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Phytochemical Analysis and Compound Profiling of *Boerhavia elegans* Stem Extract Using GC–MS

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ABSTRACT

Natural products are essential in drug discovery and have diverse applications in biotechnology. Naturally derived compounds are widely recognized as lead candidates in conventional drug development. Molecular docking has become a valuable tool for predicting interactions between small molecules and drug targets, assisting medicinal chemists in designing compounds with potential pharmacological effects. This study aimed to investigate the phytoconstituents of the methanol extract of *Boerhavia elegans* stem through gas chromatography–mass spectrometry (GC–MS) analysis and to conduct molecular docking studies of active components against the BCL2 receptor protein. Liquid–liquid extractions were performed using *n*-hexane, chloroform and butanol, followed by GC–MS analysis of the extracts. Molecular docking studies were conducted on active components identified by GC–MS against the BCL2 receptor protein. GC–MS analysis identified 22 compounds in the extracts, with prominent compounds, including hexadecenoic acid, octadec-9-enoic acid, various benzene dicarboxylic acid esters, hexadecanoic acid derivatives, 13-docosenamide and stigmasterol. Molecular docking revealed that γ-sitosterol, α-sitosterol and campesterol exhibited the lowest binding scores, indicating high affinity. These findings support the traditional use of *B. elegans* stem in treating various ailments, and the molecular docking results provide insights into potential mechanisms of action of the identified compounds.

1 | Introduction

Natural compounds are biologically active substances with diverse applications, and they originate from various sources, such as fungi, marine life, bacteria and plants [1, 2]. Historically, plants have served as a primary source for food, clothing, shelter and medicine, underlying their significance in human life. The World Health Organization (WHO) defines medicinal plants as those possessing therapeutic potential or serving as precursors for pharmaceutical drugs [3]. The phytochemical compounds derived from these plants play a pivotal role in the pharmaceutical and food industries, with many having undergone extensive research to develop them into commercial medicines. Their safety and environmental compatibility make medicinal plants a preferred choice for therapeutic applications, exhibiting a range of biological activities, including antioxidant, antifungal, cytotoxic and anti-inflammatory effects [4, 5].

The genus *Boerhavia* (family Nyctaginaceae) encompasses over 100 species predominantly found in tropical regions of Africa and Asia. These species are rich in phytochemicals such as alkaloids, flavonoids and phytosterols, which exhibit a broad spectrum of

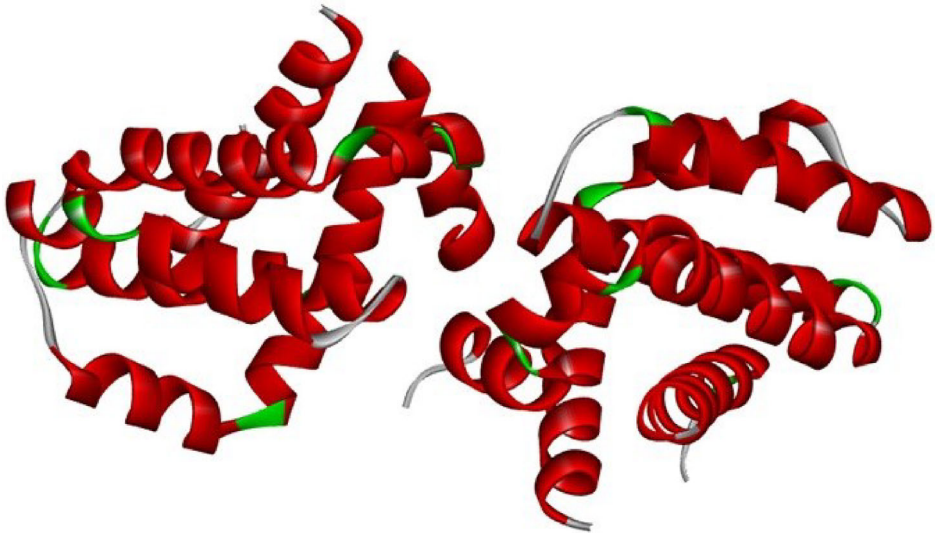


FIGURE 1 | BCl2 protein.

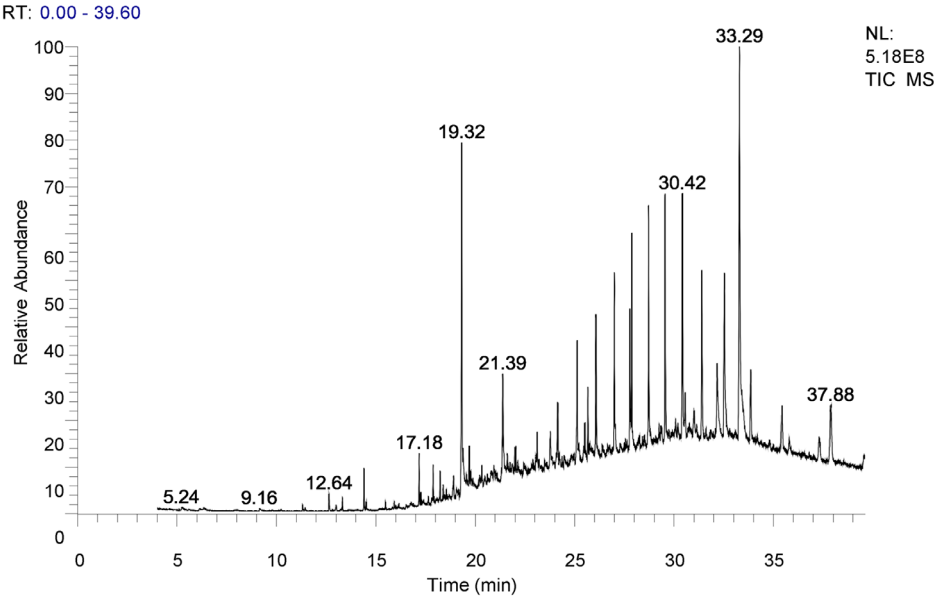


FIGURE 2 | GC–MS chromatogram of methanolic crude extract fractionated with 100% hexane.

biological and pharmacological activities [6, 7]. Among these species, *Boerhavia elegans* has gained particular interest for its therapeutic applications, demonstrating antimicrobial, anti-inflammatory and antimalarial effects [8, 9]. Morphologically, *B. elegans* is characterized by its fleshy stems, broad leaves and notable chemical composition, making it an interesting subject for phytochemical analysis [10, 11].

The analysis of phytochemical constituents typically relies on advanced analytical techniques, such as gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS), which provide high sensitivity and specificity in compound identification [12]. GC–MS, specifically, excels in separating volatile compounds based on their mass-to-charge ratios, facilitating the quantification of active principles [13]. Its robust performance is underscored by its ability to deliver reproducible results and a wide database of reference spectra, including the NIST Mass Spectral Library [14, 15]. This method analyses compounds based on their atomic and molecular weight [16]. The GC–MS instrument provides notable advantages such as efficiency, rapid analysis, minimal sample size, high resolution and increased sensitivity. It is extensively used for analysing active compounds in medicinal plants. Renowned for its robust performance, GC–MS offers consistent retention times, universal retention indices and well-resolved peaks. Additionally, it delivers highly reproducible fragmentation patterns and benefits from strong database support, including the comprehensive NIST Mass Spectral Library and the Wiley Registry [17–19].

The conventional drug discovery process remains complex and resource-intensive, prompting the integration of computational approaches like molecular docking. This technique allows researchers to simulate interactions between small molecules

TABLE 1 | Compound identification of hexane extract from *Boerhavia elegans* stem by GC–MS.

Compound	Rt	Molecular formula	Molecular weight	% Area	SI
Hydrocarbon (10 compounds)					
Tetratetracontane	30.42	$C_{44}H_{90}$	618	6.65	884
Nonacosane	26.08	$C_{29}H_{60}$	408	5.56	908
Hentriacontane	28.73	$C_{31}H_{64}$	436	9.6	889
Docosane	22.04	$C_{22}H_{46}$	310	1.49	851
Octacosane	23.11	$C_{28}H_{58}$	394	2.17	879
Pentriacontane	24.14	$C_{35}H_{72}$	492	3.4	895
1-Docosene	19.69	$C_{22}H_{44}$	308	4.25	861
1-Nonadecene	17.18	$C_{19}H_{38}$	266	2.25	880
1-Hexadecene	14.4	$C_{16}H_{32}$	224	4.51	891
1-Octadecene	17.18	$C_{18}H_{36}$	252	3.89	901
Alcohols/ether (2 compounds)					
Hexadecen-1-ol, *trans*-9-	17.18	$C_{16}H_{32}O$	240	4.55	879
4,8,12,16-Tetramethylheptadecan-4-oli	23.76	$C_{21}H_{40}O_2$	324	1.6	865
Aldehydes/ketone (2 compounds)					
3,5,7-Tris(trimethylsiloxy)-2-[3,4-di(trimethylsiloxy)phenyl]-4*H*-1-benzopyran-4-one	37.88	$C_{30}H_{50}O_7Si_5$	662	8.07	733
Bicyclo [3.3.1] nonane-2,4-dione,9,9-dimethoxy	13.32	$C_{14}H_{20}O_2$	220	2.1	992
Carboxylic//ester (10 compounds)					
Hexadecenoic acid	19.32	$C_{16}H_{32}O_2$	256	11.28	875
Octadec-9-enoic acid	21.39	$C_{18}H_{34}O_2$	282	3.27	910
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	25.67	$C_{16}H_{22}O_4$	278	2.56	912
1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester	18.23	$C_{16}H_{224}$	278	9.95	934
1,2-Benzenedicarboxylic acid, diisooctyl ester	25.67	$C_{24}H_{38}O_4$	390	1.91	914
Hexadecanoicacid,3-[(trimethylsilyl) oxy] propyl ester	24.95	$C_{22}H_{46}O_3Si$	386	1.4	645
1,2-Benzenedicarboxylic acid, diisooctyl ester	25.67	$C_{24}H_{38}O_4$	390	5.22	899
1,2-Benzenedicarboxylic acid	25.67	$C_{24}H_{38}O_4$	390	1.76	840
Hexadecenoic, methyl	18.87	$C_{17}H_{34}O_2$	270	1.88	911
1,2-Benzenedicarboxylic acid, dicyclohexyl ester	25.52	$C_{20}H_{26}O_4$	330	4.48	933
Nitrogen compounds (4 compounds)					
13-Docosenamide, (*Z*)-	27.77	$C_{22}H_{43}NO$	337	3.74	864
1,4-Methanonaphthalene-2,3-dicarbonitrile,1,4-dihydro-9,9-dimethyl-	13.32	$C_{15}H_{12}N_2$	220	2.67	986
Siloxane compound (1 compound)					
Cyclodecasiloxane, eicosamethyl-	25.75	$C_{20}H_{60}O10Si_{10}$	740	3.53	749
Steroid (4 compounds)					
Stigmasterol	32.53	$C_{29}H_{48}O$	412	4.39	621
STIGMAST-5-EN-3-OL, (3á,24S)-	33.29	$C_{29}H_{50}O$	414	14.82	710
Campesterol	32.16	$C_{28}H_{48}O$	400	6.07	610
28,33-Dinorgorgost-5-en-24-one,3-hydroxy-, (3á)-	32.53	$C_{28}H_{44}O_2$	412	6.26	596
Heterocyclic compound (1 compound)					
2*H*-1-Benzopyran,7,8-dimethoxy-2,2-dimethyl-	13.32	$C_{13}H_{16}O_3$	220	1.37	984

Abbreviation: Rt, retention time.

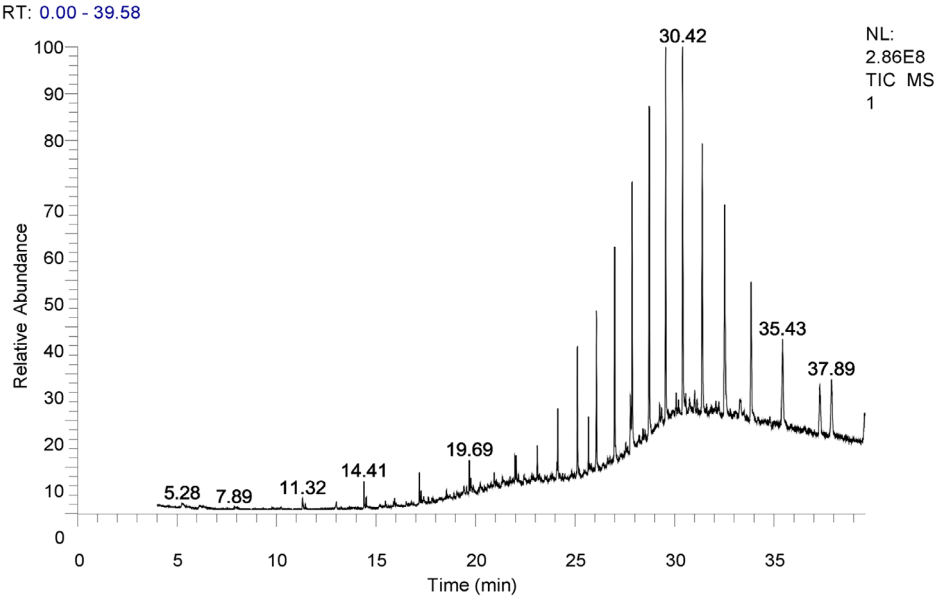


FIGURE 3 | GC–MS chromatogram of methanolic crude extract fractionated with 100% chloroform.

and protein targets, facilitating rapid screening of potential drug candidates [20, 21].

By examining binding affinities and interaction modes, molecular docking provides valuable insights into the mechanisms of action of bioactive compounds, which is often challenging to achieve through traditional experimental methods [22–25].

Despite the recognition of *B. elegans* as a promising source of phytochemicals, literature on its GC–MS analysis is limited. This study aims to bridge this gap by identifying and profiling the phytochemical constituents of *B. elegans* stem extracts through GC–MS analysis and investigating their potential biological activities via molecular docking studies against target proteins.

2 | Materials and Methods

2.1 | Chemicals

Ultrapure water delivered by a Milli *Q* system (Millipore, France) was used for dilution and preparation. All solvents were supplied by Fisher Scientific UK Ltd. The solvents were pure for analysis and HPLC-grade.

2.2 | Plant Material and Extraction Process

Stems of *B. elegans* were collected from Hadramout, Alborgat, South Yemen. The stems were cleaned with deionized water, freeze-dried and prepared for extraction. The dried samples were ground using an electric grinder and sieved through a 100-mesh standard sieve.

For extraction, 100 g of *B. elegans* stem powder was macerated in 300 mL of methanol at an ambient temperature for 3 days, with one extraction per day. After maceration, the solution was filtered through Whatman No. 41 filter paper to remove solid residues, and the filtrate was concentrated using a rotary evaporator at 40°C. This extract was then subjected to liquid–liquid extraction and repeated three times with three different solvents: *n*-hexane, chloroform and butanol. The dried samples were dissolved in a 50:50 methanol–water mixture and placed in a separating funnel. *n*-Hexane, chloroform and butanol were added sequentially, and the funnel was shaken thrice after each addition. The aqueous and organic layers were separated, and the organic layers were carefully removed for further analysis.

2.3 | Chromatographic Techniques

For column chromatography, silica gel 60 with a particle size of 230–400 mesh (Merck) was used to pack the glass columns (50 cm in length and 30 mm in diameter).

Thin layer chromatography (TLC) was performed on pre-coated silica gel plates with a layer thickness of 0.2 mm (60 F_{524}, Merck). The TLC spots were visualized under UV light at wavelengths of 254 and 366 nm, followed by treatment with a *p*-anisaldehyde-sulphuric acid reagent. This reagent was prepared by dissolving 0.05 mL of *p*-anisaldehyde in 10 mL of glacial acetic acid, followed by the addition of 85 mL of methanol and 5 mL of concentrated sulphuric acid. A 50% sulphuric acid spray reagent, composed of 50% methanol and 50% concentrated sulphuric acid, was also applied. After spraying, the TLC plates were heated to develop coloured spots, allowing for visualization and collection of the corresponding fractions.

2.4 | Gas Chromatography–Mass Spectrometry

Sample analysis was conducted using a Thermo Scientific Trace 1300 gas chromatograph and ISQ 7000 single quadrupole mass spectrometer. The oven was equipped with an Rtx-5MS column (30 m × 0.25 mm ID × 0.25 μm film thickness, Restek Corporation, Bellefonte, PA, USA). Ultra-high purity helium carrier gas was used with a constant flow rate of 1.00 mL/min.

TABLE 2 | Compound identification of chloroform extract from *Boerhavia elegans* stem by GC–MS.

Compounds	Rt	Molecular formula	Molecular weight	Area %	SI
Hydrocarbon (12 compounds)					
Tetratetracontane	24.14	$C_{24}H_{50}$	338	2.29	897
Nonacosane	28.73	$C_{29}H_{60}$	408	7.51	905
Hentriacontane	24.14	$C_{21}H_{44}$	296	2.6	879
Octacosane	25.13	$C_{28}H_{58}$	394	3.42	892
Pentriacontane	25.13	$C_{35}H_{72}$	492	3.96	863
1-Docosene	19.7	$C_{22}H_{44}$	308	8.14	891
1-Nonadecene	17.18	$C_{19}H_{38}$	266	1.01	914
1-Hexadecene	14.41	$C_{16}H_{32}$	224,	1.02	913
1-Octadecene	17.18	$C_{18}H_{36}$	252	3.39	912
1-Heptadecanol	17.18	$C_{17}H_{36O}$	256	2.08	902
Hexatriacontane	25.13	$C_{36}H_{74}$	506	4.27	910
Hexacosane	26.08	$C_{26}H_{54}$	366	5.53	914
Aldehydes/ketone					
7,9-Di-*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	18.92	$C_{17}H_{24}O_3$	276	1.01	904
Bicyclo[3.3.1]nonane-2,4-dione,3-(2,2dimethylpropylidene)-	13.32	$C_{14}H_{20}O_2$	220	1.73	984
Carboxylic/ester					
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	25.67	$C_{16}H_{22}O_4$	278	1.77	925
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	18.23	$C_{16}H_{22}O_4$	278	3.47	927
1,2-Benzenedicarboxylic acid, diisooctyl ester	25.67	$C_{24}H_{38}O_4$	390	2.05	905
1,2-Benzenedicarboxylic acid	25.67	$C_{24}H_{38}O_4$	390	11.42	904
1,2-B Benzenedicarboxylic acid, dibutyl ester	19.41	$C_{16}H_{22}O_4$	278	26.52	851
Hexanedioic acid, bis(2-ethylhexyl) ester	24.17	$C_{22}H_{42}O_4$	370	100	861
Nitrogen compounds					
13-Docosenamide, (*Z*)-	27.78	$C_{22}H_{43}NO$	337	1.92	712
Oleanitrile	25.12	$C_{18}H_{33}N$	263	4.62	687
Steroid					
á-Sitosterol	33.28	$C_{29}H_{50}O$	414	17.49	582
ç-Sitosterol	33.28	$C_{29}H_{50}O$	414	7.2	645
Lactone					
2(4*H*)-Benzofuranone,5,6,7,7A-tetrahydro-6-hydroxy-4,4,7A-trymethyl-, (6S-*cis*)-	17.03	$C_{11}H_{16}O_3$	196	2	837
Flavonoid derivative					
3,5,7-Tris(trimethylsiloxy)-2-[3,4di(trimethylsiloxy)phynyl]-4*H*-1-benzopyran-4-one	28	$C22H_{26}O_7Si$	430	1.35	737

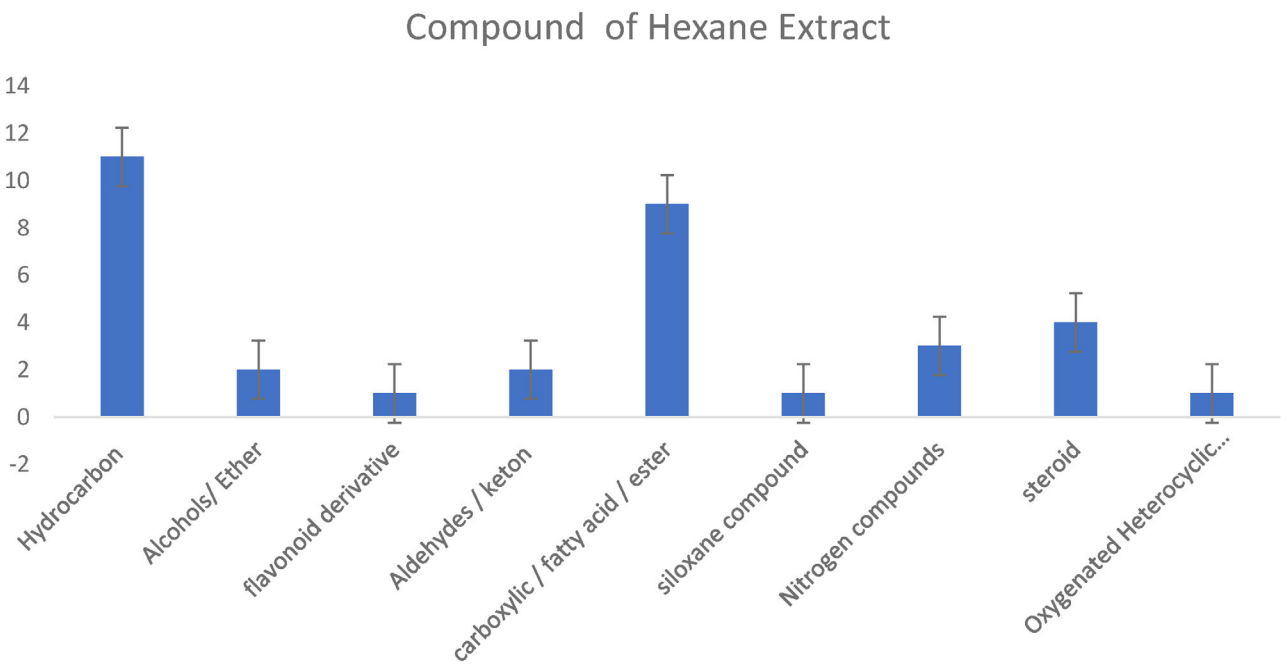
Abbreviation: Rt, retention time.

The GC oven program started at 80°C where it was held for 2 min, followed by a temperature increase of 6°C/min up to 300°C with a hold of 10 min, resulting in a total run time of 48.6 min. The transfer line and the ion source temperatures were set to 250°C. The MS, characterized by a maximum scan speed of 40,000 amu/s, was operated in the electron ionization (EI) scan mode with a mass range of 40–450 m/z, resulting in a data acquisition rate of 5 Hz.

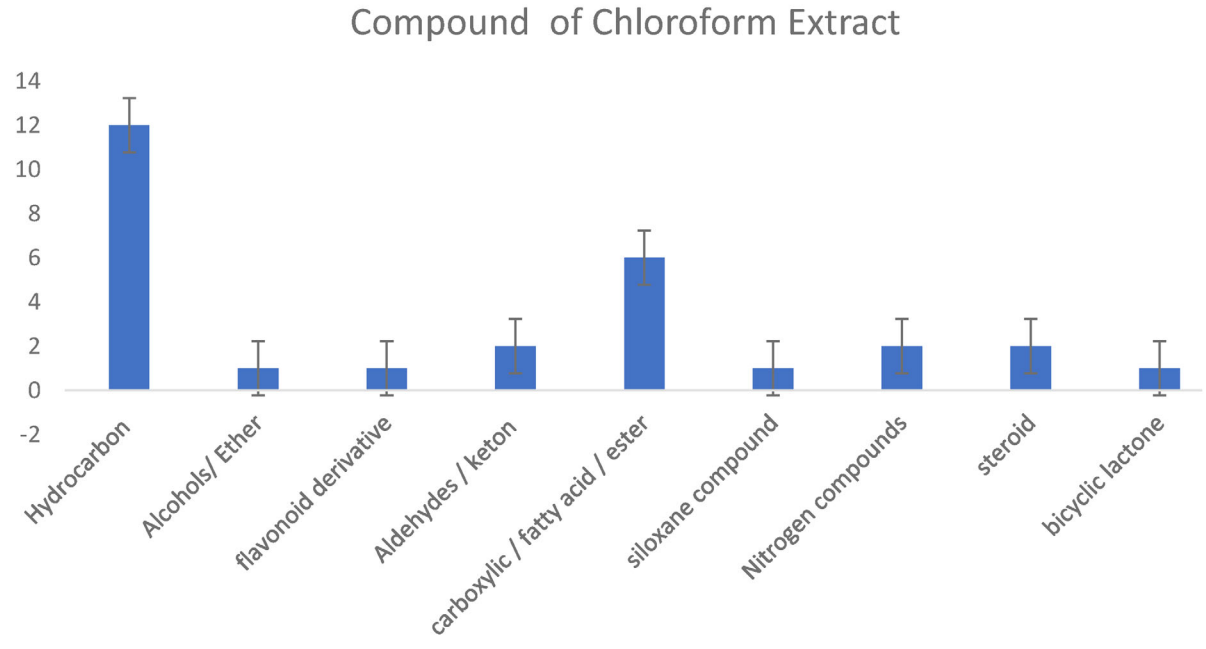
2.5 | Molecule Docking

Molecular Docking of Selected Bioactive Compounds from *B. elegans* Extracts with Target Proteins in Cancer Cells.

The chemical structures derived from the stem extracts of *B elegans* were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and saved in SDF format. The



(A)



(B)

FIGURE 4 | GC–MS profiling of *Boerhavia elegans* stem should be listed as (A) hexane fraction and (B) chloroform fraction.

structure of BCL2 protein shown in Figure 1, which is overexpressed in various cancers, was obtained from the Protein Data Bank (https://www.rcsb.org/).

Molecular docking between the ligands and protein macromolecules was performed using PyRx 0.8, whereas the three-dimensional structure, amino acid interactions and active site cavity were analysed using Discovery Studio Visualizer. The hexane and chloroform extracts from the stem were found to contain significant compounds, including stigmasterol, 1,2-benzenedicarboxylic acid, di-cyclohexyl ester, α-sitosterol, campesterol, γ-sitosterol and 7,9-di-*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione.

TABLE 3 | Molecular docking findings for compounds obtained from two extracts of *Boerhavia elegans* targeting the BCl2 protein.

Compound name	PubChem	Binding affinity (Δ*G*)
Hexadecen-1-ol, *trans*-9-	5283282	−6.1
4,8,12,16-Tetramethylheptadecan-4-oli	567149	−6.4
Bicyclo [3.3.1] nonane-2,4-dione, 9,9-dimethoxy	537288	−5.5
Hexadecanoic acid	985	−5.4
Octadec-9-enoic acid	965	−6.4
1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	21924291	−6.4
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	6782	−6.7
1,2-Benzenedicarboxylic acid, hexadecanoic	1017	−6.2
Hexadecanoic acid, methyl	8181	−4.6
1,2-Benzenedicarboxylic acid, di cyclohexyl ester	6777	−8
1,2-Benzene dicarboxylic acid, dibutyl ester	3026	−6.5
13-Docosenamide, (*Z*)-	5365369	−5.2
Olea nitrile	6420241	−4.9
Stigmasterol	5280794	−7.9
Stigmast-5-en-3-ol, (3á,24S)-	13828710	−5.8
Campesterol	173183	−7.7
ç-Sitosterol	222284	−7.9
á-Sitosterol	9548595	−8.5
2(4*H*)-benzofuranone, 5,6,7,7A-tetrahydro-6-hydroxy-4,4,7A-trimethyl-, (6S-*cis*)-	14334	−6.3
1-Heptadecanol	15076	−5.1
7,9-Di-*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione	545303	−6.9
2H-1-Benzopyran, 7,8-dimethoxy-2,2-dimethyl-	3085351	−6.3

3 | Results and Discussion

Recently, the classification of organisms has increasingly relied on markers, including morphological, anatomical, cytological, biochemical and molecular indicators. GC–MS has proven to be an effective method for accurate identification of phytocompounds. This type of GC–MS analysis serves as a fundamental step in uncovering the active ingredients of this medicinal plant and will be instrumental for further detailed investigations [26].

3.1 | Fractionation and GC–MS Analysis of the Hexane Extract

The *n*-hexane extract of *B. elegans* stems was fractionated using silica gel column chromatography. Silica gel (230–400 mesh, Merck) was packed into a glass column (50 cm length, 30 mm diameter) for gravity elusion. A stepwise gradient of hexane-diethyl ether and ethyl acetate was used as the mobile phase, beginning with 100% hexane, followed by incremental additions of diethyl ether (90:10, 95:15, 80:20 and 60:40) and then progressing with increased ethyl acetate content in diethyl ether (90:10, 80:20, 70:30, 60:40 and 50:50). Fractions of 50 mL were collected, and 189 fractions were obtained. These fractions were combined on the basis of similar retention factors observed by TLC, resulting in the following merged groups: (1–14), (15–42), (43–52), (53–62), (63–80), (81–101), (102–117), (118–128), (129–161), (162–178) and (179–189). The combined fractions were then concentrated using a rotary evaporator and analysed via GC–MS.

The compounds in the hexane fraction were identified using GC–MS analysis (Figure 2). The key components, including retention times, molecular formulas, molecular weights and concentration percentages, are listed in Table 1. The major compounds found in the hexane fraction included hexadecenoic acid, octadec-9-enoic acid, 1,2-benzenedicarboxylic acid mono(2-ethylhexyl) ester, methyl hexadecenoic acid and 13-docosenamide (*Z*)-. Diethyl ether was added in increments (10%, 20%, 30%, 40% and 50%) to increase the polarity of hexane, with the components from each extract detailed in Table S1.

3.2 | Fractionation and GC–MS Analysis of the Chloroform Extract

The chloroform extract, obtained from the second round of extraction, was fractionated using silica gel column chromatography. Elution began with 200 mL of 100% hexane, followed by a gradient of hexane-diethyl ether mixtures at ratios of 9:1, 8:2, 7:3 and 6:4. Ethyl acetate was then added to increasing concentrations of diethyl ether (90:10, 80:20, 70:30, 60:40 and 50:50). TLC analysis of the collected fractions was conducted using *n*-hexane and ethyl acetate mixtures (9:1, 8:2 and 7:3)

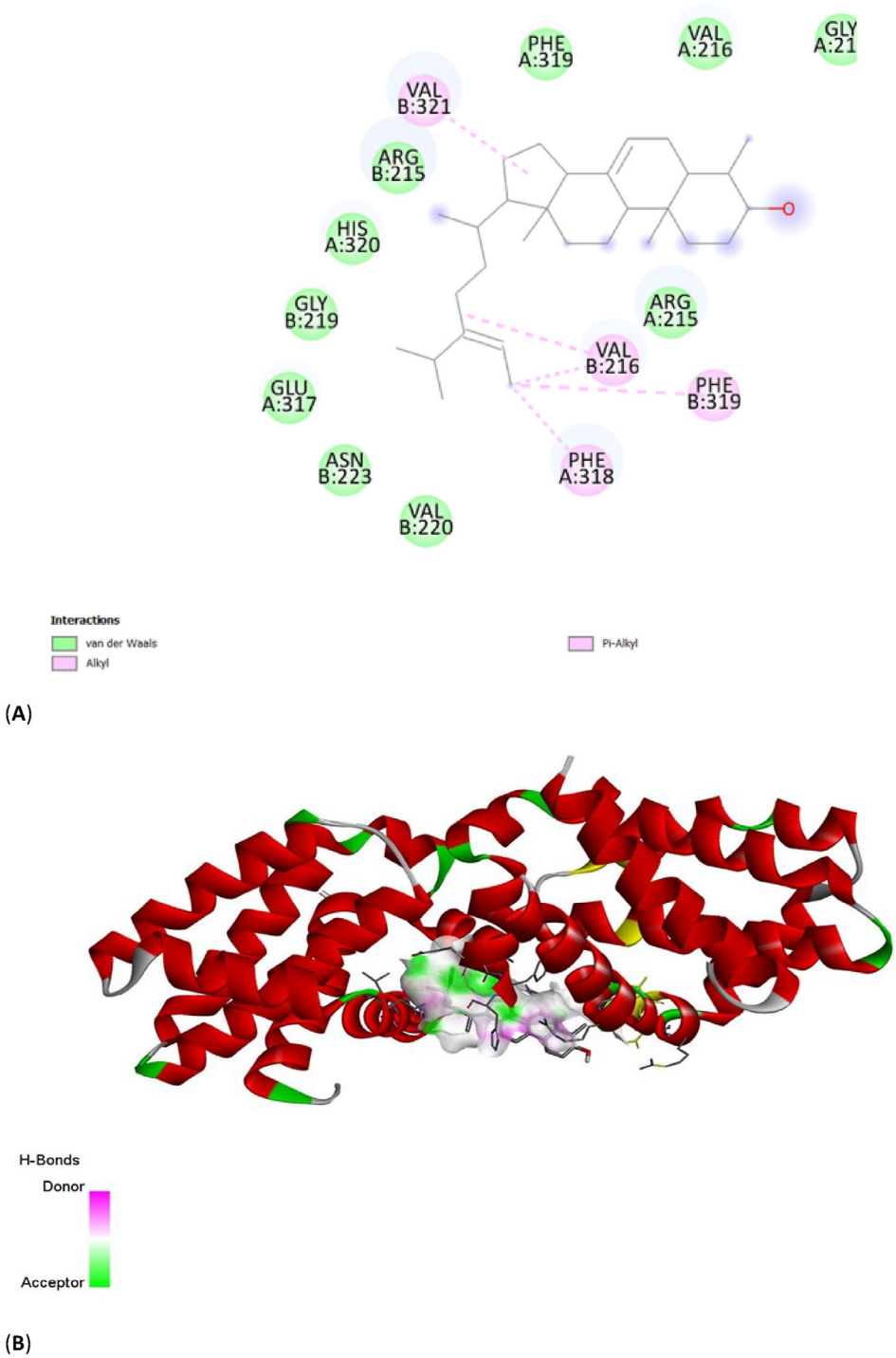


FIGURE 5 | Part (A) shows the 3D interaction between the ligand á-Sitosterol and the receptor **BCl2**; part (B) shows the interacting atoms and the bond formed between the ligand and the receptor protein in receptor.

as developing solvents. In total, 129 fractions were collected and grouped based on similar retention factors, resulting in the following merged groups: (1–14), (15–31), (32–36), (37–44), (45–53), (54–62), (63–74), (75–91), (92–111), (112–122) and (123–129). These samples were concentrated using a rotary evaporator and submitted to GC–MS analysis.

The chloroform fraction was analysed by GC–MS (Figure 3), which revealed a range of compounds. The key components, along with their retention times, molecular formulas, molecular weights and concentration percentages, are presented in Table 2. The dominant compounds in this fraction included 1,2-benzenedicarboxylic acid diisooctyl ester, 13-docosenamide (*Z*)-, 7,9-di-*tert*-butyl-1-oxaspiro (4,5) deca-6,9-diene-2,8-dione and bicyclo[3.3.1]nonane-2,4-dione,3-(2,2-dimethylpropylidene). Diethyl ether was also added in increments (10%, 20%, 30%, 40% and 50%) to increase the polarity of hexane, and the compound details are listed in Table S2.

The GC–MS analysis conducted in this study revealed that the selected plant extracts are rich in phytochemical compounds with various biological activities. Figure 4 illustrates the phytochemical constituents identified in the GC–MS profiling of *B. elegans* stem extracts for the hexane and chloroform fractions and the percentage distribution of these major compounds in the two fractions. An analysis of chloroform and hexane extracts reveals that both are predominantly composed of hydrocarbons. This is consistent with the non-polar nature of hexane and the moderate polar nature of chloroform. Additionally, both extracts contained small amounts of carboxylic acids, fatty acids, esters and other functional groups.

The diversity of compounds suggests potential varied applications or biological activities.

3.3 | Molecular Docking of Bioactive Compounds From *B. elegans* Extract Against Cancer Cell Proteins

The binding affinity (ΔG bind) indicates how well a molecule binds to its target, based on the size, structure, functional groups and interactions [27, 28]. Molecular docking is used to evaluate the likelihood and compatibility of interactions between a protein and a ligand within a complex [29]. The ΔG binding value plays a crucial role in determining the strength of ligand–receptor interactions, with lower ΔG binding values corresponding to more stable binding. The potential of natural compounds to act as inhibitors is primarily determined by their binding affinities for the target protein [30]. The ΔG values of the 22 ligands with the BCl2 receptor and the molecular docking results for the studied compounds on BCl2 are presented in Table 3.

The Table 3 indicates that the á-Sitosterol compound has the lowest binding affinity to the BCL2 receptor. It is known for its ability to reduce cholesterol levels and manage benign prostatic hypertrophy and inflammation. Campesterol and stigmasterol possess a range of beneficial properties, including antiarthritic, hepatoprotective, antiasthma, anti-inflammatory, diuretic, cancer-preventive, antioxidant and hypocholesterolemic effects [31, 32].

Figure 5 illustrates the primary interactions between the ligands and amino acids of both receptors, demonstrating hydrophobic binding within the orthosteric pocket, indicating the nonpolar nature of the compounds' identified through GC–MS analysis.

4 | Conclusion

Recognizing the therapeutic potential of *Boerhavia* species, our study on the 'Phytochemical Analysis and Compound Profiling of *B. elegans* Stem Extract Using GC–MS' investigated the phytochemical constituents of *B. elegans* stem using GC–MS. The stems were collected, prepared and extracted using solvents such as *n*-hexane and chloroform. The extracts were fractionated and analysed using GC–MS to identify various compounds, including hydrocarbons, alcohols, esters and steroids. The findings underscore the significant therapeutic potential of *Boerhavia* species and their applications in pharmaceuticals and biotechnology. Additionally, molecular docking studies of the molecules identified from the crude extract further support the relevance of *B. elegans* in medicinal research. These findings provide a foundation for further exploration of *B. elegans* in medicinal contexts.

Author Contributions

Tahreer M. ALRaddadi: investigation, writing–original draft, formal analysis. **Saleh O. Bahaffi**: project supervision, conceptualization, review and editing. **Lateefa A. ALkhateeb**: project co-supervision, editing. All authors have read and agreed to the published version of the manuscript.

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Ethics Statement

The authors have nothing to report.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data presented in this study are available upon request from the authors.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.