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Original Article

Detection of Active Chemical Compounds in Lycium Shawii Plant Using GC-MS Technique and Their Evaluation as Antioxidant

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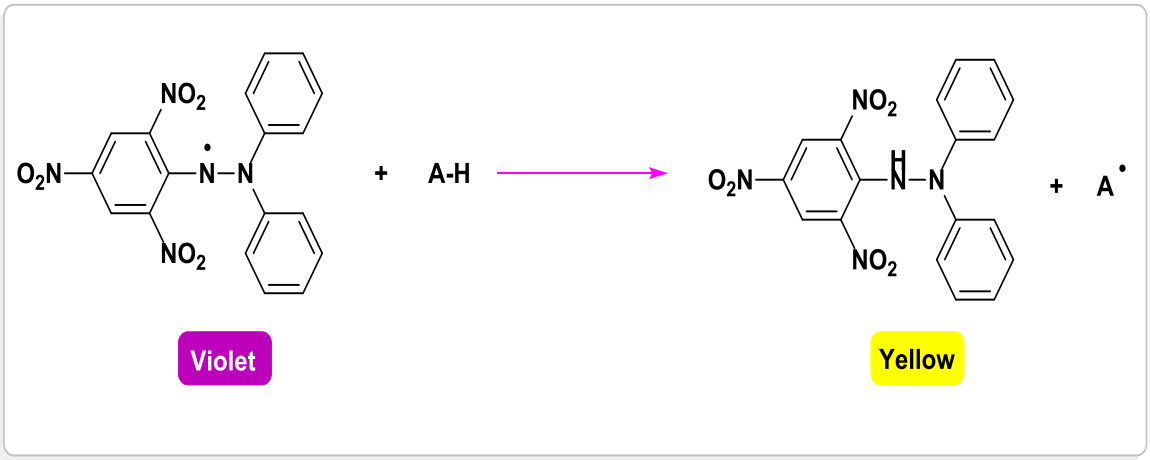
ABSTRACT

Lycium shawii is considered a thorny perennial and wild plant. Bramble usually grows in dry and hot lands because this plant lives on moisture. The height of the plant reaches one and a half meters, sometimes up to two meters, and is considered as a deciduous shrub whose leaves fall off during the months of July and August, and sometimes it continues until September in two types, L.edgworthii and L.dasystemum, while in the rest of the other species, it continues until December and may continue until February. Using the GC-MS technology has emerged many active compounds for Lycium shawii from these compounds are Butyn-1-ol, Carbonic acid, dimethyl ester, Carbonic acid, dimethyl ester, Ethene, methox, Diazene, dimethyl, Diadimethyl, Hexadecenoic acid, methyl ester Pentadecanoic acid, methyl ester, -(ethenylthio), 9,12-Octadecadienoic acid, methyl ester, 6-Octadecenoic acid, methyl ester 9-Octadecenoic acid (Z), methyl ester Octadecenoic acid, methyl ester, Phytol methyl stearate methyl ester, Heptadecanoic acid, and 16-methyl and another compounds .The results showed an evaluation of the antioxidant activity of extracts (roots, leaves, and stems) of Lycium shawii plant. For the aqueous and alcoholic extract using DPPH, the aqueous extract of leaves is the best antioxidant than other extracts.

GRAPHICAL ABSTRACT



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Introduction

Lycium shawiiL is a perennial deciduous shrub with orange-red a nightshade plant with a long history in China and has been used in medicine and functional foods. Lycium shawii contains many nutrients, such as polysaccharides, phenolic acid, carotene, betaine, and flavonoids, which possess many advantages, such as antioxidant, anti-radiation, anti-cancer, anti-aging properties, promoting hematopoiesis, brightening eyes, etc. [1]. Likewise, it is included in traditional Chinese Medicines [2]. By reviewing studies related to the use of Lycium shawii in the treatment of diabetes, it was concluded that Lycium shawii has the potential to be an effective treatment for diabetes and is an excellent alternative to chemical drugs as it does not contain any harmful effects [3]. Many studies have confirmed that food additives rich in antioxidants play an important role [4, 5]. In the prevention and treatment of many diseases, as the fruits of the Lycium shawii plant contain sugars, vitamins C and E and carotenoids, flavonoids, and betain, which are effective antioxidants that work to curb free radicals It increases the activity of enzymatic antioxidants [6, 7], and thus reduces the oxygenation of cells that cause apoptosis [8], studies indicated that alcoholic extract the leaves of Lycium shawii plant enhance antioxidants because they contain many phenolic compounds such as Chlorogenic acid and rutin, found the stems of the plant contain many compounds that act as antioxidants, such as vitamin E and flavanols such as Quercetin-3-O-rutinoside [9]. Studies have confirmed that bramble contains many mineral elements, the most important of which are potassium, sodium, phosphorus, magnesium, calcium and iron [10], and also contains organic acids [11].

Materials and Methods

Ascorbic acid, Na_2EDTA, ammonium acetate, glacial acetate acid, acetyl acetone, trichloro acetic acid, potassium dihydrogen phosphate, sodium phosphate dibasic, DMSO, and DPPH, ethanol alcohol were purchased form Sigma-Aldrich. The plant was collected from the agricultural areas in Diwaniyah.

Preparation of Lycium shawii is shown in Figure 1 [12].

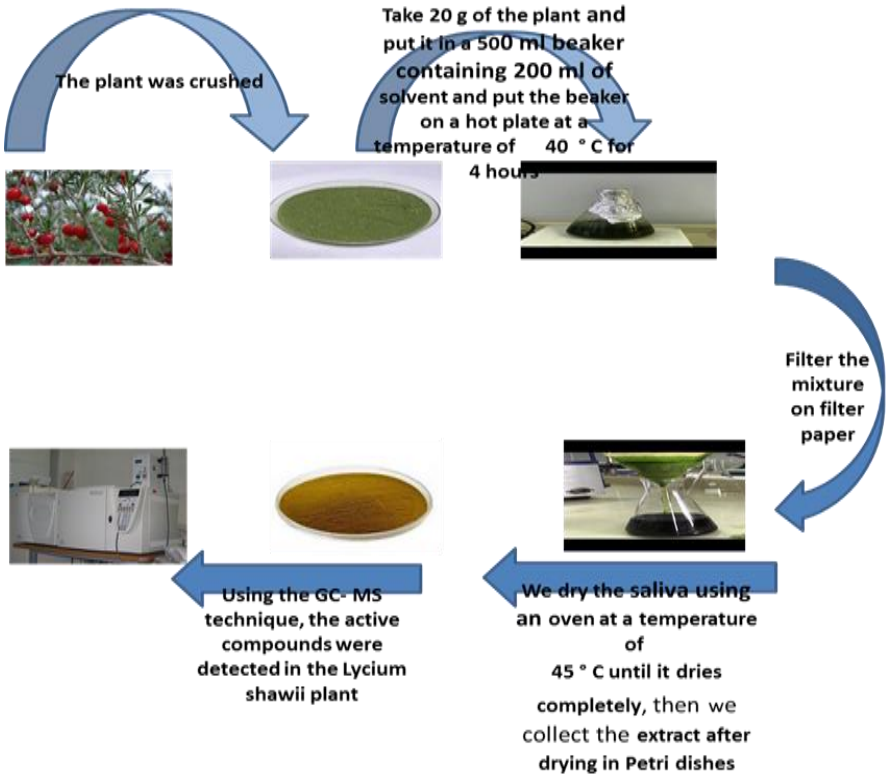

Figure 1: Steps to access the GC-MS technology [13]

Evaluation of antioxidant activity of extracts (roots, leaves, and stems) of Lycium shawii plant is depicted in Figure 2 [14].

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of the extract was carried out by Inbathamizh method [15, 16].

Procedure and principle

Various concentrations of Lycium shawii extracts were added to 1.0 ml of Na_2EDTA solution prepared by dissolving 0.13 g of ferrous ammonium sulfate and 0.26 g of Na_2EDTA in 100 ml of water and mixed with 1.0 ml of dimethyl sulfoxide 0.85%, and then the mixture was added to 0.1 M phosphate buffer (PH 7.4) to initiate the reaction by adding 0.5 ml of 0.22% ascorbic acid. The reaction mixture was kept in a water bath at 90 °C for 15 min and the reaction was quenched by adding 1.0 ml of 7.5% trichloroacetic acid. After that, 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid and 2 ml of acetyl acetone in 1.0 L of water) was added to all the test tubes and incubated for 15 min for color development. The mixture was obtained without ascorbic acid served as control. Absorbance was observed at 412 nm.

Results and Discussion

Lycium shawii root aqueous extract sample

Lycium shawii root aqueous extract sample show in Figure 3 and Table 1 show the compounds of the root aqueous extract.

Lycium shawii root ethanolic extract sample

Lycium shawii root ethanolic extract sample show in Figure 4 and Table 2 show the compounds of the root ethanolic extract.

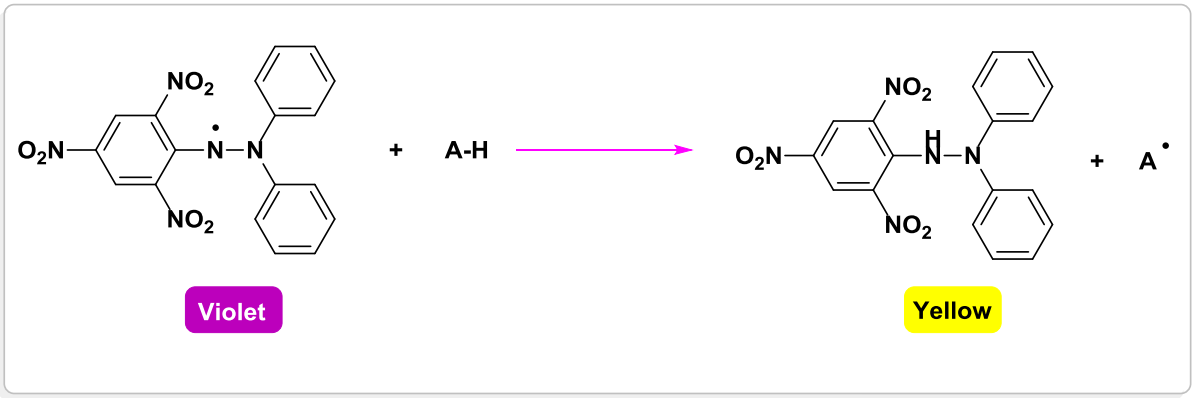

Figure 2: Reaction of DPPH with AH [15]

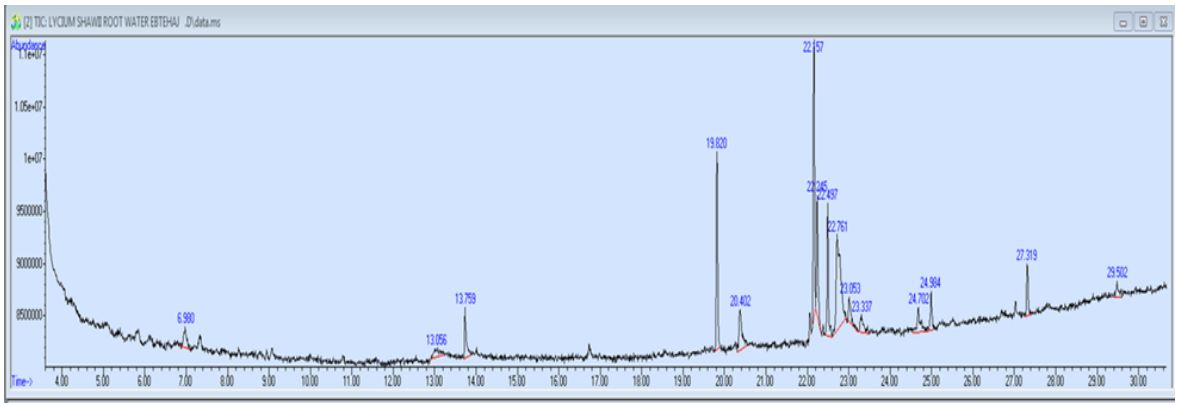

Figure 3: GC-MS results of root aqueous extract

Table 1: Compounds of the root aqueous extract

PK	RT	Area%	Compounds
1	6.98	3.05	Cis-Aconitic anhydride Pyrimidine, 4,6-dimethoxy-5-nitro Butyn-1-ol
2	13.056	2.44	3-Butyn-1-ol Diazene, dimethyl- Butyn-1-ol 3-
3	13.757	4.15	Cetrimonium_Bromide Dimantine Benzyldimethylhexadecylammonium chloride
4	19.816	12.43	Hexadecanoic acid, methyl ester
5	20.404	5.90	2-Pyrrolidinethione 1,2,2,3,4-Butanepentacarbonitrile Spirohexanone, 5,5-dichloro-4
6	22.161	16.87	9-Octadecenoic acid, methyl ester 11-Octadecenoic acid, methyl ester trans-13-Octadecenoic acid, methyl ester
7	22.248	8.10	Heptanedioic acid, dimethyl ester
8	22.499	8.86	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
9	22.758	19.33	Pyrazine, ethoxy Propanedioic acid, 2-propenyl-, dimethyl ester Spirohexan-4-one, 5,5-dimethyl
10	23.053	2.76	Pyrrolidinethione 2- 1,2,2,3,4-Butanepentacarbonitrile
11	24.706	4.21	3-Butyn-1-ol Pyrimidine, 4,6-dimethoxy-5-nitro Ethanedicarboxamide, N-allyl-N'-(2,5-Dimethylphenyl)
12	24.983	2.96	Heptanedioic acid, dimethyl ester
13	27.320	3.78	Heptanedioic acid, dimethyl ester Spiro[2.3]hexan-4-one, 5,5-dichloro-6-methyl
14	29.501	2.23	Heptanedioic acid, dimethyl ester

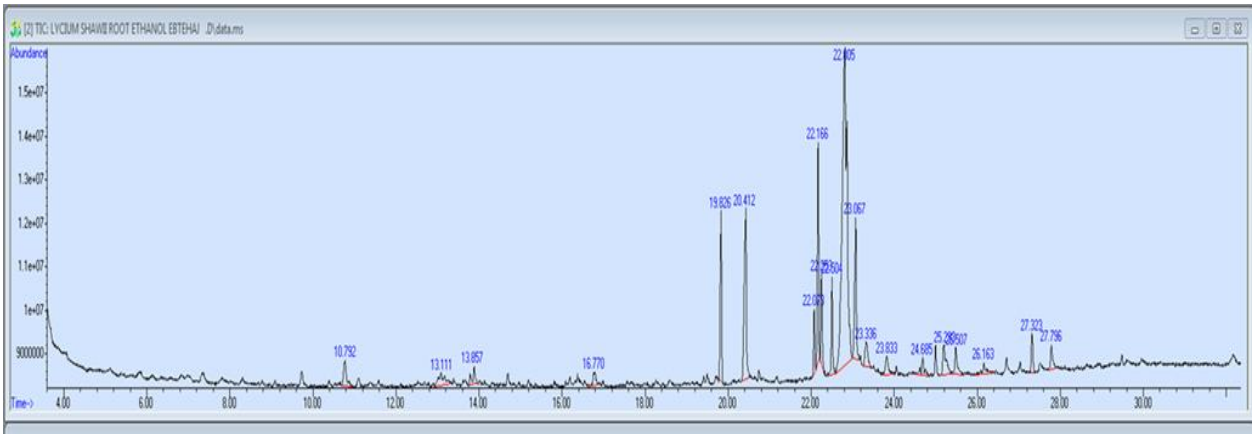

Figure 4: GC-MS results of root ethanolic extract

Table 2: Compounds of the root ethanolic extract

PK	RT	Area%	Compounds
1	13.108	2.05	Diazene, dimethyl 4-Spirohexanone, 5,5-dichloro
2	13.861	1.30	Diazene, dimethyl Ethene, methoxy
3	16.769	1.20	Dimenhydrinate 2-Propanamine, N-methyl dodecyl ester Fumaric acid, 2-dimethylaminoethyl
4	19.824	5.78	Hexadecanoic acid, methyl ester Pentadecanoic acid, methyl ester
5	20.413	9.54	N-Hexadecanoic acid Pentadecanoic acid
6	22.075	2.33	9,12-Octadecadienoic acid (Z,Z) 9,12-Octadecadienoic acid, methyl ester 9,12-Octadecadienoic acid (Z,Z),methyl ester
7	22.170	8.03	9-Octadecenoic acid, methyl ester,(E) 11-Octadecenoic acid, methyl ester Trans-13-Octadecenoic acid, methyl ester
8	22.256	3.40	)9-Octadecenoic acid, methyl ester,(E 11-Octadecenoic acid, methyl ester 9-Octadecenoic acid(Z), methyl ester
9	22.507	3.63	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
10	22.802	41.14	Oleic Acid 9-Octadecenoic acid, (E)
11	23.070	6.24	Octadecanoic acid
12	23.832	1.46	Spirohexan-4-one, 5,5-dimethyl Spirohexan-5-one) 1H)-Pyrimidinone, 2-(butylthio(4
13	25.234	2.61	N-Trifluoroacetylimidazole 3-Butyn-1-ol
14	25.511	1.76	1,2,2,3,4-Butanepentacarbonitrile
15	27.320	1.57	Docosanoic acid, methyl ester Nonadecanoic acid, methyl ester Methyl stearate

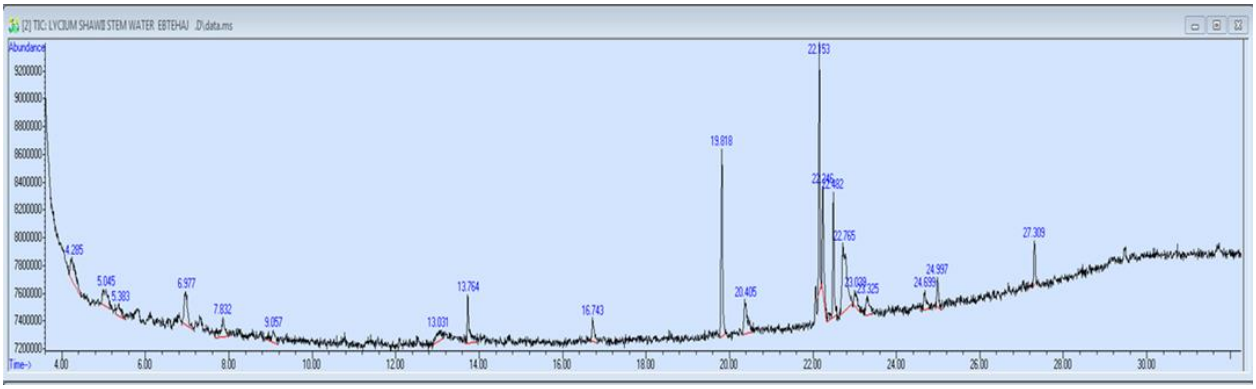

Figure 5: GC-MS results of stem aqueous extract

Lycium shawii stem aqueous extract sample

Lycium shawii stem aqueous extract sample show in Figure 5 and Table 3 show the compounds of the stem aqueous extract.

Lycium shawii stem ethanolic extract sample

Lycium shawii stem ethanolic extract sample show in Figure 6 and Table 4 show the compounds of the stem ethanolic extract.

Table 3: Compounds of the stem aqueous extract

PK	RT	Area%	Compounds
1	4.288	4.93	3-Butyn-1-ol
2	5.041	3.36	Propanenitrile, 3-(6-bromo-3,4-met hylenedioxybenzylidenhydrazino)-3-oxo- Carbonic acid, dimethyl ester
3	5.378	1.96	Ethene, methoxy Carbonic acid, dimethyl ester
4	6.980	4.87	Urea Pyrimidine, 4,6-dimethoxy-5-nitro
5	7.828	3.65	Pyrimidine, 4,6-dimethoxy-5-nitro 3-Chloro-N-[2-methyl-4(3H)-oxo-3-q Uinazolinyl]-2-thianaphthenecarbox amide Heptane, 1-(ethenylthio)
6	9.057	2.03	3-Butyn-1-ol Heptane, 1-(ethenylthio)
7	13.030	2.40	4-Spirohexanone, 5,5-dichloro
8	13.765	4.58	Diazene, dimethyl
9	16.743	2.20	Diazene, dimethyl
10	19.815	11.85	Hexadecanoic acid, methyl ester Pentadecanoic acid, methyl ester
11	20.404	4.88	2-Pyrrolidinethione 1,2,2,3,4-Butanepentacarbonitrile Heptane, 1-(ethenylthio)
12	22.152	13.08	9-Octadecenoic acid, methyl ester, (E) 11-Octadecenoic acid, methyl ester Trans-13-Octadecenoic acid, methyl ester
13	22.248	7.21	Heptanedioic acid, dimethyl ester
14	22.481	8.06	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
15	22.767	13.36	Pyrazine, ethoxy 1-Carboxycyclopropane-2-acetic acid (E), dimethyl ester N-Methyl-2-[1,1-dicyano-2- (4-methoxyphenyl)-vinylimino]pyrrolidin
16	23.035	2.00	2-Pyrrolidinethione 1,2,2,3,4-Butanepentacarbonitrile
17	25.000	2.21	Heptanedioic acid, dimethyl ester 4-Bromo-N-[(2-pyridyl)aminomethyl] phthalimide)Heptane, 1-(ethenylthio
18	27.311	2.76	Heptanedioic acid, dimethyl ester Spiro[2.3]hexan-4-one, 5,5-dichloro-6-methyl

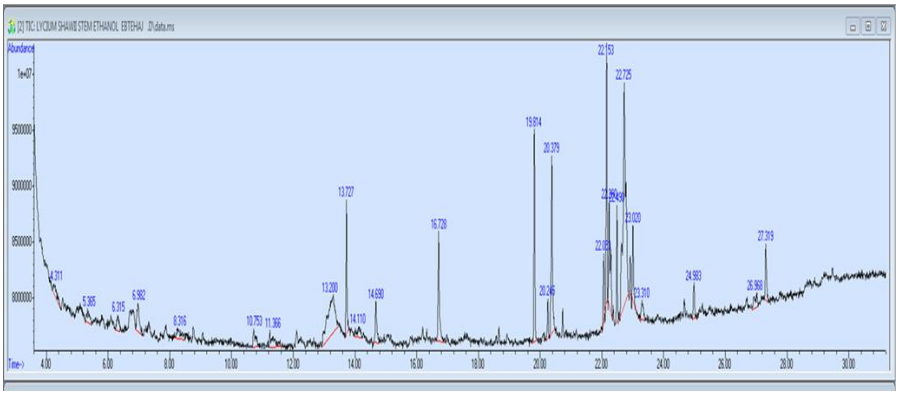

Figure 6: GC-MS results of stem ethanolic extract

Table 4: Compounds of the stem ethanolic extract

PK	RT	Area%	Compounds
1	4.314	1.32	3-Butyn-1-ol
2	5.361	1.19	Ethene, methoxy Diazene, dimethyl
3	6.313	1.24	Ethene, methoxy Diazene, dimethyl
4	6.980	2.41	Urea Cis-Aconitic anhydride
5	8.313	1.56	3-Butyn-1-ol Diazene, dimethyl
6	10.754	1.43	Spirohexan-4-one, 5,5-dichloro-6,6 –dimethyl
7	11.368	2.09	Pyrazine, methoxy-, 1-oxide
8	13.203	9.99	3-Butyn-1-ol Carbonic acid, dimethyl ester
9	13.731	4.19	Dimethyl palmitamine Cetrimonium Bromide Dodecyltrimethylammonium bromide
10	14.112	1.23	2-Pyrrolidinethione
11	16.726	5.05	Tetradonium Bromide 1-Hexanol, 6-(dimethylamino)
12	19.816	7.08	Hexadecanoic acid, methyl ester Pentadecanoic acid, methyl ester
13	20.248	1.39	Phthalic acid, isobutyl octyl este Dibutyl phthalate
14	20.378	7.97	N-Hexadecanoic acid Pentadecanoic acid
15	22.049	1.97	Spirohexan-4-one, 5,5-dimethyl)1H)-Pyrimidinone, 2-(butylthio(4)1H)-Pyrimidinone, 2-(propylthio (4
16	22.153	8.07	)9-Octadecenoic acid, methyl ester, (E 11-Octadecenoic acid, methyl ester 9-Octadecenoic acid (Z)-, methyl ester
17	22.490	3.56	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
18	22.724	21.16	Oleic Acid Cis-9-Hexadecenoic acid Cis-Vaccenic acid
19	23.018	2.58	)Heptane, 1-(ethenylthio
20	26.965	1.40	Spirohexan-4-one, 5,5-dichloro-6,6-dimethyl
21	27.320	2.36	Heptanedioic acid, dimethyl ester

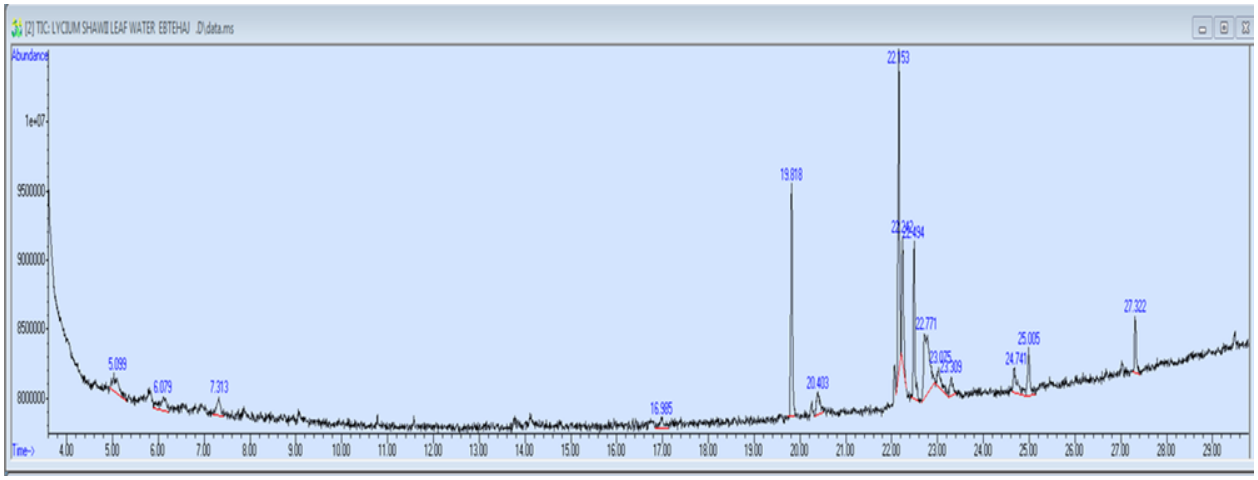

Figure 7: GC-MS results of leave aqueous extract

Table 5: Compounds of the leave aqueous extract

PK	RT	Area%	Compounds
1	5.101	4.15	Ethene, methoxy 3-Butyn-1-ol
2	7.317	2.68	4-Bromo-N-[(2-pyridyl)aminomethyl] phthalimide Heptanedioic acid, dimethyl ester Heptane, 1-(ethenylthio)
3	16.985	2.00	3-Butyn-1-ol
4	19.816	14.53	Hexadecanoic acid, methyl ester Pentadecanoic acid, methyl ester
5	20.404	3.24	2-Pyrrolidinethione 1,2,2,3,4-Butanepentacarbonitrile)Heptane, 1-(ethenylthio
6	22.152	19.03	9-Octadecenoic acid (Z)-, methyl ester 9-Octadecenoic acid, methyl ester, (E 11-Octadecenoic acid, methyl ester
7	22.490	10.01	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
8	22.767	14.53	1,2-Cyclobutanedicarboxylic acid, 3-methyl-, dimethyl ester
9	24.740	4.07	Pyrimidine, 4,6-dimethoxy-5-nitro
10	25.009	4.50	Heptanedioic acid, dimethyl ester

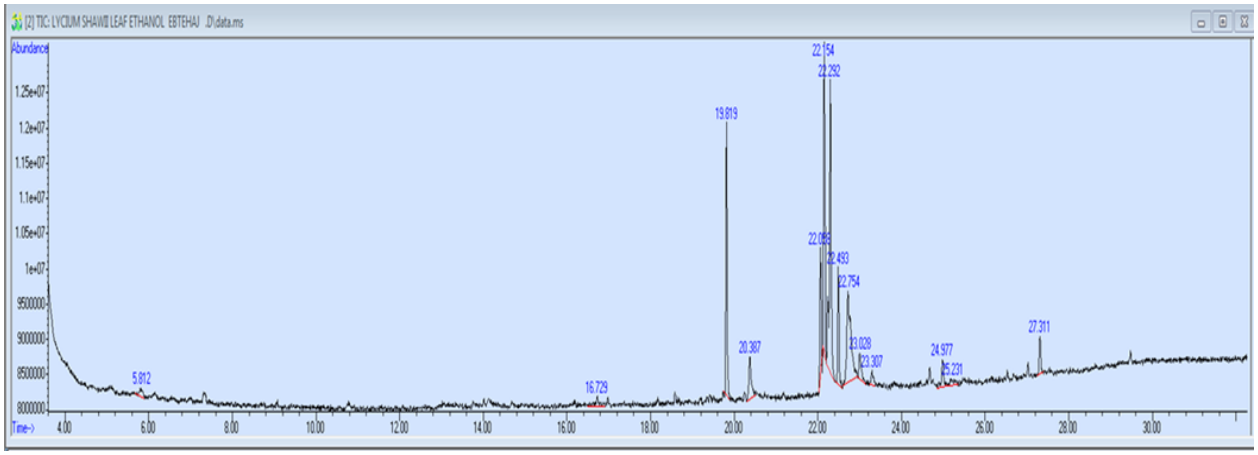

Figure 8: GC-MS results of leave ethanolic extract.

Table 6: Compounds of the leave ethanolic extract

PK	RT	Area%	Compounds
1	5.811	1.17	3-Butyn-1-ol Carbonic acid, dimethyl ester
2	16.726	1.74	Ethene, methoxy Diazene, dimethyl
3	19.816	14.71	Hexadecanoic acid, methyl ester Pentadecanoic acid, methyl ester
4	20.387	4.57	)Heptane, 1-(ethenylthio
5	22.057	6.21	9,12-Octadecadienoic acid, methyl ester)9,12-Octadecadienoic acid (Z,Z 9,12-Octadecadienoic acid (Z,Z)-,methyl ester
6	22.152	17.29	6-Octadecenoic acid, methyl ester 9-Octadecenoic acid (Z)-, methyl ester 11-Octadecenoic acid, methyl ester
7	22.291	22.12	Phytol
8	22.490	6.10	Methyl stearate Heptadecanoic acid, 16-methyl-, methyl ester
9	22.758	15.98	)1H)-Pyrimidinone, 2-(butylthio(4)1H)-Pyrimidinone, 2-(propylthio(4)1H)-Pyrimidinone, 2-(ethylthio(4
10	23.027	2.47	Heptane, 1-(ethenylthio)- N-Trifluoroacetylimidazole
11	23.304	1.38	Spirohexan-4-one, 5,5-dimethyl)1H)-Pyrimidinone, 2-(butylthio(4)1H)-Pyrimidinone, 2-(propylthio(4
12	24.974	2.62	Heptanedioic acid, dimethyl ester
13	25.234	1.25	Spirohexan-5-one
14	27.311	2.39	4-Bromo-N-[(2-pyridyl)aminomethyl] phthalimide

Lycium shawii leave aqueous extract sample

Lycium shawii leaves aqueous extract sample show in Figure 7 and Table 5 show the compounds of the leaves aqueous extract.

Lycium shawii leave ethanolic extract sample

Lycium shawii leaves ethanolic extract sample show in Figure 8 and Table 6 show the compounds of the leaves ethanolic extract.

Evaluation of the antioxidant activity of extracts (roots, leaves, and stems) of Lycium shawii

Results evaluation of the antioxidant activity of extracts (roots, leaves, stems) of Lycium shawii show in Table 7.

The results showed that the best antioxidant out of the six extracts used for the Lycium shawii was the aqueous extract of the leaves, where the most antioxidant appeared, and also as a result of appearance of the following active compounds in it using the GC-MAS technique are Ethene, methoxy, 3-Butyn-1-ol, 4-Bromo-N-[(2-pyridyl)aminomethyl] phthalimide, Heptanedioic acid, dimethyl ester, Heptane, 1-(ethenylthio), Hexadecanoic acid, methyl ester, Pentadecanoic acid, methyl ester, 2-Pyrrolidinethione, 1,2,2,3,4-Butanepentacarbonitrile, 9-Octadecenoic acid (Z)-, methyl ester, 9-Octadecenoic acid, methyl ester, (E), 11-Octadecenoic acid, methyl ester, Methyl stearate, Heptadecanoic acid, 16-methyl-, methyl ester, 1,2Cyclobutanedicarboxylic acid, 3-methyl-, dimethyl ester, Pyrimidine, 4,6-dimethoxy-5-nitro, Heptanedioic acid, and dimethyl ester.

Results evaluation of the antioxidant activity of extracts (roots, leaves, stems) of Lycium shawii show in Figure 9.

Table 7: Evaluation of the antioxidant activity of extracts (roots, leaves, stems) of Lycium shawii

Groups	S.D±mean	P-value
REAQ	59.28± 36.91	REAQ vs. REAL 0.015 REAQ vs. SEAQ 0.434 REAQ vs. SEAL 0.020 REAQ vs. LEAQ 0.012 REAQ vs. LEAL 0.116
REAL	92.44± 3.76	REAL vs. SEAQ 0.082 REAL vs. SEAL 0.905 REAL vs. LEAQ 0.911 REAL vs. LEAL 0.337
SEAQ	69.40± 30.29	SEAQ vs. SEAL 0.103 SEAQ vs. LEAQ 0.066 SEAQ vs. LEAL 0.412
SEAL	90.91± 4.59	SEAL vs. LEAQ 0.817 SEAL vs. LEAL 0.399
LEAQ	93.87± 0.84	LEAQ vs. LEAL 0.286
LEAL	80.01± 10.32	

**The mean difference is significant at (P≤0.05)*

**The mean difference is not significant at (P>0.05).*

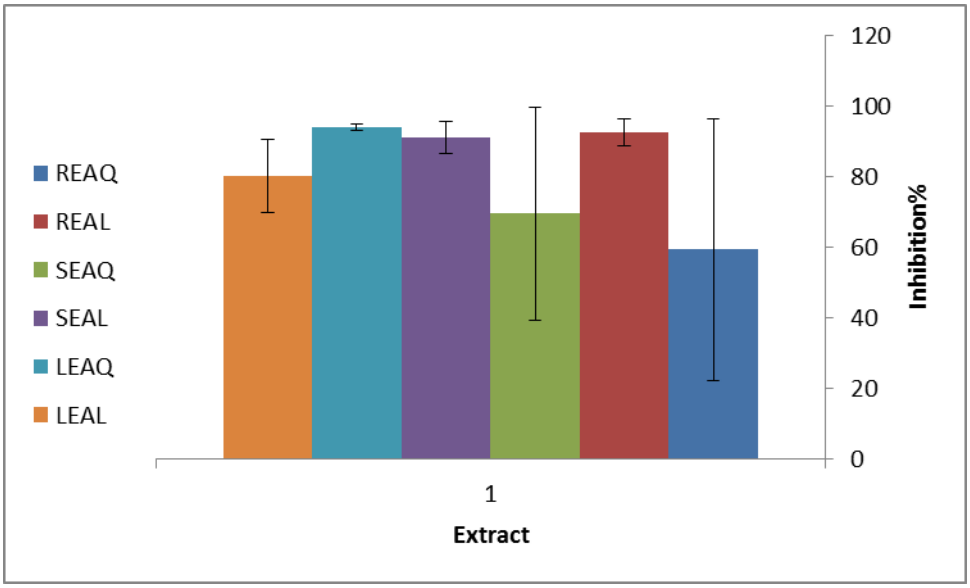

Figure 9: Inhibition ratios of DPPH for aqueous extract and alcoholic extract of Lycium shawii plant parts (root, leaves, and steam)

Hydroxyl radical scavenging activity

The results showed that the best antioxidant out of the six extracts used for the Lycium shawii was the aqueous extract of the leaves, where the most antioxidant appeared, and also as a result of appearance of the following active compounds in it using the GC-MAS technique are (3-Butyn-1-ol, Carbonic acid, dimethyl ester, Ethene, methoxy, Diazene, dimethyl, Hexadecanoic acid, methyl ester, Heptane, 1-(ethenylthio), 9,12-Octadecadienoic acid, methyl ester, 9,12-Octadecadienoic acid (Z,Z), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, Phytol , 6-Octadecenoic acid, methyl ester, 9-Octadecenoic acid (Z)-, methyl ester, 11-Octadecenoic acid, methyl ester. Results Hydroxyl radical scavenging activity show in Table 8 and results Hydroxyl radical scavenging activity show in Figure 10.

Table 8: Hydroxyl radical scavenging activity

Groups	Mean± S.D	P-value
REAQ	83.51± 10.06	REAQ vs. REAL 0.000 REAQ vs. SEAQ 0.801 REAQ vs. SEAL 0.099 REAQ vs. LEAQ 0.939 REAQ vs. LEAL 0.041
REAL	37.61± 26.69	REAL vs. SEAQ 0.001 REAL vs. SEAL 0.022 REAL vs. LEAQ 0.000 REAL vs. LEAL 0.057
SEAQ	80.70 ±9.45	SEAQ vs SEAL 0.0157 SEAQ vs LEAQ 0.743 SEAQ vs LEAL 0.068
SEAL	64.61± 23.09	SEAL vs. LEAQ 0.086 SEAL vs. LEAL 0.657
LEAQ	84.36± 9.93	LEAQ vs. LEAL 0.034
LEAL	59.66 ± 16.88	

**The mean difference is significant at (P≤0.05).*

**The mean difference is not significant at (P>0.05).*

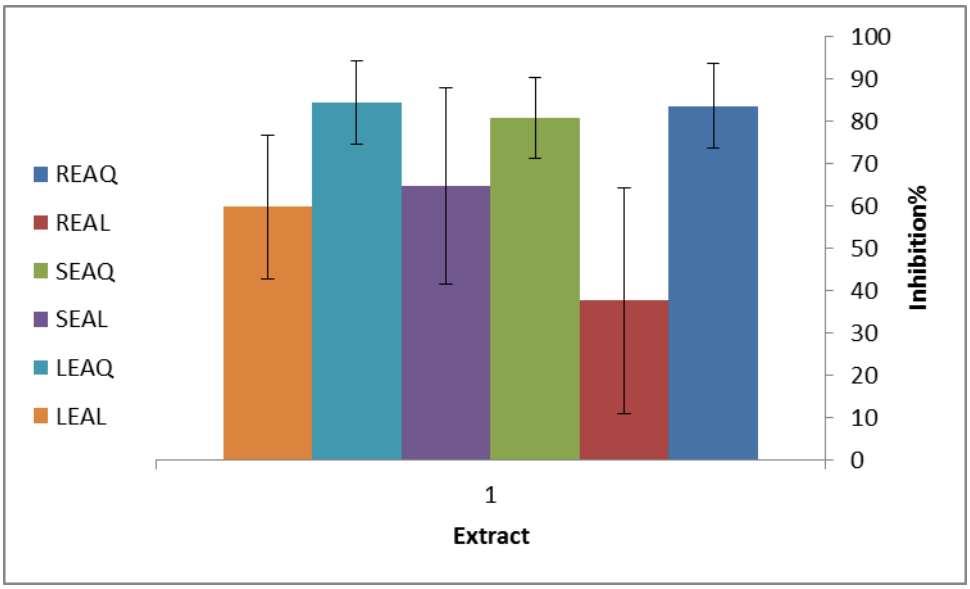

Figure 10: Inhibition ratios of OH for aqueous extract and alcoholic extract of Lycium shawii plant parts (root, leaves, and steam)

Conclusion

It was concluded from this study that the bramble plant is considered a strong antioxidant that inhibits free radicals because it contains many effective compounds that make it a powerful antioxidant, so this plant can be used in many medical applications.

Disclosure Statement

No potential conflict of interest was reported by the authors.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

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