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Research Article

Microwave-Assisted Extraction and Phytochemical Profile of *Nonea pulmonarioides* and Its Antifungal, Antibacterial, and Antioxidant Activities

Haval H. Mohammed and Fuad O. Abdullah

Department of Chemistry, College of Science, Salahaddin University, Erbil, Kurdistan, Iraq

Correspondence should be addressed to Fuad O. Abdullah; fuad.abdullah@su.edu.krd

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Microwave-assisted extraction (MAE) was chosen to isolate secondary natural compounds from *Nonea pulmonarioides*. MAE is an efficient approach compared to maceration. In our study of *N. pulmonarioides,* the Folin–Ciocalteu and trichloroaluminum techniques were used to determine the total phenolic and flavonoid contents. Antioxidant activity was determined by the β-carotene/linoleic acid test. The minimum inhibitory concentration (MIC) was measured using the microwell dilution assay method. Overall, MAE may represent a substantially faster extraction system (5 min) with an even greater extraction yield than maceration extraction (24 h). Phytochemical screening indicated the existence of classes of several secondary metabolites. The methanol extract had a high total phenolic content (TPC) of $113.33 \pm 1.06\,\mu$g of gallic acid equivalents (GAE)/mg of dry plant weight, while the total flavonoid content (TFC) in the acetone extract was $44.26 \pm 0.88\,\mu$g of quercetin equivalent (QE)/mg of the dry plant. The antioxidant activity data correlated well with the acetone extract (32.41 ± 0.93, I%) compared to the other extracts. The antimicrobial assay (MIC) results showed high activity of the extract against *Candida albicans* for the petroleum ether extract, which was significantly higher than against other microorganisms. The quantitative gas chromatography-mass spectrophotometry (GC-MS) analysis of the extracts confirmed that the most abundant compound in the methanol extract was ethyl tridecanoate (45.94%), while nonadecane was the predominant compound in the acetone (35.7%) and the petroleum ether (18.34%) extracts.

1. Introduction

Medicinal plants produce secondary metabolites with remarkable and diverse biological functions that are widely used as food additives and pharmaceutical ingredients for therapeutic, aromatic, and culinary purposes [1, 2]. In southern Kurdistan, medicinal plant usage is based on hundreds of years of experience and findings and a rich medicinal history [3]. Traditional Kurdish medicine remains the first choice for treating many diseases, especially for those who cannot afford high-priced synthetic drugs; additionally, this traditional medicine is used in remote villages, such as those on Kodo mountain. Various methods found in local herbal traditions have provided medicinal remedies for centuries among the Kurdish people [4, 5].

In developing countries, a very high number of people depend on traditional medicinal plants for their primary health care [6]. In addition, medicinal plant researchers are interested in the most important parts of plants, the bioactive compounds [7]. Recently, many researchers have focused on phytochemical studies to find the bioactive compounds used in medicine [8]. Different techniques are available to extract the secondary metabolites, such as the modern methods of microwave- and ultrasonic-assisted extraction and the conventional methods of maceration, infusion, decoction, and Soxhlet extraction [9]. According to previous studies, modern processes are preferred over traditional methods for manufacturing medications. The multiple advantages of modern methods include higher yields in less time and the reduced use of solvent and energy [10, 11].

The genus *Nonea*, also known as monkswort, is a large group of plants in the Boraginaceae family; there are about 156 genera and 2700 species in this family, which is a source of many bioactive constituents. Boraginaceae species have been used in folk medicine worldwide to treat burns and wounds [12, 13]. Previous studies have illustrated the potent wound healing properties of these plants, which are linked to their anti-inflammatory, antioxidant, antiviral, and antibacterial properties. These properties are directly based on their phenolic compounds, for instance, naphthoquinones, phenolic acids, and flavonoids [14].

The genus *Nonea* contains 55 species distinguished by their accrescent fruiting calyx, hairy appendages at the throat of the corolla, and glandular hairs on the branches, stems, or cymes. The flowers on the plant are five-petaled and come in various colors, including red, purple, white, and pink. The leaves are oval or tapered at the ends [15, 16]. Five species from this genus grow in Iraq: *N. melanocarpa*, *N. pulla*, *N. Ventricose*, *N. obtusifolia,* and *N. pulmonarioides*. Most *Nonea* species grow from the southern Atlantic to west-central Asia, northern Africa, and Europe [15]. *N. pulmonarioides*, which grows on the Kodo mountain in Kurdistan, Iraq, has long been used to treat inflammatory diseases [5].

Medicinal plants have been used for numerous biological, clinical, pharmaceutical, and medicinal purposes for many years; however, their use as biofactories for preparing desired pharmaceutical and biomedical compounds is relatively modern [2, 17]. The presence of phytochemicals, such as terpenoids, alkaloids, and phenols, supports the use of medicinal plants in alternative and traditional medicines because phytochemicals found in roots, leaves, stems, seeds, flowers, and fruits are generally known for being responsible for protecting the plant, animals, and humans [17, 18]. The phenolic compounds, including flavonoids, contribute to human health through antioxidant activity, free radical scavenging, and antimicrobial properties [19]. This investigation aimed to test green extraction methods and perform qualitative phytochemical analysis by assessing total phenol and flavonoid contents and the antimicrobial and antioxidant activities of *N. pulmonarioides*.

2. Experimental Sections

2.1. Collection of Plant Material. The plant materials (aerial parts) of *N. pulmonarioides,* as shown in Figure 1, were collected during a ripe stage from Kodo mountain, Kurdistan, Iraq, on May 14, 2021. A voucher specimen (No. 7486) was deposited at Salahaddin University-Erbil.

2.2. Preparation of Plant Extracts. After the *N. pulmonarioides* (NP) samples were collected, the fresh aerial parts were dried in the shade for 2 weeks and then ground to a fine powder with an electric grinder. In this study, two methods were used to prepare crude extracts: the conventional method of maceration and the modern method of microwave-assisted extraction (MAE).



Figure 1: Aerial parts of *N. pulmonarioides.*

2.3. Microwave-Assisted Extraction (MAE). MAE was performed using a multimodal household microwave oven (Panasonic P90N28AP-S3) at 900 W: 250 g of aerial parts was soaked in separate round bottom flasks in petroleum ether, acetone, and methanol. Each mixture of solvent and sample was extracted for 5 min using an irradiation cycle of 20 s intervals. The solvent in the round bottom flasks was evaporated and cooled by a condenser. After filtration, a rotary evaporator was used at 38–40°C to obtain powder for each extract in triplicate.

2.4. Maceration Extraction (ME). The powdered aerial parts of the plant (250 g) were dissolved in conical flasks with corresponding amounts of the solvent's petroleum ether, acetone, and methanol. After that, the sample was macerated in a dark area at room temperature for 24 h and taken in triplicate.

2.5. Phytochemical Screening. Qualitative secondary metabolites analysis was conducted using standard methods for the petroleum ether, acetone, and methanol extracts of NP to measure the steroid, terpenoids, phenol, tannin, flavonoid, saponins, quinine, phlobatannin, and *Anthraquinone glycoside* content [20–25].

2.6. Total Flavonoid Content (TFC). The TFC was determined using the $AlCl_3$ technique [26] for estimating the crude extracts (petroleum ether, acetone, and methanol) of the *N. pulmonarioides* with few modifications. A stock solution was prepared by placing 0.1 mg of quercetin in 10 ml of methanol in a volumetric flask (10 ml); then, the solution was diluted to six separate concentrations (10, 20, 40, 60, 80, and 100 mcg/ml). For each of these solutions, a 1 ml aliquot was added to a test tube and diluted to 10 ml with methanol; simultaneously, 0.3 ml of 5% $NaNO_2$ was added, followed by 10% $AlCl_3$ after 5 min. After 6 min, 2 ml of (1 M) NaOH was added, along with sufficient distilled water to make up 10 ml of the solution. Also, for each extract of *N. pulmonarioides* prepared like quercetin, the absorbance was measured for each concentration of quercetin and SPE extract at 510 nm using a spectrophotometer in triplicate. Then, the average absorbance was used to construct a calibration curve of the TFC.

$$\text{Absorbance} = 0.0078 \times \text{quercetin}\,(\mu g) + 0.0206. \quad (1)$$

2.7. Total Phenolic Content (TPC). The total phenolic content (TPC) of the petroleum ether, acetone, and methanol crude extracts of *N. pulmonarioides* was quantified using the Folin–Ciocalteu technique and gallic acid [27]. A 0.1 ml solution was mixed containing 1000 μg of each extract placed in a 50 ml volumetric flask with 1 ml of Folin–Ciocalteu reagent and 46 ml distilled water and vigorously agitated. After standing for 3 min, 3 ml 2% $NaNO_3$ was added, and the mixture was left to stand with intermittent shaking for 2 h. Also, five different gallic acid solutions were prepared following the same procedure as the extracts. The absorbance was obtained in triplicate for each concentration of gallic acid and the extracts at 760 nm using a spectrophotometer. Then, the average absorbances were used to construct a calibration curve of the TPC and the following equation.

$$\text{Absorbance} = 0.0012 \times \text{gallic acid}\,(\mu g) + 0.0128. \quad (2)$$

2.8. Antioxidant Activities

2.8.1. β-Carotene/linoleic Acid Bleaching. The antioxidant activity was tested using β-carotene with BHT as a standard for the three extracts of the *N. pulmonarioides* (petroleum ether, acetone, and methanol). This technique was used as described in [28] with a few modifications. A mixture was prepared with 500 mg of β-carotene, 25 μg of linoleic acid, and 200 mg Tween 40 emulsifier in 1 ml chloroform. Then, the chloroform was removed, and 100 ml of oxygenated distilled water was added to the residue. Solutions of the extract and BHT (standard) were made up to a concentration of 2 g/L individually, and 350 μl of each was added to 2.5 ml of the above emulsion in test tubes with vigorous agitation. The samples were incubated with a blank (methanol) in a water bath at 50°C for 2 h. The absorbance was measured in triplicate at 470 nm using a UV-Vis spectrometer (Cintra 6, GBC, Dandenong, Australia). The antioxidant activities (inhibition percentages, I%) of the samples and BHT (standard) are as follows. The relative antioxidant activities of the extracts were calculated from the following equation.

$$I\% = \left(\frac{A_{\beta-\text{caroteneafter2h}}}{A_{\text{initialâ}\beta-\text{carotene}}}\right) \times 100, \quad (3)$$

where $A_{\beta\text{-carotene after 2 h}}$ is the absorbance value of the β-carotene remaining in the samples after the 2 h assay, and $A_{\text{initial}\beta\text{-carotene}}$ is the absorbance value of β-carotene at the beginning of the experiments. All experiments were performed in triplicate, and ±SD is the standard deviation.

2.9. Bacterial and Fungal Strains. The three extracts (petroleum ether, acetone, and methanol) of the *N. pulmonarioides* were evaluated separately against a panel of 11 microorganisms. The panel included *Candida albicans* (ATCC 10231) and *Aspergillus Niger* (ATCC 16404), two common fungal strains, and the other microbial strains *Pseudomonas aeruginosa* (ATCC 27853), *Shigella dysenteriae* (PTCC 1188), *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 10031), *Salmonella paratyphi A* serotype (ATCC 5702), *Staphylococcus aureus* (ATCC 29737), *Proteus vulgaris* (PTCC 1182), and *Staphylococcus epidermidis* (ATCC 12228). The bacterial strains were cultured overnight in nutrient agar (NA) at 37°C, whereas the fungi were cultured overnight in Sabouraud dextrose agar at 30°C.

2.9.1. Microwell Dilution Assay. Using the microwell dilution assay method, the bacterial strains and yeasts sensitive to the plant extract and essential oil were studied to determine their minimum inhibitory concentration (MIC) values [29]. The inocula of the microbial strains were cultured in broth for 12 h, and the suspensions were adjusted to 0.5 McFarland standard turbidity. The extracts of *N. pulmonarioides* were dissolved in 10% DMSO solution and diluted to the highest concentration (5 mg/ml) to be tested. Subsequently, serial twofold dilutions were made to produce a concentration range from 0.078 to 5 mg/ml in 10 ml sterile test tubes containing brain heart infusion (BHI) broth for bacterial strains and *Sabouraud dextrose* (SD) broth for yeast. The 96-well plates were prepared by dispensing 95 μl of the media and 5 μl of the inoculum into each well. A 100 μl aliquot from the stock solutions of the 5 mg/ml plant extracts was added to the first row of wells. Then, 100 μl of the serial dilutions was transferred into the next six consecutive wells. The last well of each column contained 195 μl of the media without the test materials, and 5 μl of the inoculum and was used as the negative control. The final volume in each well was 200 μl. Gentamicin and Rifampin for bacteria and nystatin for yeast were used as standard drugs for positive controls in conditions identical to the test materials. The plates were covered with sterile plate sealers. The contents of each well were mixed on a plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by the presence of a white pellet on the well bottom and confirmed by plating 5 μl samples from clear wells on an NA medium. The MIC value was defined as the lowest concentration of the plant extracts required to inhibit the growth of microorganisms. All tests were repeated two times.

2.10. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis. GC-MS analyses of the essential oils were carried out on an Agilent HP-6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) with an HP-5MS 5% phenyl methyl siloxane capillary column (30 m × 0.25 mm, 0.25 μm film thickness; Restek, Bellefonte, PA) equipped with an Agilent HP-5973 mass selective detector (MSD) in the electron impact mode (ionization energy: 70 eV) operating. Retention indices (RIs) were calculated for all compounds using a homologous series of n-alkanes injected under conditions equivalent to those of the samples. The identification of the components of the volatile fraction was

based on retention indices relative to n-alkanes and computer-matching with the Wiley275.L and Wiley7 n.L libraries, as well as comparisons of the fragmentation patterns of the mass spectra with the data published in the literature [30].

3. Results and Discussion

3.1. Extraction Yields of Aerial Plant Parts. The MAE and ME processes were used in this study. The green MAE technique has many benefits for extracting phytochemicals from plants, as it can extract phytochemicals in a short period. It requires a small amount of solvent and so is less costly. Compared to ME, there are great differences in the required time and solvent volume because ME requires 2500 ml of solvent and 24 h to extract 250 g, but MAE requires a few min and less solvent (Table 1).

Table 1: Comparison of yields of maceration and microwave extraction with different solvents from *N. pulmonarioides.*

Method	Time	Solvent extraction	Yield %	Amount in g
Maceration (ME)	24 h	NPPE	3.3	8.25
		NPAE	2.86	7.15
		NPME	6	15
Microwave (MAE)	5 min	NPPE	3.628	9.07
		NPAE	2	5
		NPME	6.64	16.6

NPPE: petroleum ether extract; NPAE: acetone extract; NPME methanol extract.

Table 2: Qualitative tests for phytochemical screening of *Nonea pulmonarioides.*

Test	NPPE	NPAE	NPME
Steroids	+	+	+
Tannins	−	−	+
Flavonoids	−	+	+
Terpenoids	+	+	+
Phlobatannins	−	−	−
Quinines	−	−	−
Saponins	+	+	+
Phenolic compounds	−	−	+
Anthraquinone glycosides	−	−	−

(+) presence, (−) absence; NPPE: petroleum ether extract; NPAE: acetone extract; NPME: methanol extract.

3.2. Qualitative Secondary Metabolite Compounds. Phytochemical screening has previously been performed on plants from the same genus, that is, *N. vesicaria*, *N. setosa* [31], *N. rossica* [32], and *N. edgeworthii* [33]. These plants contain phytochemicals mainly known for biological activity and the treatment of cancer, oxidation, and neurological diseases and have fewer side effects than synthetic products. In the current study, the phytochemicals in *N. pulmonarioides* have more important biological activity and can be useful for treating other diseases [7, 33]. The phytochemical results from the petroleum ether, acetone, and methanol extracts prepared from *N. pulmonarioides* indicated the presence of some secondary metabolic products. Steroids, terpenoids, and saponins were found in all extracts. In addition, the methanolic extract contains a greater variety of phytochemicals compared to the acetone and petroleum ether extracts (Table 2).

3.3. Total Phenolic and Flavonoid Contents. Phenolic compounds are important plant constituents whose redox properties are responsible for antioxidant activity. For quantitative determination, the phenolic acid content was measured using the Folin–Ciocalteu reagent in each extract. Phenolic acids are more important and correlate with bioactivity. The flavonoid contents in selected plant extracts were determined using the aluminum chloride method. Investigations have shown that flavonoids are very active, with antiviral, anti-inflammatory, anticancer, and anti-allergic activities [34]. The results of this study show that not all polyphenols in crude extracts are necessarily flavonoids but may be tannins or phenolic acids. Many species of this genus have been studied and reported on previously, including *N. caspica* [35], *N. micrantha* [36], and *N. lutea* [37]. Compared to the current study, they had greater amounts of polyphenols and flavonoids. Furthermore, compared to the methanolic extract of *N. pulmonarioides*, the species *N. melanocarpa* has a lower concentration of polyphenols (22.2 μg/mg) [38]. Additionally, the rate of flavonoids in the methanol extract in *N. pulmonarioides* is higher than in *N. vesicaria* [7]. The TPC and TFC vary between species of the same genus due to various biotic and abiotic factors, such as the genetic potential, environmental conditions, and storage period. The TPC was determined using gallic acid equivalents and the calibration curve ($y = 0.0012\times + 0.0128$; $R^2 = 0.9976$), while the TFC was determined using quercetin equivalents and the calibration curve ($y = 0.0078\times - 0.0206$; $R^2 = 0.999$) and the results are shown in Table 3.

3.4. Antioxidant Activity. This test assesses the ability of the plant extract to inhibit the formation of conjugated diene hydroperoxides from linoleic acid oxidation [39]. The potent antioxidant activities of petroleum ether, acetone, and methanol extracts prepared from the plant *N. pulmonarioides* were investigated using a β-carotene/linoleic acid bleaching assay. The results (Table 4) showed that the acetone extract exhibited stronger antioxidant activities than the other extracts because the linoleic acid oxidation of the acetone extract inhibited 32.44% ± 0.93%, but the methanol and petroleum ether extracts inhibited 29.02% ± 0.52% and 26.94% ± 0.65%, respectively. Nevertheless, all the tested extracts have less antioxidant activity than BHT (81.39% ± 1.05%).

3.5. Antimicrobial and Antifungal Activities In Vitro Assay. The antibacterial and antifungal activities of *N. pulmonarioides* extracts against bacteria and fungi were evaluated in this work. The results from this study against Gram-positive bacteria (*B. subtilis* (ATCC 6633), *S. aureus* (ATCC 29737), and *S. epidermidis* (ATCC 12228)), Gram-

Table 3: Total phenolic and flavonoid contents of the petroleum ether, acetone, and methanol extracts from *N. pulmonarioides.*

NO.	Extracts	Total flavonoid content Quercetin equivalent (μg/mg)	Total phenolic contents Gallic acid equivalent (μg/mg)
1	NPPE	15.34 ± 1.30	22.41 ± 0.95
2	NPAE	44.26 ± 0.88	32.8 ± 1.20
3	NPME	38.14 ± 0.90	113.33 ± 1.06

Expressed ± standard deviation; NPPE: petroleum ether extract; NPAE: acetone extract; NPME methanol extract.

Table 4: Antioxidant activity/β-carotene/linoleic acid bleaching assay.

No.	Sample	Absorbance average	β-carotene assay (%I), ±SD
1	NPAE	0.1542	32.41 ± 0.93
2	NPME	0.1381	29.02 ± 0.52
3	NPPE	0.1282	26.94 ± 0.65
4	BHT	0.3872	81.39 ± 1.05

NPPE: petroleum ether extract; NPAE: acetone extract; NPME: methanol extract; BHT: butylated hydroxytoluene.

Table 5: Antimicrobial and antifungal (MIC) results of extracts and commercial antibiotics.

Test microorganism	NPPE MIC	NPAE MIC	NPME MIC	Rifampin MIC	Gentamicin MIC	Nystatin MIC
Aspergillus Niger (ATCC 9029)	—	—	—	—	—	—
Candida albicans (ATCC 10231)	0.5	2	4	—	—	0.0312
Bacillus subtilis (ATCC 6633)	2	4	8	0.0313	0.0039	—
Escherichia coli (ATCC 25922)	4	4	8	0.0039	0.0039	—
Klebsiella pneumonia (ATCC 10031)	4	4	16	0.01563	0.0039	—
Pseudomonas aeruginosa (ATCC 27853)	4	4	16	0.0313	7.81	—
Salmonella paratyphi-A serotype (ATCC 5702)	8	4	16	0.01563	0.0039	—
Shigella dysenteriae (PTCC 1188)	8	4	16	0.01563	0.0039	—
Staphylococcus aureus (ATCC 29737)	8	4	16	0.0313	0.00195	—
Staphylococcus epidermidis (ATCC 12228)	8	8	16	0.00195	0.00195	—

MIC: according to a milligram per milliliter and (—) NO detected; NPPE: petroleum ether extract; NPAE: acetone extract; NPME: methanol extract.

negative bacteria (*E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 10031), *P. aeruginosa* (ATCC 27853), *S. paratyphi-A* serotype (ATCC 5702), *S. dysenteriae* (PTCC 1188)), and against the fungi *A. Niger* (ATCC 9029, mould) and *C. albicans* (ATCC 10231, yeast) showed that the three extracts (petroleum, acetone, and methanol) of *N. pulmonarioide*s displayed only weak MIC antimicrobial activities, except to *A. Niger* (Table 5). The MIC is defined as the minimum inhibition concentration that prevents the growth of microbes and is expressed in terms of mg/ml.

In this study, the *N. pulmonarioides* plant extract showed interesting activity against the *C. albicans* (ATCC 10231) fungi compared to the other micrograms in Table 5 and additionally showed high activity against the Gram-positive bacteria *B. subtilis* (ATCC 6633). Petroleum ether was the best extracting solvent for MIC antimicrobial activities. However, in vitro results cannot be directly applied to clinical applications or disease therapies.

3.6. GC-MS Phytochemical Profile. The chemical components of plants are very important in finding new, more effective drugs. The GC-MS phytochemical screening results of the three extracts (petroleum, acetone, and methanol) of *N. pulmonarioides* showed 55 different compounds with retention times of 24, 20, and 49 min, respectively. According to this study, some compounds are present in all three extracts in varying amounts, which affects their antimicrobial properties. However, some of the peaks in the chromatograms remain unidentified. GC-MS analysis for the three extracts (petroleum, acetone, and methanol) were identified by their retention time (min), concentration (%), and similarity (%) and are shown in Table 6. This type of GC-MS analysis is the first step toward understanding the nature of active compounds in this medicinal plant. The results of the GC-MS analyses showed that nonadecane was the major compound in the petroleum ether (18.34%) and acetone (35.7%) extracts, but ethyl tridecanoate was the major compound (45.94%) in the methanol extract, as shown in Figures 2, 3, and 4. Additionally, according to a previous study, ethyl tridecanoate has antibacterial and antifungal properties [40]. In addition to these two compounds, multiple other major components were present Figure 5. Some compounds were present in very low amounts, as shown in Figure 6. Nonadecane has been used as an anti-HIV, antioxidant, antibacterial, antimicrobial, cytotoxic effect, antimicrobial, and antimalarial treatment [41]. The large amount of polyunsaturated fatty acids (PUFAs) detected by GC-MS confirms that *N. pulmonarioides,* like *N. setosa*, has useful anti-inflammation properties, and

Table 6: GC-MS analysis of petroleum ether, acetone, and methanol extracts from *N. pulmonarioides*.

No.	*Name compound*	T_R[a]	*Similarity (%)*[b]	*NPPE*[c]	*NPAE*[c]	*NPME*[c]
				% Concentration		
1	Heptane	5.830	84	0.2	0.84	0.51
2	Unknown	9.865	38	0.47	1.52	1.02
3	D-limonene	17.615	99	0.32	1.17	0.9
4	Sec-butyl propenyl disulfide	24.354	96	0.2	—	—
5	Citronellol	27.034	98	3.53	1.74	—
6	Geraniol	28.160	94	0.48	—	—
7	Citronellyl formate	32.578	87	0.47	—	—
8	Eugenol	32.887	98	0.23	—	—
9	Geranyl acetate	33.841	91	0.35	—	—
10	Methyleugenol	34.853	98	0.5	—	—
11	Caryophyllene	36.053	99	0.37	—	—
12	A-Guaiene	36.602	99	0.3	—	—
13	A.-humulene	37.579	99	0.36	—	—
14	Germacrene D	38.619	98	0.56	1.03	—
15	Pentadecane	39.082	97	0.93	1.43	0.67
16	A-bulnesene	39.419	99	0.12	—	—
17	Dodecanoic acid	41.734	99	6.76	3.85	3.09
18	Dodecanoic acid, ethyl ester	42.780	64	0.16	—	—
19	Hexadecane	43.077	97	0.47	0.94	0.77
20	Tridecanoic acid	45.397	99	0.69	—	1.01
21	Ethyl tridecanoate	45.603	99	—	—	45.94
22	8-Heptadecene	46.038	98	0.56	—	—
23	Heptadecane	46.878	98	4.34	6.29	0.85
24	Farnesol	47.541	90	0.28	—	—
25	Unknown	48.472	64	—	—	3.76
26	Dodecanoyl chloride	48.644	50	0.53	—	—
27	Tetradecanoic acid	49.101	99	2.69	1.5	1.32
28	Dibenzothiophene	49.290	96	—	—	0.77
29	Benzyl benzoate	49.558	96	0.39	—	—
30	Octadecane	50.467	98	0.61	0.9	0.73
31	Unknown	50.850	38	—	—	0.77
32	Unknown	51.073	74	0.21	—	0.67
33	Neophytadiene	51.736	76	0.64	2.39	—
34	Unknown	51.901	53	0.24	—	—
35	Benzoic acid, 2-phenylethyl ester	52.627	80	0.62	—	—
36	Unknown	52.862	38	—	—	0.70
37	Z-5-nonadecene	53.056	97	4.95	6.79	—
38	Unknown	53.307	64	0.78	—	—
39	Nonadecane	53.959	98	18.34	35.74	1.32
40	Methyl palmitate	54.696	99	0.91	—	—
41	Unknown	55.559	25	0.17	—	—
42	Dibutyl phthalate	55.714	97	0.2	0.82	—
43	N-Hexadecanoic acid	55.976	99	6.16	3.77	5.40
44	Unknown	56.331	58	0.47	—	—
45	Hexadecanoic acid, ethyl ester	56.914	83	0.23	—	0.68
46	Eicosane	57.177	96	1.79	3.21	—
47	Henicos-1-ene	59.491	97	0.37	—	—
48	Linoleic acid, methyl ester	60.057	99	1.75	—	0.57
49	Heneicosane	60.314	99	8.62	15.68	—
50	Gamolenic acid	60.892	99	1.25	—	—
51	Methyl stearate	61.057	99	0.84	—	—
52	Linoleic acid	61.389	99	7.05	2.09	5.27
53	Oleic acid	61.606	99	15.89	8.30	13.72
54	Octadecanoic acid	62.206	99	1.64	—	1.16
55	Pentacosane	62.452	98	—	—	8.41

NO.	*Compound type total in (NPPE, NPAE and NPME)*	*% And (number) in NPPE*	*% And (number) in NPAE*	*% And (number) in NPME*
1	Sesquiterpenoids	2.35(6)	3.42(2)	—
2	Monoterpene	0.32(1)	1.17(1)	0.9(1)
3	Ester	5.92 (10)	0.82(1)	47.19(3)

Table 6: Continued.

No.	*Name compound*	T_R[a]	*Similarity (%)*[b]	*NPPE*[c]	*NPAE*[c]	*NPME*[c]
				% Concentration		
4	Monoterpenoid	4.01 (2)	1.74(1)	—		
5	Acyclic sesquiterpene	0.28(1)	—	—		
6	Fatty acid	42.13(8)	19.51(5)	30.97(7)		
7	Hydrocarbon	41.19(11)	71.82(9)	13.26(7)		
8	Unknown	2.34(6)	1.52(1)	6.91(5)		
9	Other	1.46(4)	—	0.77(1)		
10	Total	100	100	100		

[a]Retention time and [b]mass spectra were compared to MS databases that were accessible and [c]peak area percentage was calculated from the GC-FID chromatogram; *NPPE: petroleum ether extract; NPAE: acetone extract; NPME: methanol extract.*

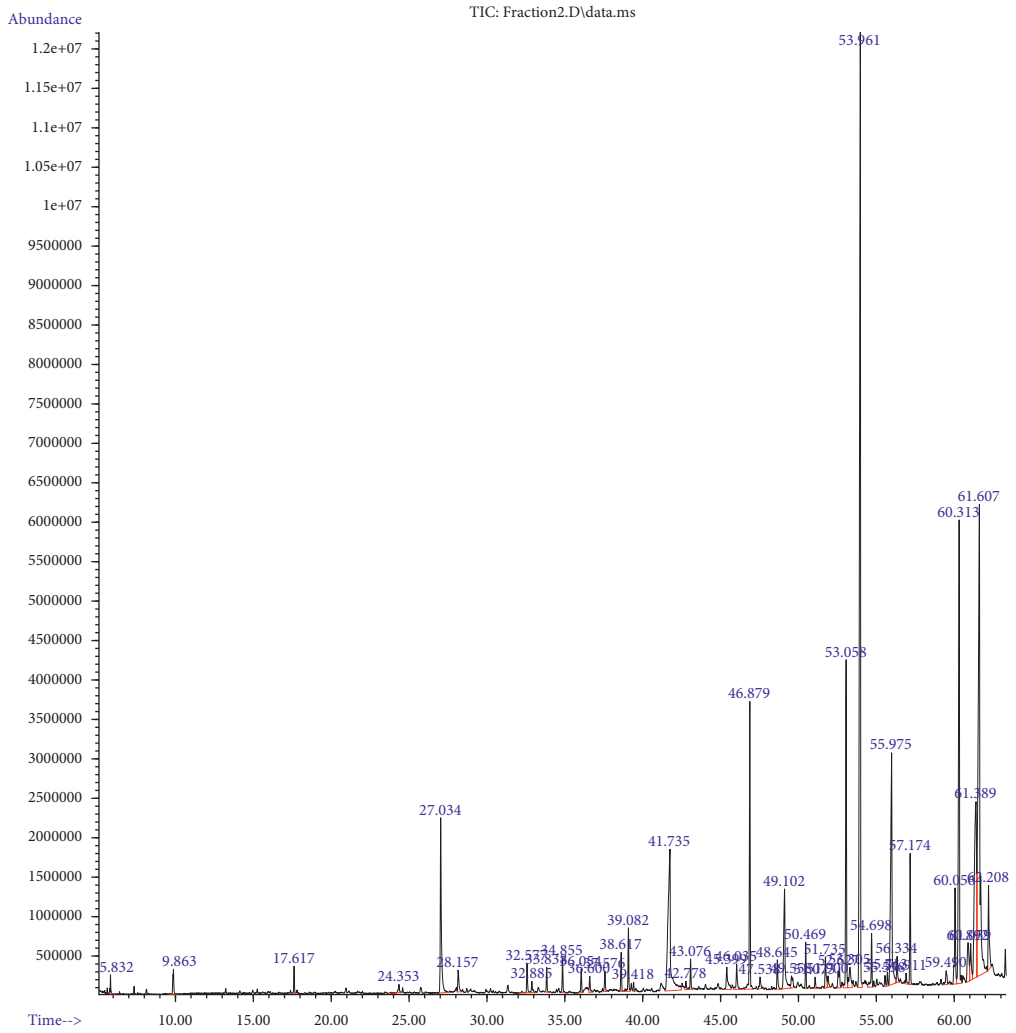


Figure 2: GC-MS chromatogram of petroleum ether extract of *N pulmonarioides.*

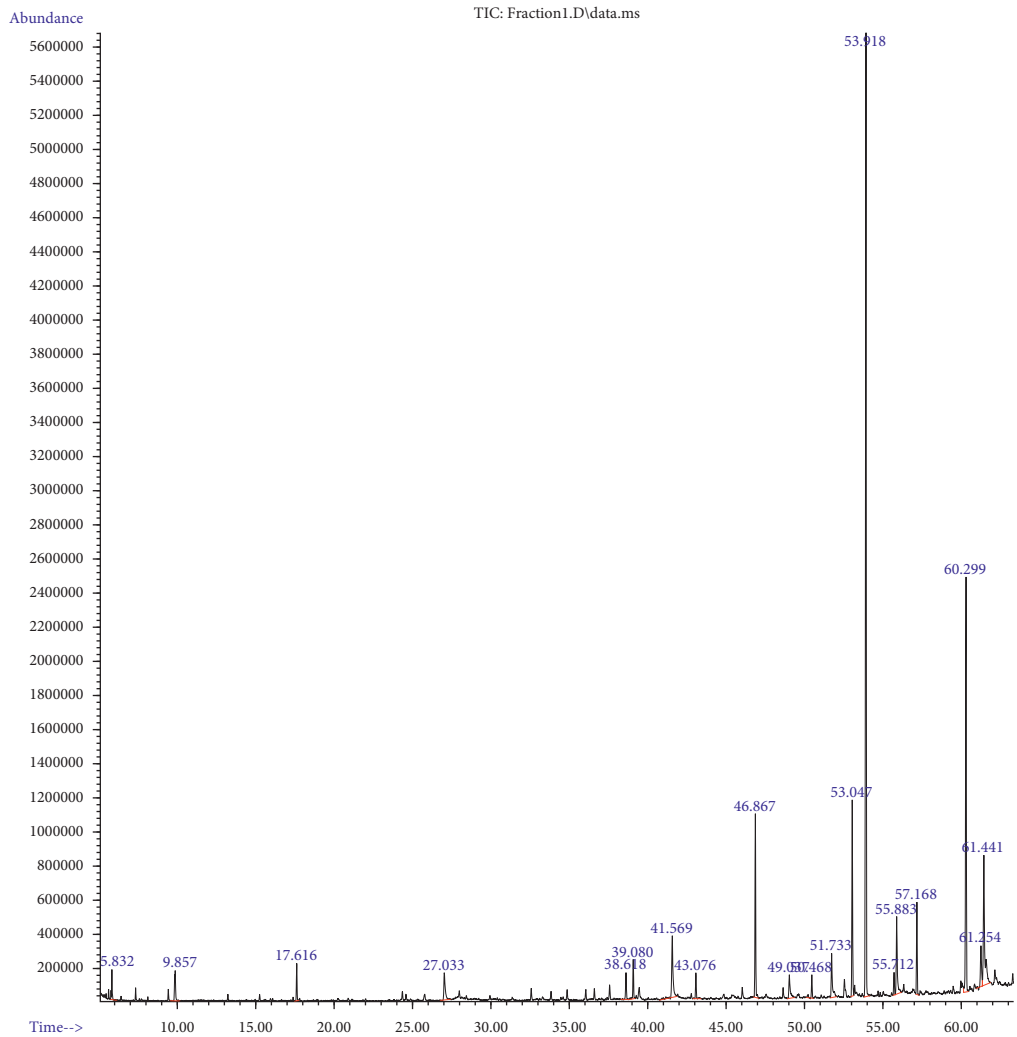


Figure 3: GC-MS chromatogram of acetone extract of *N. pulmonarioides*.

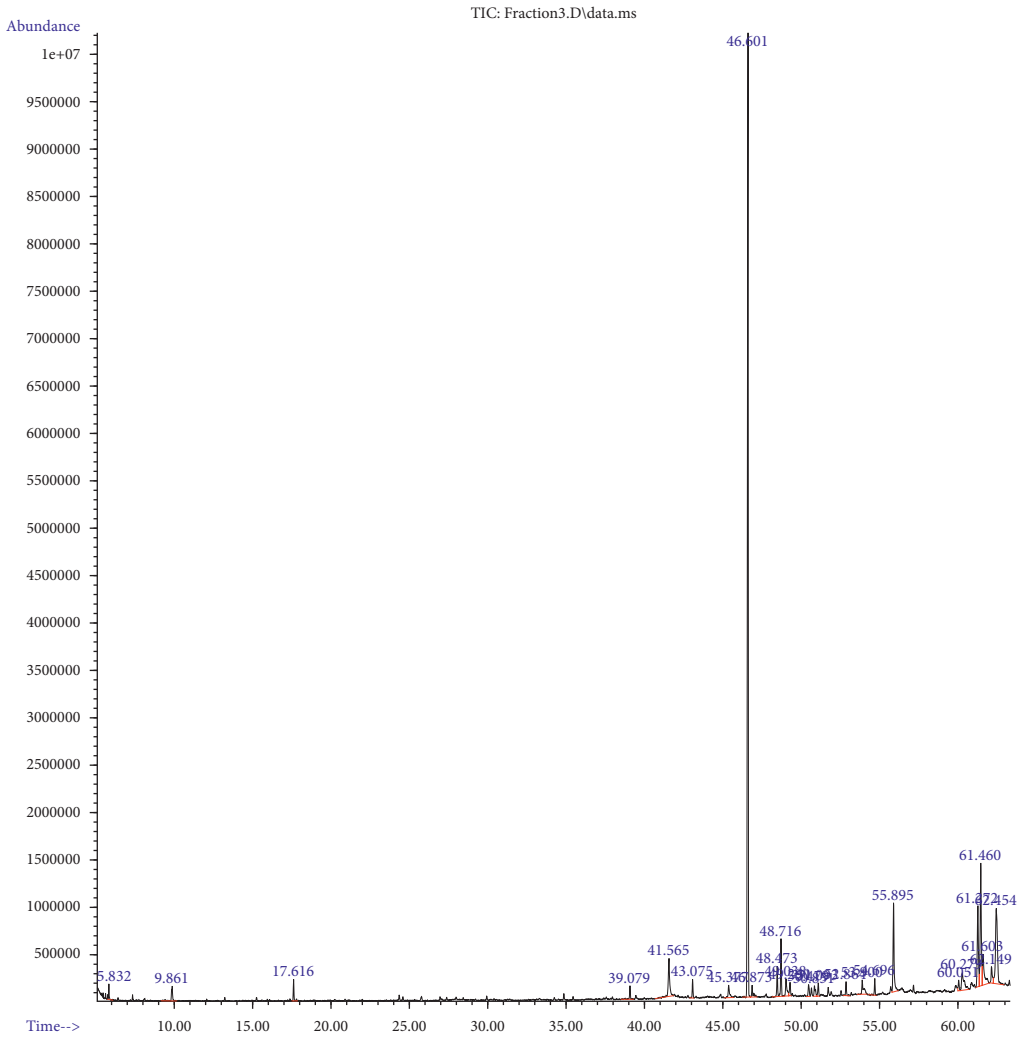


Figure 4: GC-MS chromatogram of methanol extract of *N. pulmonarioides*.

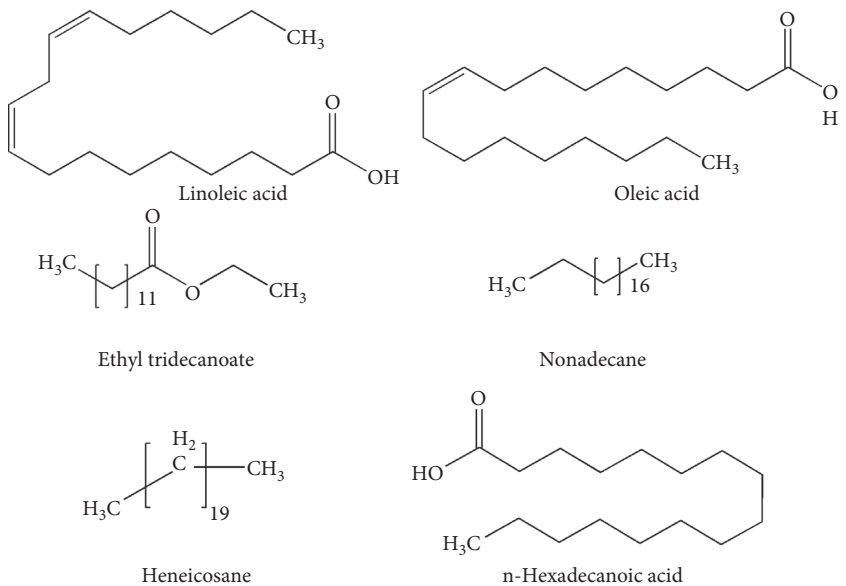


Figure 5: The most abundant compounds detected by GC-MS analysis.

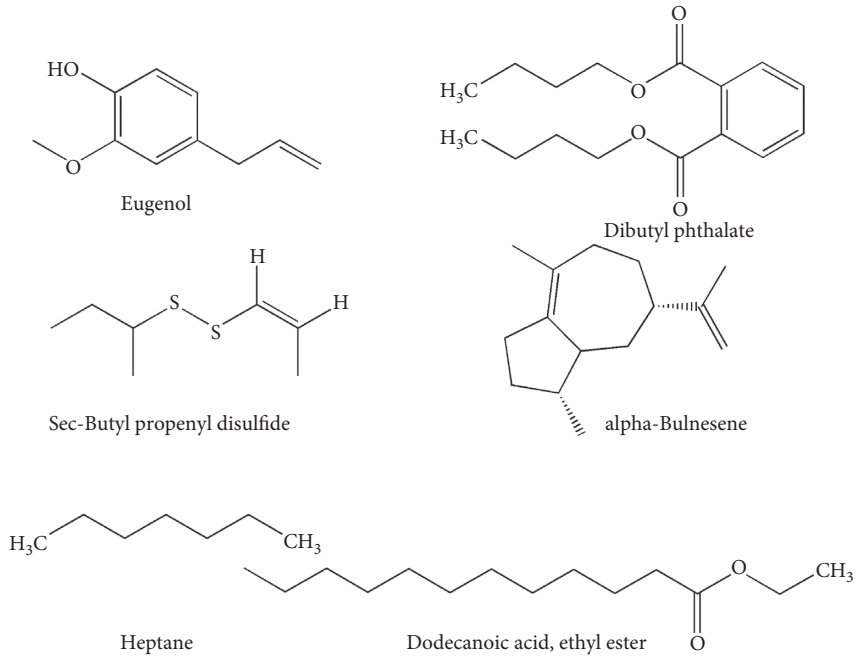


Figure 6: Some less-common compounds detected by GC-MS analysis.

according to previous research, PUFAs are the predominant chemical in the Boraginaceae family [42].

4. Conclusions

The current study evaluated the bioactive properties of the species *N. pulmonarioides*, which grows in Kurdistan. The phytochemical screening of the three extracts identified the bioactive compounds with interesting pharmacological activities. Additionally, the TPC was higher in the methanol extract, and the TFC was higher in the acetone extract. The antioxidant activity data showed that extraction in acetone had a significant impact compared to other solvents. The MIC of the antifungal activity of the petroleum ether extract against *C. albicans* was significantly higher compared to other microorganisms. Additionally, the GC-MS data showed that *N. pulmonarioides* contained a large number of two compounds, nonadecane, and ethyl tridecanoate, with biological activity.

Data Availability

The data used are included in this article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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