

Research Article

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Analysis of bioactive compounds present in *Boerhavia elegans* seeds by GC-MS

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Abstract: *Boerhavia elegans* L. (Nyctaginaceae) is a tropical plant widely distributed in the eastern part of Yemen, Oman, and many other countries. *B. elegans* is used in traditional medicine to treat inflammation, anemia, and urinary tract disorders. The seeds, called Alhydawan, are used as food additives to prepare porridge, one of the most popular foods in Yemen. The present study aims to analyze the bioactive constituents of the methanolic extract of *B. elegans* seeds after fractionation on silica gel column chromatography. The methanolic extract was subjected to column chromatography and eluted with a hexane and hexane diethyl ether mixture of different compositions. The eluted fractions were tested with thin-layer chromatography. The gas chromatography-mass spectrometry analysis of fractions indicated some compounds such as dodecane, 2,6,11-trimethyl, butylated hydroxytoluene, 2,6,10-trimethyltridecane, hexadecane, 2,6,11,15-tetramethyl, nonadecane, 2-methyl, 7,9-di-tert-butyl-1-oxaspiro(4,5) deca-6,9-diene-2,8-dione, *n*-hexadecenoic acid, octadecanoic acid, bis(2-ethylhexyl) phthalate, 13-docosenamide, (Z)-, and phenol, 2,4-bis(1,1-dimethyl ethyl) phosphite (3:1). Hence, *B. elegans* may have antimicrobial, anticancer, antioxidant, and antidiabetic activities due to the presence of secondary metabolites in the extract.

Keywords: *Boerhavia elegans* seeds, GC-MS analysis, medicinal plants, bioactive compounds

1 Introduction

Herbs have been the primary source of practically all medical remedies from ancient times until the introduction of synthetic pharmaceuticals in the nineteenth century [1]. Because of their long history of medicinal use and minimal carcinogenic risk compared to synthetic alternatives, medicinal plants are highly esteemed in many cultures [2]. Researchers currently source many traditional drugs from natural sources [3]. Utilizing therapeutic plants in conventional medical practices has created opportunities for further investigation, and biodiversity preservation is now widely acknowledged [4]. The designated genus of *Boerhavia* is in honor of Hermann Boerhaave, an eighteenth-century Dutch botanist affiliated with the University of Leiden in Germany. Scientists frequently mention the generic name *Boerhaavia* in scientific texts [5]. However, Linnaeus adapted Boerhaave's name to *Boerhavia* when establishing the genus; thus, *Boerhavia* is the correct spelling for the plant [6]. *Boerhavia*, often spelled as “Boerhaavia,” is a diverse genus of Nyctaginaceae, commonly referred to as the four o'clock family because the majority of species in this genus bloom their flowers 4 h after noon, namely in the early evening or morning. The family Nyctaginaceae comprises 300–400 species classified into around 30 genera. These include trees, shrubs, and herbs found widely across tropical and subtropical regions of Asia, Africa, America, and Australia [7,8]. The genus *Boerhavia* within this family contains approximately 40 species, also distributed widely in the tropics and subtropics of these same continents [9,10]. *Boerhavia* is known for its unique alkaloid mix, including flavonoids, phenolic glycosides, phenolic acids, sterols, and organic acids [11]. Quinolizidine alkaloids like *punarnavine* are one example. India contains six major *Boerhavia* species: *B. diffusa*, *B. repens*, *B. chinensis*, *B. erecta*, *B. elegans*, and *B. reniformis*. The medicinal value of plants from the *Boerhavia* genus has long been acknowledged across various traditional medicine systems globally. *Boerhavia* species find therapeutic applications in Indian practices like Ayurveda, Siddha, and Unani medicine. They are also prominently featured in folk remedies, such as traditional

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Chinese prescriptions and African herbalism. Moreover, *B. elegans* are recognized in the pharmacopeias of Brazil and India for their healing properties, classifying *B. elegans* as belonging to the *Boerhavia* genus, the Nyctaginaceae plant family. Botanists classify Choisy as belonging to the Nyctaginaceae family and place it under the genus *Boerhavia*, which includes a wide range of more than 100 species. The tropical and subtropical zones are the most common habitats for this blooming plant; it is also in West India, Iran, Pakistan, Oman, and Saudi Arabia [12–15]. In 2015, Al-Farga *et al.* examined the physicochemical properties, proximate composition, amino acid, mineral, vitamin concentrations, phenolic acids, and volatile oil components extracted from dry *B. elegans* seeds [15]. The edible herbaceous plant from which *B. elegans* seeds come is a member of a family that native tribes in southern Yemen frequently use in traditional dishes. They are a primary ingredient for making porridge and are also in bread and cake mixtures [16].

Nevertheless, there are many prepared traditional medicines and there are only a few chemical and pharmacological studies from *Boerhavia* genera, including *Boerhavia plumbaginea*, *Boerhavia chinensis*, *Boerhavia repens*, *Boerhavia diffusa*, *Boerhavia erecta* and *B. elegans*. Therefore, modern pharmacochemical research is needed to reveal the bioactivity claimed by traditional knowledge. Hence, this work aims to fractionate methanolic extract on a silica gel column to examine the chemical constituents of the seeds by separation and qualitative determination of *B. elegans* chemical constituents using gas chromatography-mass spectrometry (GC-MS) and identify the constituents responsible for any observed bioactivity.

1.1 Bioactivity of *Boerhavia* genus plants

Boerhavia has received growing attention from physiochemists because previous research has documented its diverse pharmacological and biological activities, indicating therapeutic potential. Research has shown that a methanolic extract of *B. diffusa* leaves has antibacterial properties and significant activity against *Staphylococcus aureus*, one of numerous harmful bacteria [17]. In their 2010 work, Ramazani *et al.* reported on the antimalarial effects of *B. elegans*, representing one of the initial studies to demonstrate this plant's effectiveness against malaria [18]. Traditional medicine practices have suggested that *B. elegans* can remedy numerous other health issues, such as painful menstruation, urinary tract problems, intestinal infections, inflammation, jaundice, and general weakness [19].

2 Materials and methods

2.1 Chemicals and reagents

The Direct-Q water purification system was utilized to acquire ultrapure water. The Milli-Q® water purification system (Merck) used solvents such as methanol, chloroform, ethyl acetate, diethyl ether, and hexane obtained from Thermo Fisher Scientific (Waltham, MA, USA). p-Anisaldehyde-sulfuric acid and 50% sulfuric acid were used as spray chemicals to determine the terpenes, steroids, and phenols.

2.2 Thin-layer chromatography (TLC)

TLC was performed using pre-coated silica gel plates with a 0.2 mm layer (Merck 60F 254). TLC spot visualization was carried out under UV light at 254 and 366 nm. Then, the TLC plate was sprayed with a 50% sulfuric acid spray reagent prepared with 50% methanol, and 50% concentrated sulfuric acid was added slowly [20]. After that, the TLC plates were sprayed and heated at 150°C for 1 min, and the plates revealed color spots.

2.3 Plant material and extract preparation

Dried *B. elegans* seeds were originally imported from the Hadramout region of South Yemen. They were then milled into powder using an electric grinder. After that, the powder was passed through a 100-mesh sieve and stored in a refrigerator at 4°C until use. One kilogram of the *B. elegans* powder was extracted three times (once daily) over 3 days of maceration in 1.5 L MeOH at ambient temperature, followed by filtration using Whatman No. 41 filter paper to remove the solid residue. The extract was evaporated in a rotary evaporator (Stuart RE300 Rotary Evaporator) at 40°C, yielding 41.5 g of oily material.

2.4 Column chromatography

An open glass column 80 cm in length and 3.5 cm in diameter was manufactured in the glassware workshop of the Chemistry Department at King Abdulaziz University. First, a small amount of glass wool was placed at the bottom of the chromatography column, and then the column was filled 2/3 with silica gel 60 (70–230 mesh, Grade 60) by slurry packing using *n*-hexane. After homogenization

Table 1: Hexane diethyl ether column fraction

Hexane %	Diethyl ether %	Fractions obtained	Weight (mg)	Color
100	0	1–21 (F1)	924.9	Dark brown
90	10	22–38 (F2)	811.5	Light yellow
80	20	39–57 (F3)	782.3	Black
70	30	58–71 (F4)	386.2	Yellow
60	40	72–82 (F5)	842.6	Yellow
50	50	84–97 (F6)	355.1	Dark green

with an appropriate quantity of silica gel, the oily extract (41.5 g) was added to the top of the column [21]. The initial eluent was *n*-hexane, and then the polarity increased gradually using diethyl ether, ethyl acetate, and methanol. The volume of the collected fractions was 30 mL for each. The amount of solvent consumed varied depending on TLC observations. To follow the fractionation, TLC and UV light were used, and spray reagents were collected, as shown in Table 1.

2.5 GC-MS

The bioactive compounds from hexane and hexane diethyl ether extracts of *B. elegans* seeds were analyzed using GC-MS with a GC TRACE 1300-TSQ 8000 EVO mass spectrometer from Thermo Scientific equipped with a capillary non-polar-5%-phenyl-column (Thermo-17MS, 30 m in length, 0.25 mm id, 0.15 μ m film thickness). The GC-MS detection involved an electron ionization system (70 eV). Sampling split was performed with an injection volume of 1 μ L and a pulsed split less (pulse:

25 psi, unit 1 min). Helium was used as the carrier gas, with a purity of 99.9995% at a 1 mL/min flow rate. The injection temperature was 280°C, and the heated ion source was 250°C. The oven's initial temperature was at 70°C (held for 2 min), then raised to 150°C at 25°C/min (held for 2 min), then raised to 200°C (3°C/min) (held for 2 min), and the final temperature was 280°C (6.07°C/min) with a hold time of 10 min – the total run time for a 49.03-min measurement period. NIST library mass spectra were used to identify and interpret bioactive compounds' GC-MS mass spectra.

3 Results and discussion

3.1 Fractionation of the hexane extract

Approximately 2 L of 100% *n*-hex was used for the initial elution, and 2 L of hexane and diethyl ether mixtures were added in the following proportions: (*n*-hex 9: Et2O 1), (*n*-hex

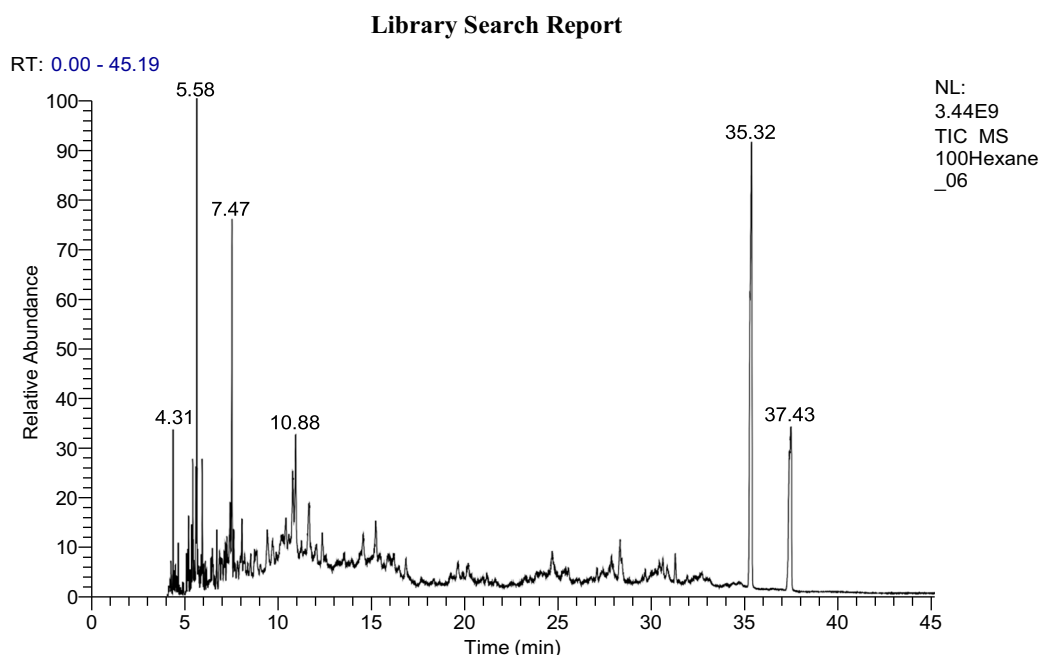
**Figure 1:** GC-MS chromatogram of methanolic crude extract fractionated with 100% hexane.

Table 2: GC-MS components of the methanolic extract fractionated using 100% hexane

No.	<i>t</i> _R *	Compound name	Molecular formula	Molecular weight	SI	RSI	Area %
1	4.31	Dodecane,2,6,11-trimethyl-	C ₁₅ H ₃₂	212	877	882	1.87
2	5.37	2,6,10-Trimethyltridecane	C ₁₆ H ₃₄	226	876	878	3.27
3	5.58	Tetradecane	C ₁₄ H ₃₀	198	848	856	9.88
4	5.88	Heptadecane,2,6,10,15-tetramethyl	C ₂₁ H ₄₄	269	854	856	2.11
5	7.47	Hexadecane,2,6,11,15-tetramethyl-	C ₂₀ H ₄₂	282	845	866	11.5
6	10.88	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C ₁₇ H ₂₄ O ₃	267	859	927	19.25
7	11.59	Phthalic acid, butyl tetradecyl ester	C ₂₃ H ₃₆ O ₄	376	797	838	3.39
8	35.31	Phenol,2,4-bis(1,1-dimethylethyl)-phosphite(3:1)	C ₄₂ H ₆₃ O ₃ P	646	869	881	32.48
9	37.42	Tris(2,4-di-tert-butylphenyl)phosphate	C ₄₂ H ₃₆ O ₄ P	662	537	551	16.26

**t*_R: retention time.

8: Et2O 2), (*n*-hex 7: Et2O 3), (*n*-hex 6: Et2O 4), and (*n*-hex 5: Et2O 5). Using 10 ml of petroleum ether and EtOAc mixtures as the mobile phase, petroleum ether 9:EtOAc 1 was used for fractions F1, F2, and F3, while for fractions F5 and F6, petroleum ether 8:EtOAc 2 was used. The fractions whose retention factors were comparable to the gathered fractions were merged and analyzed by TLC. GC-MS was used to further examine the fractions F1, F2, F3, F4, F5, and F6. Table 1 summarizes all fractions.

3.2 GC-MS analysis

3.2.1 Hexane extract

Figure 1 shows the GC-MS chromatogram of the *B. elegans* seed eluted by 100% hexane and the probable bioactive compounds present in the extract with their retention time *t*_R. Table 2 provides detailed information about the component's names, retention time, molecular formula,

Table 3: GC-MS components of the methanolic extract fractionated using hexane and diethyl ether

RT	Compound name	100% hexane	90% hexane	80% hexane	70% hexane	60% hexane	50% hexane
4.31	Dodecane,2,6,11-trimethyl-	✓	✓	✓	✓	ND	✓
5.37	2,6,10-Trimethyltridecane	✓	✓	ND	ND	ND	✓
5.58	Tetradecane	✓	✓	ND	ND	ND	✓
5.77	Butylated hydroxytoluene	✓	ND	ND	✓	ND	ND
5.89	Hexadecane,2,6,11,15-tetramethyl	✓	✓	ND	✓	ND	✓
7.46	Nonadecane,2-methyl	✓	✓	✓	ND	ND	✓
7.53	Heptadecane,2,6,10,15-tetramethyl	✓	✓	✓	✓	ND	ND
11.02	7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	✓	✓	✓	✓	ND	✓
12.37	<i>n</i> -Hexadecanoic acid	✓	ND	✓	ND	✓	✓
15.69	Oleic acid	ND	ND	✓	ND	ND	ND
16.86	Octadecanoic acid	✓	ND	✓	ND	ND	✓
24.67	Bis(2-ethylhexyl) phthalate	ND	ND	✓	ND	ND	ND
28.33	13-Docosenamide, (Z)-	ND	ND	✓	ND	✓	✓
35.31	Phenol,2,4-bis(1,1-dimethylethyl) phosphite (3:1)	✓	✓	ND	ND	ND	ND
37.36	Tris(2,4-di-tert-butylphenyl) phosphate	✓	✓	✓	✓	ND	✓

ND: not detected.

Table 4: SD and 95% confidence limit based on the retention time for isolated compounds from *B. elegans* seeds

Compound name	Mean	SD	95% confidence interval	Number of tests
Dodecane,2,6,11-trimethyl-	4.3140	0.0055	4.3140 ± 0.0048	5
2,6,10-Trimethyltridecane	5.3733	0.0058	5.3733 ± 0.0065	3
Tetradecane	5.6400	0.1039	5.6400 ± 0.1176	3
Butylated hydroxytoluene	5.7650	0.0071	5.7650 ± 0.0098	2
Hexadecane,2,6,11,15-tetramethyl	5.7400	0.1732	5.7400 ± 0.1697	4
Nonadecane,2-methyl	7.4850	0.0311	7.4850 ± 0.0305	4
Heptadecane,2,6,10,15-tetramethyl	6.2225	0.8827	6.2225 ± 0.8650	4
7,9-Di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	10.9620	0.0610	10.9620 ± 0.0535	5
<i>n</i> -Hexadecanoic acid	12.0100	0.7742	12.0100 ± 0.7587	4
Octadecanoic acid	16.4500	0.6589	16.4500 ± 0.7456	3
13-Docosenamide, (Z)-	28.2967	0.0071	28.2967 ± 0.0080	3
Phenol,2,4-bis(1,1-dimethylethyl) phosphite (3:1)	35.3350	0.0354	35.3350 ± 0.0490	2
Tris(2,4-di-tert-butylphenyl) phosphate	37.4880	0.0746	37.4880 ± 0.0654	5

molecular weight, match factor (SI), reversed match factor (RSI), and % peak area. The primary ingredients consisted of phenol, 2,4-bis(1,1-dimethylethyl)-phosphite (3:1) (32.48%), 7,9-di-tert-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione (19.25%), and tris(2,4-di-tert-butylphenyl) phosphate (16.26%). These three components represent 67.99%, while the other six components were 32.01%. A library spectrum search was conducted to yield three numerical values for each shown spectrum and a single value for the overall search. The three numbers consist of (1) a match factor (SI) between the unknown and the library spectrum, taking into account

all peaks; (2) a match factor (RSI) between the unknown and the library spectrum, disregarding any peaks in the unknown that are not present in the library spectrum; and (3) a probability value.

3.2.2 Hexane-diethyl ether extract

Diethyl ether was added to hexane in increments of 10, 20, 30, 40, and 50% to increase its polarity. The detected components in each extract are shown in Table 3, associated

Table 5: GC-MS bioactive components isolated using hexane and diethyl ether

No	Compound name	Nature of the compound	Activity	References
1	Dodecane,2,6,11-trimethyl-	Alkane	No activity reported	
2	2,6,10-Trimethyltridecane	Alkane	No activity reported	
3	Tetradecane	Hydrocarbons	Antimicrobial	[22]
4	Butylated hydroxytoluene	Phenols	Antioxidant	[23]
5	Hexadecane,2,6,11,15-tetramethyl	Alkane	Lipid biomarker, catalyst	[24]
6	Nonadecane,2-methyl	Volatile heterocyclic hydrocarbon	Antioxidant	[22,25]
7	Heptadecane,2,6,10,15-tetramethyl	Alkane	Dyeing, anti-HIV, anticancer	[24]
8	7,9-Di-tert-butyl-1-oxaspiro (4, 5) deca-6,9-diene-2,8-dione	Flavonoid	Antimicrobial, antifungal	[22,26]
9	<i>n</i> -Hexadecanoic acid	Palmitic acid (saturated fatty acid)	Anti-inflammatory, antiandrogenic, antioxidant, antibacterial, hypocholesterolemic, nematocide, pesticide, lubricant, antiandrogenic, hemolytic, 5-alpha reductase inhibitor, antipsychotic	[27,28]
10	Oleic acid	Primary amide	Antifungal	[29]
11	Octadecanoic acid	Fatty acid	Antifungal, antitumor activity, antibacterial	[30,31]
12	Bis(2-ethylhexyl) phthalate	Esters of phthalic acid	Antibacterial, larvicidal activity	[32]
13	13-Docosenamide, (Z)-	Amide compound	Antimicrobial, anti-nociceptive	[33]
14	Phenol,2,4-bis (1,1 dimethyl ethyl) phosphite (3:1)	Phenol	Anti-enterococcal, antioxidant, anticancer, antiviral, antifungal, additive	[24,34]
15	Tris (2,4-di-tert-butyl phenyl) phosphate	Phenol	Anti-enterococcal, antioxidant activities	[34]

with the corresponding retention period. Certain chemicals, such as oleic acid, were only in specific percentages. In contrast, primary chemicals persisted in all percentages despite increased polarity concentration, indicating much of it in *B. elegans*. Standard deviation (SD) and 95% confidence limit based on the retention time of chemical compounds identified in Table 3 are tabulated in Table 4, considering that oleic acid (RT = 15.69) has only one reading.

Table 5 shows the chemical compounds eluted with hexane diethyl ether and their biological activities. Phosphatene, 2,6,10,15-tetramethyl, 7,9-di-tert-butyl-1-oxaspiro (4, 5) DECA-6,9-diene-2,8-dione, *n*-hexadecenoic acid, oleic acid, octadecanoic acid, 13-docosenamide, (*Z*)-, phenol, 2,4-bis(1,1 dimethyl ethyl) phosphite (3:1), and Tris(2,4-di-tert-butylphenyl) phosphate are among these bioactive compounds, and categorized chemicals into various groups, including phenols, flavonoids, amides, and fatty acids.

4 Conclusions

GC-MS analysis of methanolic extracts of the seeds of *B. elegans* fractionated on column chromatography with hexane and hexane diethyl ether showed a composite profile of different natural compounds such as alkane hydrocarbons, phenols, flavonoids, phosphates, and fatty acids. Most of these compounds have biological activity. 2,4-Bis(1,1-dimethylethyl)-phosphite (3:1) had the highest peak area (32.48%), and heptadecane 2,6,10,15-tetramethyl had the lowest peak area (2.11%). Ethyl acetate and methanol extracts will be subject to further analysis and investigation. According to the literature survey, *B. elegans* treats inflammatory, anticancer, antiviral, antifungal, and antioxidant conditions due to the significant biological activity exhibited by these substances.

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FLateefa ALkhateeb: project co-supervisor. All authors have read and agreed to the published version of the manuscript.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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