Archives of Pharmaceutical Sciences Ain Shams University 2023; Vol. 7(1): 31-40

OPEN ACCESS

Pharmacognosy

Research Article

GC/Ms analysis and antimicrobial activities of different extracts of Egyptian sprouting Broccoli leaves (*Brassica oleracea L. var. italica*) family Brassicaceae

Mira A. Abdelhalim[a], Eman Al-Sayed[b], Khaled A. Shams[c], and Omayma A. Eldahshan[b*]

[a]*Badr Medical District, Directorate of Health Affairs, Egyptian Ministry of Health, Cairo 11829, Egypt*
[b]*Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt*
[c]*Chemistry of Medicinal Plants Department, Pharmaceutical and Drug Industries Reserach Institiute, National Reserach Center, Dokki, Giza, 12622, Egypt*

Abstract

This study aimed to profile the phytochemical components of the hexane and dichloromethane (DCM) extracts obtained from the fresh Egyptian Broccoli leaves (*Brassica oleracea* L. *var. italica*), together with their biological activity. The extracts were analyzed by GC-MS, whereby 25 and 12 compounds were obtained from the hexane and DCM extracts, respectively. The major constituents of the hexane extract were 3-methyloctacosane (58.76%) followed by 15-methyltriacontane (15.09%) while the major constituents of the DCM extract were 3, 3, 17, 17-tetraethylnonadecane (33.71%), benzyl isothiocyanate (16.44%) and 13-methylnonacosane (13.38%). The two extracts were investigated for anti-*Helicobacter pylori* and antimicrobial activities using the agar-well diffusion method. The hexane and DCM extracts possessed significant antimicrobial activities against *H. pylori*, with an activity index of 1.25 and 1.35, respectively, and a minimum inhibitory concentration (MIC) of 7.8 µg/mL for both extracts. The hexane extract showed strong activity on *Escherichia coli* (ATCC8739) and *Staphylococcus aureus* (ATCC6538) (with MIC = 62.5 and 31.125 µg/mL, respectively), moderate activity on *Bacillus subtilis* (ATCC6633), *Klebsiella pneumoniae* (ATCC13883) and *Candida albicans (*ATCC10221) (with a MIC = 15.62 µg/mL) for the three strains. While DCM extract showed strong activity against *S. aureus* and *E. coli* (MIC =1.38 and 1.36 µg/ mL, respectively), moderate activity on *B. subtilis*, *K. pneumoniae*, and *C. albicans* (MIC = 3.9, 62.5 and 7.8 µg/mL, respectively). Both extracts showed low activity against *Aspergillus niger* with an activity index of 0.57 and 0.66, respectively.

Keywords: *Broccoli; nonacosane; monoterpenes; benzyl isothiocyanate; antimicrobial activities; H. pylori.*

***Correspondence** | Omayma A. Eldahshan; Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Cairo 11566, Egypt. Email: oeldahshan@pharma.asu.edu.eg

Citation | Abdelhalim MA, Al-Sayed E, Shams KA, Eldahshan OA, 2023. GC/Ms analysis and antimicrobial activities of different extracts of Egyptian sprouting Broccoli leaves *(Brassica oleracea L. var. italica)* family Brassicaceae. Arch Pharm Sci ASU 7(1): 31-40
DOI: 10.21608/aps.2023.189998.1107
Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.
Received 29 January 2023. **Accepted** 24 March 2023.

Published by: Ain Shams University, Faculty of Pharmacy

1. Introduction

The Brassicaceae or Cruciferae is a medium-sized family of flowering plants classified into 338 genera with 3,709 species **[1-2]**. The family is commonly known as the mustards, the crucifers, or the cabbage family. The tribe Brassicaceae is one of the 13-19 tribes which were recognized within the family, **[3-5]** and the most important economically **[5-7]**. Plants belonging to Brassicaceae have a large worldwide distribution but are not found in Antarctica **[8]**. The genus *Brassica* is one of 51 genera in the tribe Brassicaceae **[9]**. It is the most

economically important genus within this tribe **[10]**. It contains 37 different species **[6]**. *Brassica* is a genus of pungent herbs and isothiocyanates (ITC_S) are responsible for their pungent taste **[11]**. The Brassicaceae family is a rich source of bioactive phytochemicals such as terpenes, Phyto-steroids, glucosinolates, phenolics, and tocopherols **[11]** in both edible and non-edible parts.

Glucosinolates and their byproducts obtained after enzymatic hydrolysis have shown potent anticancer, **[12]** antifungal **[13]**, and antimicrobial activities **[14]**. *B. oleracea* L. *variety italica* commonly known as broccoli has flower heads, usually dark green, arranged in a tree-like structure branching out from a thick stalk while sprouting Broccoli produces multiple heads and thinner stalks. The mass of flower heads is surrounded by leaves. Phenolic components from leaves and seeds have reported antioxidant activity **[15-16]**. Flavonoids (mainly flavonols) and glucosinolates (mainly glucoraphanin) are the most predominant components of *B. oleracea* **[17]**. The main flavonols are kaempferol and quercetin, both of which exist as glycosides **[18]**. Broccoli was commonly used in traditional Chinese medicine for the treatment of dyslexia, fibromyalgia, sore throat, hyperlipidemia, and hypercholesterolemia.

The demand for safe and effective natural antimicrobial compounds has increased recently due to the increase in bacterial resistance to antibiotics. Many plant-derived compounds have been investigated for their antibacterial and antifungal actions **[19]**. Previous literature showed that hexane and DCM are the solvents of choice for successive extractions of the leaves from *B. oleracea* L. sprouts **[20, 21]**. Esters and alcoholic compounds from *B. oleracea* L. extracts have a potent antimicrobial effect against pathogenic fungi and bacteria **[19]**. The hexane extract of *B. oleracea* L. has a potent effect against *H-pylori* **[20]**. Broccoli is produced in large quantities but a high percentage of harvest (mainly leaves) is considered waste. This study aims to profile the phytochemical components of *B. oleracea* L. leaves and investigate their antimicrobial effects.

2. Experimental Section

2.1. Plant Material

Fresh leaves of *B. oleracea* L sprouts were obtained from Obour market, located about 30 Km outside of Cairo, Egypt. The plant was identified by the taxonomists at National Research Center (N.R.C), Egypt. A voucher specimen (PHG-P-Bo-236) was kept in the herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Ain Shams University.

2.2. Preparation of Hexane Extract

The intact fresh leaves (0.5 kg) of *B. oleracea* L. sprouts were cut into small pieces and then extracted with one liter of absolute hexane till exhaustion. The hexane extract was filtered through the Whatman filter paper. The filtrate was completely evaporated in a vacuum ≈ at 40 °C till dryness using a rotary evaporator apparatus. The process finally yielded about 24.92 g.

2.3. Preparation of the DCM Extract

The plant marc remained after hexane extraction was macerated in one liter of DCM for 7 days, and then washed 3 successive times with DCM. The DCM macerate was evaporated in a vacuum≈ **at** 40 °C till dryness using a rotary evaporator apparatus to yield the DCM powder extract (≈ 1.73 g).

2.4. Analysis of Hexane and DCM Extracts by GC-MS

GC Analysis was carried out using a GC HP 5890 Hewlett Packard equipped with FID and Rtx-5MS fused silica capillary column (30 m ×

0.25 mm internal diameter (I.D.), film thickness 0.25 μm) using a sample volume of 0.03 μL. The oven temperature was programmed from 50 °C to 280 °C at 3 °C/min, the injector temperature was set at 250 °C, the detector temperature was 280 °C, carrier gas, He (1.0 mL/min), automatic sample injection (Autosampler) is 0.02 μL of the extract; split: 1:30 for hexane and 1: 15 for DCM. The relative proportions of the active constituents were expressed as percentages obtained by peak area normalization. GC/MS analysis was performed on a PerkinElmer quadrupole MS system (Model 5) coupled with the GC HP 5972, equipped with an Rtx-5MS capillary column. The oven temperature was programmed from 50 °C to 280 °C at 3 °C/min, injector temperature was set at 250 °C, carrier gas, He (0.5 mL/min); Autosampler, 0.02 μL of the extract; split: 1:30 for hexane and 1:15 for DCM. The MS operating parameters were as follows: interface temperature: 280 °C, ion source temperature: 220 °C, EI mode: 70 eV, scan range: 35–500 amu.

2.5. Identification of Compounds

Mass spectra of the individual GC peaks were identified by comparison of their mass spectra and Kovats indices with those reported in the literature (Adams 2007) and GC libraries (NIST).

2.6. Antimicrobial Activity and *H. pylori* Activities of *B. oleracea* Extracts

The susceptibility of *B. oleracea* L. extracts against gram-negative, and gram-positive bacteria and fungi were determined using the agar well diffusion method, the agar plate surface was inoculated by spreading a volume of the microbial inoculum over the entire agar surface. Then, a hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer or a tip, and a volume (20–100 μL) of extract solution at the desired concentration was introduced into each well. Then, the agar plates were incubated under suitable conditions depending on the tested microorganism. The diameter of the inhibition zones was measured to the nearest mm. The anti-*H. pylori* activities were determined by the agar diffusion method. Briefly, a total volume of 100 μL of *H. pylori* suspension (1.0×10^8 colony forming units (CFUs/mL) was spread onto Mueller Hinton agar plates (BBL) containing 10% sheep blood. Then, a hole with a diameter of 6 to 8 mm is punched aseptically and a volume (100 μL) of the antimicrobial agent or extract solution at the desired concentration was introduced into the well. DMSO was used as a negative control and antibiotics amoxicillin **[27]** (0.05 mg/mL), clarithromycin **[28]** (0.05 mg/mL), and metronidazole (0.8 mg/mL) was used as a positive control. After 72 h of incubation at 37 °C under a microaerophilic condition. The inhibition zone was determined.

3. Results and Discussion

3.1. Analysis of Hexane and DCM Extracts

The hexane and DCM extracts obtained from the fresh Egyptian Broccoli leaves (*B. oleracea* L.) were subjected to GS/MS analysis **(Fig. 1)**. The yield/kg of plant material was 49.84 and 3.46, respectively. 37 compounds were identified from both extracts representing 96.18% **(Table 1)** and 89.75% **(Table 2)** of the total detected components, respectively. The major constituents of the hexane extract were 3-methyloctacosane (58.76%) followed by 15-methyltriacontane (15.09%). The hexane extract was characterized by the predominance of long-chain alkanes and fatty acids derivatives as well as triterpenes hydrocarbons, while the DCM extract was characterized by monoterpenes, long-chain alkanes derivatives, and benzyl isothiocyanate. The major constituents of the DCM extract were 3,3,17,17-tetraethylnonadecane (33.71%), benzyl isothiocyanate (16.44 %), and 13-methylnonacosane (13.38%).

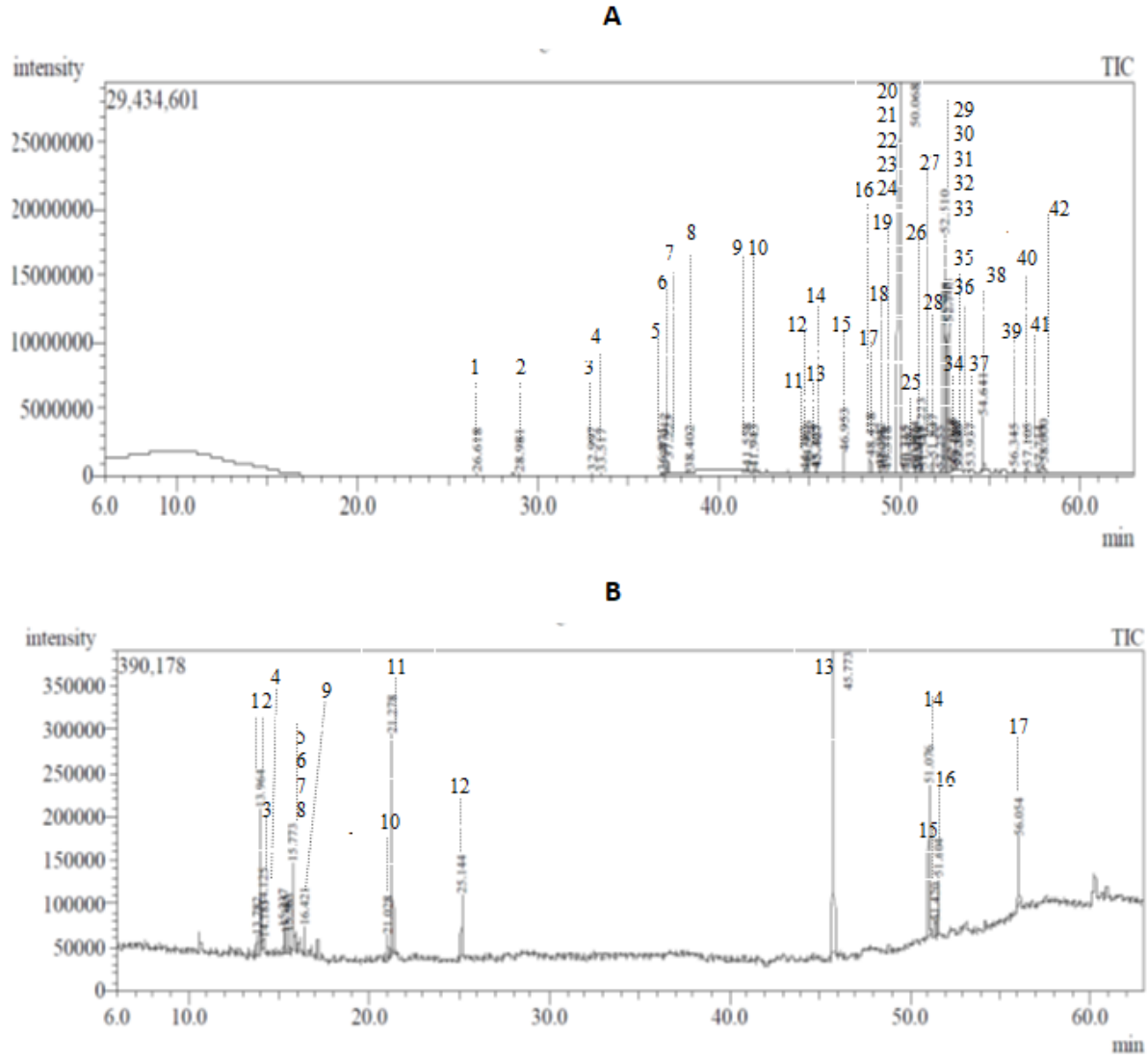

Fig. 1. GC/MS chromatogram of the fresh Egyptian Broccoli leaves (*B. oleracea* L.var. italica) hexane; A and DCM; B extracts on a DB-5 column

Table1.Tentative identified components of the hexane extract of fresh Egyptian Broccoli leaves.

NO.	Molecular weight	Formula	Compounds	Area [%] [a]	KI Calculated	Reported
1	212	$C_{14}H_{28}O$	*n*-Tetradecanal	0.07	1597	1601
2	226	$C_{15}H_{30}O$	*n*-Pentadecanal	0.06	1699	1695
3	268	$C_{17}H_{32}O_2$	Palmitoleic acid, methyl ester	0.15	1881	1879
4	270	$C_{17}H_{34}O_2$	Palmitic acid, methyl ester	0.07	1905	1910
5	294	$C_{19}H_{34}O_2$	Linoleic acid, methyl ester	0.08	2076	2076
6	-	-	u.i.	0.65	2083	-
7	310	$C_{22}H_{46}$	6,6-Diethyloctadecane	0.54	2095	2094
8	310	$C_{22}H_{46}$	3-Ethyl-3-methylnonadecane	0.09	2159	2158
9	326	$C_{21}H_{42}O_2$	Arachidic acid, methyl ester	0.14	2333	2333
10	-	-	u.i.	0.06	2354	-

11	366	$C_{26}H_{54}$	11-Methylpentacosane	0.40	2528	2529
13	366	$C_{26}H_{54}$	3-Ethyl-3-methyltricosane	0.11	2566	2565
14	-	-	u.i.	0.12	2570	-
15	380	$C_{27}H_{56}$	3,3-Diethyltricosane	0.85	2668	2673
16	394	$C_{28}H_{58}$	2-Methylheptacosane	0.98	2762	2761
17	408	$C_{29}H_{60}$	3,3,19,19-Tetraethylhenicosane	0.20	2795	2795
18	-	-	u.i.	0.07	2801	-
19	-	-	u.i.	0.01	2820	-
20	**408**	**$C_{29}H_{60}$**	**3-Methyloctacosane**	**58.76**	**2868**	**2871**
21	400	$C_{29}H_{52}$	Nonacos-1-ene	0.02	2884	2884
22	422	$C_{30}H_{62}$	4,8-Dimethyloctacosane	0.08	2895	2890
23	-	-	u.i.	0.23	2920	-
24	-	-	u.i.	0.08	2926	-
25	422	$C_{30}H_{62}$	13-Methylnonacosane	0.07	2933	2931
26	394	$C_{27}H_{54}O$	Heptacosanal	1.14	2942	2944
27	-	-	u.i.	0.09	2950	-
28	-	-	u.i.	0.57	2982	-
29	-	-	u.i.	0.14	3008	-
30	**436**	**$C_{31}H_{64}$**	**15-Methyltriacontane**	**15.09**	**3025**	**3025**
31	**436**	**$C_{31}H_{64}$**	**9-Methyltriacontane**	**6.66**	**3031**	**3034**
32	**408**	**$C_{28}H_{56}O$**	**Octacosanal**	**7.20**	**3040**	**3039**
33	-	-	u.i.	0.54	3051	-
34	-	-	u.i.	0.20	3064	-
35	-	-	u.i.	0.22	3068	-
36	386	$C_{27}H_{46}O$	Cholesterol	0.07	3075	3075
37	-	-	u.i.	0.12	3116	-
38	450	$C_{32}H_{66}$	4-Methylhentriacontane	2.71	3162	3158
39	-	-	u.i.	0.20	3271	-
40	-	-	u.i.	0.30	3320	-
41	-	-	u.i.	0.12	3360	-
42	424	$C_{30}H_{48}O$	*α*-Amyrone	0.64	3377	3373

Functional group	Total peak [%]	No. of identified compounds
Monoterpene hydrocarbons	-	-
Sesquiterpene hydrocarbons	-	-
Triterpene hydrocarbons	0.64	1
Oxygenated monoterpenes	-	-
Oxygenated sesquiterpenes	-	-
Oxygenated triterpenes	0.64	1
Total hydrocarbon compounds	86.56	
Total oxygenated compounds	9.62	
Total	96.18	

u.i., unidentified. [a] Values are expressed as relative area percentages; the major components are highlighted in bold. (Values were expressed as relative area percentages to the total identified components).

Table 2. Tentative identified components of the DCM extract of fresh Egyptian Broccoli leaves.

NO.	Molecular weight	Formula	Compounds	Area [%] [a]	KI Calculated	Reported
1	152	$C_{10}H_{16}O$	*cis*-Limonene oxide	6.1	1135	1134
3	-	$C_{10}H_{16}O$	*cis-p*-Mentha-2,8-dien-1-ol	1.92	1146	1149
4	-	-	-	0.38	1148	-
5	152	-	-	1.87	1181	-
6	-	$C_{10}H_{16}O$	*trans-p*-Mentha-1(7),8-dien-2-ol	2.03	1186	1185
7	152	-	-	0.97	1191	-
8	-	$C_{10}H_{16}O$	*trans*-Carveol	3.83	1198	1196
9	152	-	-	1.34	1220	-
10	**149**	$C_8H_8O_3$	Vanillin	1.58	1381	1382
11	226	**C_8H_7NS**	**Benzyl isothiocyanate**	**16.44**	**1389**	**1389**
12	**380**	$C_{16}H_{34}$	8-Methylpentadecane	4.51	1539	1539
13	**422**	**$C_{27}H_{56}$**	**3,3,17,17-Tetraethylnonadecane**	**33.71**	**2592**	**2592**
14	422	**$C_{30}H_{62}$**	**13-Methylnonacosane**	**13.38**	**2933**	**2931**
15	436	$C_{30}H_{62}$	3-Methylnonacosane	1.02	2955	2958
16	-	$C_{31}H_{64}$	9,15-Dimethylnonacosane	5.05	2967	2967
17	152	-	-	5.87	3252	-

Functional group	Total peak [%]	No. of identified compounds
Monoterpene hydrocarbons	13.88	4
Sesquiterpene hydrocarbons	-	-
Triterpene hydrocarbons	-	-
Oxygenated monoterpenes	13.88	4
Oxygenated sesquiterpenes	-	-
Oxygenated triterpenes	-	-
Total hydrocarbon compounds	57.67	
Total oxygenated compounds	15.46	
Total isothiocyanate *(TIC)*	16.44	1
Total	89.57	

Previous literature showed that the cutting, chewing, or processing of *Brassica* parts releases the myrosinase enzyme that gives the ITCs **[22]**. The DCM fraction of *B. juncea var. raya* contained several sulfur-nitrogen compounds, and most of the sulfur compounds were ITCs **[21]**. The DCM extract from *B. oleracea* L. has benzyl-isothiocyanate which is one of the most important secondary metabolites. Benzyl-isothiocyanate has a broad-spectrum antibacterial effect **[23]**.

Monoterpenes are common phytochemical compounds that occur in cabbage **[24]**. The current work showed that the DCM extract has four different monoterpenes (*cis*-limonene oxide, *cis-p*-mentha-2,8-dien-1-ol, *trans-p*-mentha-1(7),8-dien-2-ol and *trans*-carveol). The results were identified by comparison of their mass spectra and Kovats indices with those reported in the literature (Adams 2007) and GC libraries (NIST). Previous literature showed that the oxygenated monoterpenes have broad-spectrum antibacterial activities depending on the susceptibility of tested bacteria **[23]**. The hexane extract in the current study has fatty acids methyl esters (FAME). Previous literature showed that FAME-containing extracts have the greatest antifungal and antibacterial activities among the tested extracts **[26]**.

3.2. Antimicrobial and *H. pylori* Activity

The antimicrobial activities of *B. oleracea* L. *var. italica* leaves were tested against two gram-positive bacteria (*B. subtilis* and *S. aureus*), three gram-negative bacteria (*E. coli, K. pneumoniae*, and *H. pylori)*, and two fungi (*C. albicans* and *A. niger)*. The results of inhibition zones, activity indices (AI), minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and MBC/MIC indices for hexane and DCM extracts were shown in **Tables 3, 4, 5, and 6**. The extracts were effective against *B. subtilis*, *S. aureus*, *E.coli* and *K. pneumoniae.* Moreover, both extracts showed significant anti-fungal activity against C. *albicans* but they showed low activity against *A. niger* with an activity index less than one (with AI= 0.57 and 0.66, respectively). These results were evidenced by a higher inhibitory zone than that of the reference antibiotics and activity indices of more than one. The hexane and DCM extracts possessed significant antimicrobial activities against *H. pylori* with an activity index of 1.25 and 1.35, respectively, and MIC of 7.8 µg/mL for both extracts. This was correlated with previous literature showing the antimicrobial activity of Broccoli is mainly due to isothiocyanates, fatty acid esters, oxygenated hydrocarbons, and hydrocarbons **[29-31]**.

Table 3. Zone of inhibition [mm] of extracts of fresh Egyptian Broccoli leaves regarding reference antibiotics

Microorganism	Hexane	DCM	Control[c]	Hexane Activity Index[g]	DCM Activity Index[g]
B. subtilis (ATCC6633) [e]	24± 0.1	27±0	22±0	1.09	1.22
S. aureus (ATCC6538) [e]	23± 0.1	25±0.1	18±0.1	1.27	1.38
E. coli (ATCC8739) [d]	23± 0	26±0	19±0	1.21	1.36
K. pneumoniae (ATCC13883) [d]	25± 0.1	24±0.1	21±0.1	1.19	1.14
C. albicans (ATCC10221) [f]	26± 0	26±0	20±0	1.3	1.3
A. niger [f]	12± 0.1	14±0	21±0.1	0.57	0.66
H. pylori	25.33±0.58	27±0	20±1	1.25	1.35

The test was done using the agar diffusion technique. [b]Results are Mean ± SD of triplicate values. [c]Reference antibiotics used were gentamycin for gram-negative[d] and gram-positive [e] bacteria, fluconazole for fungi[f], amoxicillin (0.05 mg/mL), clarithromycin (0.05 mg/mL), and metronidazole (0.8 mg/mL) for *H. pylori.* Activity index[g] = (Zone of inhibition of extract/zone of inhibition of reference antibiotic. 6–9 mm: no activity; 12–15 mm: low activity; 15–18 mm: good activity; above 18 mm: significant activity.

Table 4. MIC [µg/mL] of extracts of fresh Egyptian Broccoli leaves regarding reference antibiotics

Microorganism	**Hexane**	**DCM**	**Control [a]**
B. subtilis (ATCC6633)	15.62	3.9	7.8
S. aureus (ATCC6538)	31.125	7.8	3.9
E. coli (ATCC8739)	62.5	7.8	15.52
K. pneumoniae (ATCC13883)	15.62	62.5	7.8
C. albicans (ATCC10221)	15.62	7.8	7.8
H. pylori	7.8	7.8	15.62

[a] References antibiotics used were gentamycin for bacteria, fluconazole for fungi, amoxicillin (0.05 mg/mL), clarithromycin (0.05 mg/mL), and metronidazole (0.8 mg/mL). Results are represented as ug/mL.

Table 5. MBC of extracts of fresh Egyptian Broccoli leaves regarding reference antibiotics

Microorganism	Hexane	DCM	Control [a]
B. subtilis (ATCC6633)	62.5	7.8	7.8
S. aureus (ATCC6538)	62.5	7.8	3.9
E. coli (ATCC8739)	125	15.62	15.52
K. pneumoniae (ATCC13883)	15.62	62.5	7.8
C. albicans (ATCC10221)	31.12	15.62	7.8
H. pylori	15.62	7.8	15.62

Reference antibiotics used were gentamycin for bacteria, fluconazole for fungi, amoxicillin (0.05 mg/mL), clarithromycin (0.05 mg/mL), and metronidazole (0.8 mg/mL). Results are represented as ug/mL.

Table 6. MBC/MIC of extracts of fresh Egyptian Broccoli leaves concerning reference antibiotics [a]

Microorganism	Hexane	DCM	Control
B. subtilis (ATCC6633)	4	2	1
S. aureus (ATCC6538)	2	1	1
E. coli (ATCC8739)	2	2	1
K. pneumoniae (ATCC13883)	1	1	1
C. albicans (ATCC10221)	1.99	2	1
H. pylori	2	1	1

The MBC/MIC index of the samples ≤ 4 suggested their bactericidal activity, while the MBC/MIC index of the samples > 4 demonstrated their bacteriostatic activity.

Conclusions and Future Vision

The different extracts obtained from Broccoli leaves showed a variety of phytochemical constituents which might have potential antimicrobial activity against *H. pylori, B. subtilis, S. aureus, E. coli, K. pneumoniae*, and *C. albicans.* Further in-depth phytochemical studies on hexane and DCM extracts are required to determine the active components.

Declarations

Ethics approval

Not applicable

Data availability statement

All the data supporting the findings are included in the manuscript.

Competing interests

No competing interests

Funding statement

No funding source was received

Authors' contributions statement

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by [Mira Abdellateff Abdelhalim. The first draft of the manuscript was written by [Mira Abdellateff Abdelhalim] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

4. References

1. Warwick SI, Francis, A, and Al-Shehbaz IA. Brassicaceae: Species Checklist. Plant Systematics and Evolution 2006; 259:249-258.

2. Rich TC. Crucifers of Great Britain and Ireland. Botanical Society of the British Isles; 1991.

3. Hedge IC. A systematic and geographical survey of the Old World Cruciferae; 1976.

4. Al-Shehbaz IA. The tribes of Cruciferae (Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum 1984; 65: 343-373.

5. Al-Shehbaz IA. The genera of Brassiceae (Cruciferae; Brassicaceae) in the southeastern United States. Journal of the Arnold Arboretum1985; 66: 279-351.

6. Gómez-Campo C. Anales del Instituto Botánico AJ Cavanilles 1980; 35: 165-176.

7. Gómez-Campo C. (Ed.). Biology of Brassica coenospecies. Elsevier; 1999.

8. Branca F, Chiarenza GL, Ragusa L, Argento S. Morphological characterization of the ecpgr wild brassica species COLLECTION. Acta Hortic 2013; 1005: 157-163.

9. Friend DJ. Brassica. In CRC Handbook of Flowering 2019; 48-77. CRC Press.

10. Rakow G. Species origin and economic importance of Brassica. In Brassica 2004; 3-11. Springer, Berlin, Heidelberg.

11. Ramirez D, Abellán-Victorio A, Beretta V, Camargo A, Moreno DA. Functional ingredients from Brassicaceae species: Overview and perspectives. International Journal of Molecular Sciences 2020; 21.

12. Ávila FW, Faquin V, Yang Y, Ramos SJ, Guilherme LRG, Thannhauser TW, Li L. Assessment of the anticancer compounds Se-methyl selenocysteine and glucosinolates in Se-biofortified broccoli (Brassica oleracea L. var. italica) sprouts and florets. Journal of Agricultural and food chemistry 2013; 61: 6216-6223.

13. Sisti M, Amagliani G, Brandi G. Antifungal activity of Brassica oleracea var. botrytis fresh aqueous juice. Fitoterapia 2003; 74: 453-458.

14. Ambreen A, Hira K, Ruqqia A, Sultana V. Evaluation of biochemical component and antimicrobial activity of some seaweeds occurring at Karachi coast. Pakistan Journal of Botany 2012; 44: 1799-1803.

15. Badoni H, Sharma P, Waheed SM, Singh S. Phytochemical Analysis, Antioxidant Activity and Acute Toxicity Study of Cicerarietinum and Brassica oleracea var. italica. Journal of Graphic Era University 2018; 20-29.

16. .Azhar AN, Panirselvam M, Amran NA, Ruslan MS, Samsuri S. Retention of total phenolic content and antioxidant activity in the concentration of broccoli extract by progressive freeze concentration. International Journal of Food Engineering 2020; 16.[17] Ares AM, Nozal MJ, Bernal J. Extraction, chemical characterization and biological activity determination of broccoli health-promoting compounds. Journal of Chromatography A 2013; 1313: 78-95.

18. Vallejo F, Gil-Izquierdo A, Pérez-Vicente A, & García-Viguera C. In vitro gastrointestinal digestion study of broccoli inflorescence phenolic compounds, glucosinolates, and vitamin C. Journal of Agricultural and food chemistry 2004; 52: 135-138.

19. Abdel-Shafi S, Al-Mohammadi AR, Sitohy M, Mosa B, Ismaiel A, Enan G, & Osman A. Antimicrobial activity and chemical constitution of the crude, phenolic-rich extracts of Hibiscus sabdariffa, Brassica oleracea, and Beta vulgaris. *Molecules* 2019; 24(23): 4280.

20. Moon JK, Kim JR, AhnYJ, & Shibamoto T.

Analysis and anti-Helicobacter activity of sulforaphane and related compounds present in broccoli (Brassica oleracea L.) sprouts. *Journal of Agricultural and Food Chemistry* 2010; 58(11): 6672-6677.

21. Bassan P, Bhushan S, Kaur T, Arora R, Arora S, & Vig AP. Extraction, profiling, and bioactivity analysis of volatile glucosinolates present in oil extract of Brassica juncea var. raya. *Physiology and Molecular Biology of Plants* 2018; 24: 399-409.

22. Blažević I, & Mastelić J. Free and bound volatiles of rocket (Eruca sativa Mill.). *Flavor and fragrance journal* 2008; 23(4): 278-285.

23. Li P, Zhao YM, Wang C, & Zhu HP. Antibacterial activity and main action pathway of benzyl isothiocyanate extracted from papaya seeds. *Journal of Food Science* 2021; 86(1):169-176.

24. Pinto DM, Tiiva P, Miettinen P, Joutsensaari J, Kokkola H, Nerg AM & Holopainen,J K. The effects of increasing atmospheric ozone on biogenic monoterpene profiles and the formation of secondary aerosols. *Atmospheric Environment* 2007; 41(23): 4877-4887.

25. Kotan R, Kordali S, & Cakir A. Screening of antibacterial activities of twenty-one oxygenated monoterpenes. *Zeitschrift für Naturforschung C* 2007; 62(7-8): 507-513.

26. Chandrasekaran M, Kannathasan K, & Venkatesalu V. Antimicrobial activity of fatty acid methyl esters of some members of Chenopodiaceae. *Zeitschrift für Naturforschung C* 2008; 63(5-6):331-336.

27. Mezmale L, Coelho LG, Bordin D, Leja M. Epidemiology of Helicobacter pylori. *Helicobacter* 2020; *25*: e12734.

28. O'Connor A, Furuta T, Gisbert JP, O'Morain C. Review–treatment of Helicobacter pylori infection. Helicobacter 2020; 25: e12743.

29. LeTN, Sakulsataporn N, Chiu CH, Hsieh PC. Polyphenolic Profile and Varied Bioactivities of Processed Taiwanese Grown Broccoli: A Comparative Study of Edible and Non-Edible Parts. Pharmaceuticals 2020; 13: 82.

30. Patel J, Yin HB, Bauchan G, Mowery J. Inhibition of Escherichia coli O157: H7 and Salmonella enterica virulence factors by benzyl isothiocyanate. Food microbiology2020; 86:103303.

31. Favela-González KM, Hernández-Almanza AY, De la Fuente-Salcido NM. The value of bioactive compounds of cruciferous vegetables (Brassica) as antimicrobials and antioxidants: A review. Journal of Food Biochemistry 2020; 44: e13414.