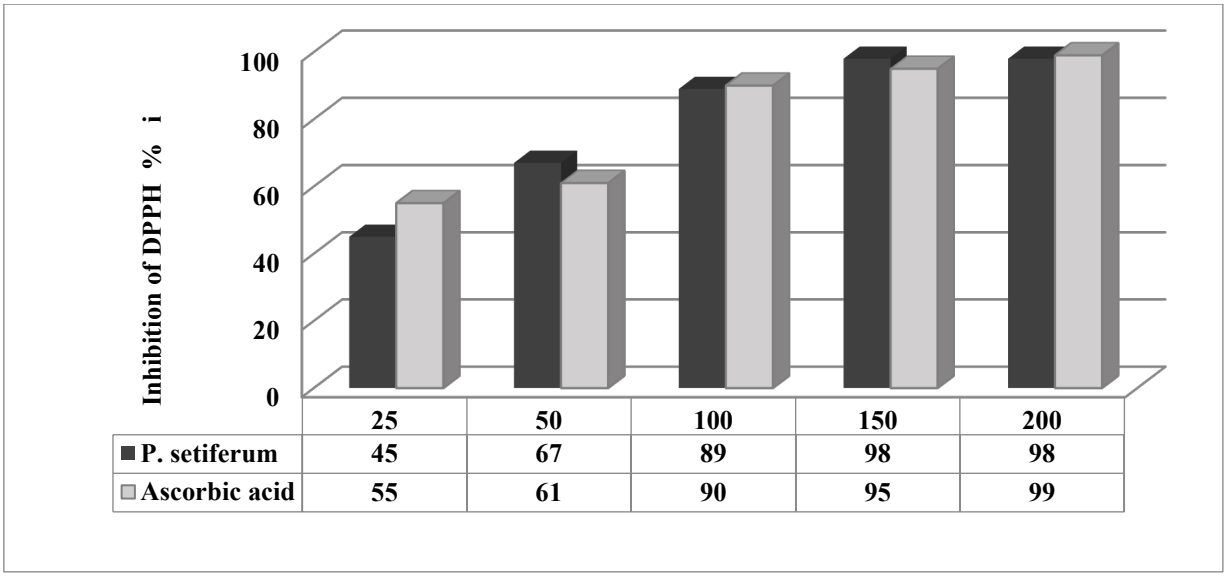

Fig. (2): The percentage inhibition of extracts from *P. setiferum* leaves by the antioxidant ascorbic acid.

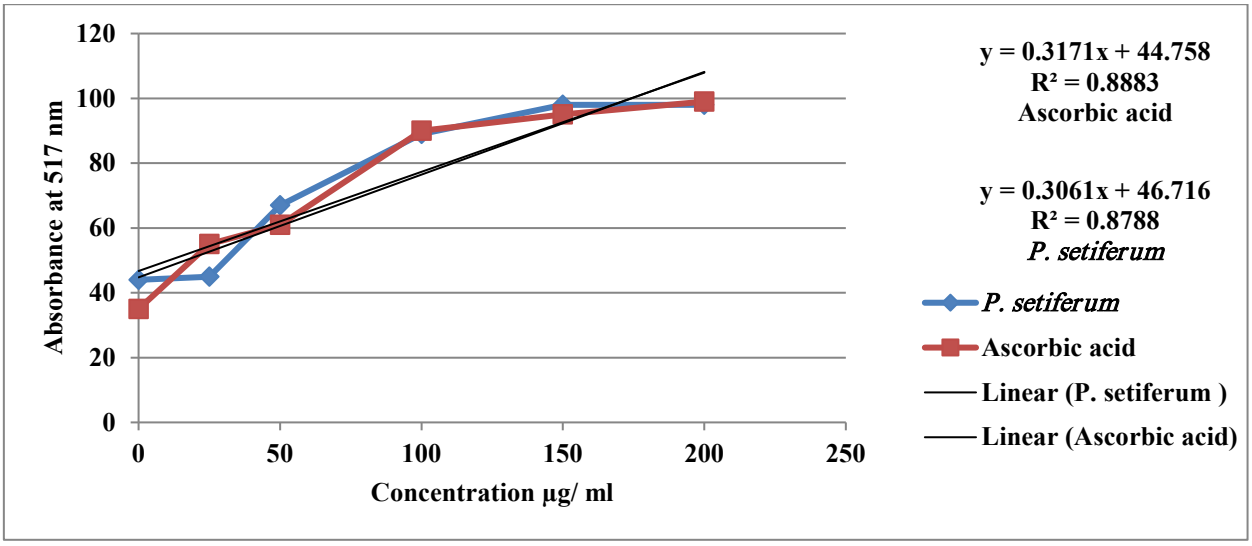

Fig. (3:) The calibration curve for the percentage suppression of the free radical by ascorbic acid and *P. setiferum* leaves.

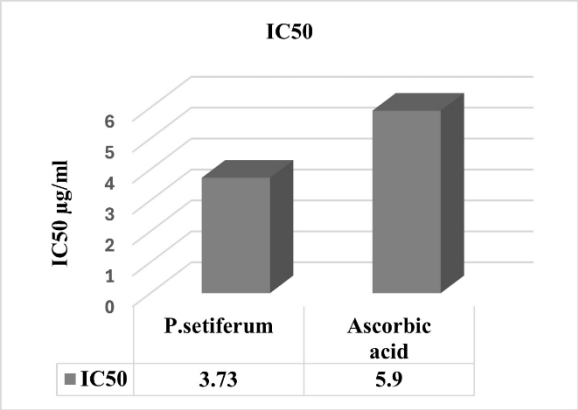

Fig. (4): Ascorbic acid and *P. setiferum* extracts' IC50 values.

Conclusion

Because *P. setiferum* extracts are excellent at preventing pharmacological effects, researchers are investigating its phyto-drugs, and antioxidant qualities. This is because bioactive substances such as n-hexadecanoic acid Hexamethyl -7, 12– di (3', 5', 5' -trimethyl hexyl) octadecane, Octadecatrienoic acid, and Methyl mannofuranoside are present. These findings revealed the potential avenues for future investigation into the bioactive components of fern plants, which could lead to the development of more potent medications with fewer adverse effects and more effective anti-disease properties

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Contributions of authors

K.O.Q: Collection of specimens. Laboratory techniques and identification of the plant.

S.A.M.A.: Suggestion the proposal of the article, Laboratory techniques and identification of the plant. Also, wrote and revised the manuscript,

D.F. A.: Suggestion the proposal of the article, Laboratory techniques., GC-MS analysis.

ORCID

K.O. Q.: https://orcid.org/0000-0002-9173-3001

S. A. A.: https://orcid.org/0000-0003-2782-3907

D. F. A.: https://orcid.org/0000-0002-7510-5881

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this research.

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دراسة المركبات الكيميائية والفعالية المضادة للأكسدة في النوع *Polystichum setiferum* (Dryopteridaceae)

كارزان عمر قادر[1] وسحر عبد العباس مالك السعدي[2] وضياء فالح الفكيكي[3]

[1]قسم علوم الحياة، كلية العلوم، جامعة السليمانية، العراق

[2]قسم علوم الحياة، كلية العلوم، جامعة البصرة، العراق

[3]قسم علوم الأغذية، كلية الزراعة، جامعة البصرة، العراق

المستخلص: تضمنت الدراسة تحليل وتشخيص المركبات الفعالة والتاثير المضاد للاكسدة للمستخلص الكحولي للسرخس *Polystichum setiferum* خلال العام 2023. اذ شخصت المركبات الكيميائية بتقنية الغاز المتصل بمطياف الكتلة GC–MS. واتضح من النتائج إلى أن النوع *P. setiferum* يحتوي على 45 مركبًا كيميائيًا، وأن اعلى نسبة مئوية سجلت في المركب n–Hexadecanoic وبلغت 8.53 %، يليه المركب 9, 12, 15–octadecatrienoic acid (7.42%) و methyl mannofuranoside (6.82%)، و–(butoxymethyl) oxirane (6.02%) و cis–13–octadecenoic acid (5.61%) أما gamma–sitosterol بلغ (2.66%) والفيتول phytol (1.29%). قدرت الفعالية المضادة للاكسدة باستخدام (DPPH) 2,2– Diphenyl–1–picrylhydrazyle واستخدمت ست تراكيزمختلفة من أوراق النوع *P. setiferum* (0، 25، 50، 100، 150، 200) ميكروغرام مل$^{-1}$ وبينت النتائج كلما ازداد التركيز زادت الفعالية التثبيطية للمستخلص الكحولي في كسح الجذر الحر DPPH. وأن اعلى قيمة سجلت عند التركيز 200 مايكروغرام مل$^{-1}$. وقد حددت الفعالية المضادة للاكسدة بالمقارنة مع حامض الاسكوربيك سجل($y = 0.3061x +$ 0.8883) بينما في النوع *P. setiferum* سجل ($y = 0.3171x + 44.758$, $R^2 = 0.8788$) 46.716. واستنادًا إلى قيم IC50 فإن االنوع ابدى فعالية مضادة للاكسدة أفضل من عينة السيطرة حامض الاسكوربيك اذ بلغت في حمض الأسكوربيك (IC50 = 5.90 مايكروغرام مل$^{-1}$) والذي كان أعلى من النوع *setiferum*.P (3.73 مايكروغرام مل$^{-1}$).

الكلمات المفتاحية: مضادات اكسدة، سراخس، تحليلGC–MS، Hexadecanoic acid، *Polystichum setiferum*.