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HPLC and GC-MS Analysis of Five Medicinal Plants Used in Folk Medicine to Treat Respiratory Diseases in Jeddah, Saudi Arabia

Amal Y. Aldhebiani[1,2,*], Afnan Alqethami[3], Aljawharah A. Alqathama[4], Mohammed Alarjah[4] and Omeima A. Abdullah[4]

[1]Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O.Box 80141, Jeddah,21589, Saudi Arabia.
[2]Princess Doctor Najla Bint Saud Al Saud Distinguished Research Center for Biotechnology, King Abdulaziz University. P.O.Box 80141, Jeddah,21589 Saudi Arabia.
[3]Department of Biology, College of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia.
[4]Department of Pharmaceutical Sciences, College of Pharmacy, Umm Al-Qura University, Makkah 21955, Saudi Arabia.

Corresponding author: Amal Y. Aldhebiani (e-mail: aaldhebiani@kau.edu.sa).

Abstract Very few phytochemical studies based on ethnobotanical works were conducted in Saudi Arabia. In Jeddah, medicinal plants play a major role in healthcare. Here and for the first time, the chemical reasons behind the use of 5 medicinal plants in folk medicine in Jeddah to treat respiratory diseases were highlighted. **Objective:** The current research aims to investigate the chemical composition of 5 medicinal plants commonly used to treat respiratory diseases in folk medicine in Jeddah, by the analysis methods of High-performance liquid chromatography (HPLC) with Gas chromatography-mass spectrometry (GC-MS) analysis. **Material and methods:** Based on ethnobotanical fieldwork conducted in Jeddah over a year from August 2018 to September 2019, many plants were collected. Five plant species were analyzed first by High-Performance Liquid Chromatography (HPLC) and second by Gas chromatography-mass spectrometry (GC-MS) method. **Results:** all these five medicinal plant species contained antioxidants. A total of five standards (quercetin, rutin, caffeic acid, cinnamic acid and gallic acid) were recognized in these plants. **Conclusion:** Therefore, it can be concluded that the chemical composition of these therapeutic plants and their ethnomedicinal significance are consistent. Additionally, the outcomes showed that although *Helianthus annuus* L. *and Anethum graveolens* L. both contained antioxidants, they were rarely used in Jeddah's traditional medicine. Due to its medical value, it is crucial to call attention to it. To complete research into traditional medicine, which leads to the development of new medications, phytochemical screening must be focused on ethnobotanical investigations.

Key Words antioxidants, pharmacology, ethnomedicine, traditional knowledge, herbal medicine

1. Introduction

Saudi Arabia has one of the oldest and most extensive herbal medicine traditions, and the locals have significant knowledge of medicinal plants. Due to the significance of these plants in Prophetic medicine [1] and the Middle East's long history of medicinal plant study [2].

The researchers counted more than 400 species belonging to 89 families that were used in traditional medicine in Saudi Arabia [1]. For example, over a hundred medicinal plants were used in Makkah [3], and 85 medicinal plants were used in Jeddah [2], 124 medicinal plants were used in popular medicine in Jazan region [4]. More than 60 herbs were frequently used to treat and prevent respiratory illnesses and more than 20 plants were commonly used to immune system [3].

The ethnobotanical expertise might aid in developing a different strategy or identifying novel pharmacological compounds derived from therapeutic plants. Potential antiviral treatments can be made from the extracts or formulations of these plants. Additionally, they contain a variety of phytochemicals and other metabolites with immunity-boosting characteristics. Enhancing the human body's immune system to combat viral diseases like the Coronavirus disease (COVID-19) and its variants [5].

Herbal traditional remedies have been employed since the beginning of the COVID-19 outbreak in December 2019,

and they have had a good impact on the health of COVID-19 patients [6], [7]. For instance, a team of doctors from Wuhan University's Zhongnan Hospital highlighted the use of traditional remedies in their recommendations for the care and prevention of COVID-19. For the prevention of COVID-19, several strategies utilizing medicinal plants were suggested [6], [8].

According to a recent study by Alqethami et al. [2] 85 medicinal herbs have been utilized in Jeddah traditionally. Sixty-one of them are used to treat respiratory diseases. Here and for the first time, the chemical composition of 5 medicinal plants commonly used to treat respiratory diseases in folk medicine in Jeddah was investigated, by the analysis methods of High-performance liquid chromatography (HPLC) with Gas chromatography-mass spectrometry (GC-MS) analysis. The objectives of this research focus on revealing the chemical constituents of these plants which will help in developing new drugs or improve natural formulations to help the immune system in fighting COVID-19 and its mutations.

2. Material and Methods

A. Study area

The city of Jeddah is situated in the center of the Red Sea's eastern side on the west coast of Saudi Arabia. On the Red Sea, it boasts the biggest seaport. The low heights of the Hejaz region and the Tihama plains are to the east of the city. The arid climate of this area, with its high summer temperatures and humidity, has an immediate impact on Jeddah. The main entry point for pilgrims to Makkah and Medina, two of Islam's holiest towns and well-liked tourist destinations, is Jeddah. With a population of more than 4,082,184, Jeddah is the second-largest city in Saudi Arabia (2016 estimates). Population growth in Jeddah is 3.8% annually, which is faster than the national average. Immigration from rural areas and abroad is to blame for this increase in population [9].

B. Collection and identification of plants

Based on ethnobotanical fieldwork conducted in Jeddah over a year from August 2018 to September 2019, many plants were collected. Based on the results of phytochemical screening [10] that showed a favorable result with the test of flavonoids, samples of five plant species were chosen (Table 1). Approval was received from King Abdulaziz University (KAU), Unit of Biomedical Ethics Research Committee, Ethics Committee (Reference No 671-19). The ethical guidelines of the International Society of Ethnobiology (ISE) Code of Ethics [11] and the American Anthropological Association [10] were adopted. Ethnomedicinal data was published in [2]. In KAU herbarium (KAUH) specimens were mounted and identified using herbarium specimens, Flora of KSA [12], [13]. The authors verified the identity. Families and nomenclature adhere to the Catalogue of Life [14]. Voucher specimens were preserved in KAUH.

C. Plant extraction

Plant extracts were hydrolyzed and subjected to analysis using the method previously described by [15]. In 10 ml of 1.2 mol HCl in 50% aqueous methanol, one gram of dried plant material was dissolved. Before being utilized for high-performance liquid chromatography, the mixture was refluxed at 80 °C for two hours, cooled, made to 10 ml in the same solvent, and filtered (HPLC).

D. Standard preparation

As a standard, quercetin, rutin, caffeic acid, cinnamic acid, and gallic acid were chosen. 25 mL of HPLC-grade methanol was used to dissolve 25 mg of each standard, resulting in a stock concentration of 1 mg/mL. Later, while still using the same mobile phase, they were dissolved in a variety of concentrations to construct the standard curve. For each standard, a calibration curve was created, and the relative standard deviation (RSD) was computed. Then, the amount of any phenolic or flavonoid acids present in each sample was determined as follows:

"Concentration of standard in the sample= " "Area under the peak of detected standard in the sample X standard concentration" /"Area under the peak of standard "

E. HPLC

The HPLC model 1260 Infinity II from Agilent Technology served as the liquid chromatography facility. The Zorbbax (SB)-C18 column, with dimensions of 150 mm, 4.6 mm, and 3.5 m (USA), was used for separation, and the column oven was set to room temperature. The solvent gradient program was as follows, using a mobile phase consisting of (A mobile phase: Methanol, B mobile phase: 0.7% Acetic acid HOAC) (Table 2). Ninety-minute runtime at a flow rate of 0.8 ml/min. The injection has a 10 L volume. At wavelengths of 254nm, 268-280nm, and 320-370nm, quantitation was carried out. At room temperature, a diode array UV detector (10X sensitive, 1260 DAD HS, G7117C) and an auto-sampler (1260 Vial-sampler, G7129A) were used. Openlab CDS-Chemstation Rev. C. 01.08 [210] is the HPLC program.

F. GC-MS

Clarus 500 GC-MS (Perkin Elmer, Shelton, CT, USA) was used throughout the investigations. Gas chromatography-mass spectrometry. Version 5.4.2.1617 of TurboMass was the software controller/integrator. A Crossbond® 100% dimethyl polysiloxane (30-meter 0.25 mmID 0.25 m df), Optima® 1 GC capillary column, manufactured by Macherey-Nagel GMBH, Duren, Germany, was utilized. Helium (quality 99.9999%) served as the carrier gas, and the flow rate was 0.90 ml/min. Source (EI+): The source was 180 °C whereas the GC inlet line was 210 °C. The trap emission was 100 v, and the electron energy was 70 eV. 275 °C, injector. The oven was set to heat up from 80 °C (hold for 3 minutes) to 170 °C (rate 10 °C/min, hold for 11.0 min), and then to 280 °C (rate 10 °C/min, hold for 5.0 min). The split ratio was 50:1, and the injection volume was 1 L. By using a complete MS scan

Sample number	Scientific name	Family	Voucher specimen number	Common name	Plant part
3	*Anethum graveolens* L.	Apiaceae	AQJ_5	Dill	Seed
5	*Cinnamomum cassia* (L.) Presl	Lauraceae	AQJ_15	Cinnamon	Bark
12	*Lepidium sativum* L.	Brassicaceae	AQJ_39	Garden cress	Seed
14	*Helianthus annuus* L.	Asteraceae	AQJ_33	Common sunflower	Seed
26	*Cuminum cyminum* L.	Apiaceae	AQJ_25	Cumin	Seed

Table 1: List of plant samples including scientific name, family, voucher specimen number, common name(s), and plant part(s)

Time [min]	Methanol [%]	0.7%HOAC [%]
0.00	15	85
70.00	85	15
80.00	85	15
85.00	90	10
90.00	15	85

Table 2: A gradient program for compositions of the mobile phase with time

from 40 to 500 m/z (500 scan/sec), samples were obtained. The eluted chemicals were characterized using NIST 2008.

3. Results

A. HPLC

Flavonoids are present in all 5 plant species that were examined using the HPLC technique. Utilizing five distinct standards (Table 3, Figure 1). The findings revealed that five different plant species included the antioxidants quercetin, rutin, caffeic acid, cinnamic acid, and gallic acid (Table 4). Four samples (3,5,14,26) had rutin, two samples (12,26) contained cinnamic acid, two samples (12,14) contained gallic acid, two samples (5,26) contained quercetin, and one sample contained caffeic acid (14).

Rutin concentration in *Anethum graveolens L.* seeds was 0.105 mg/mL. (Table 4, Figure 2). Rutin and quercetin concentrations in *Cinnamomum cassia (L.)* Presl bark were 0.121 mg/mL and 0.022 mg/mL, respectively (Table 4; Figure 3). About 0.309 mg/mL of cinnamic acid and 0.304 mg/mL of gallic acid were present in the seeds of *Lepidium sativum L.* (Table 4; Figure 4). About 0.030 mg/mL of rutin, 0.112 mg/mL of gallic acid, and 0.017 mg/mL of caffeic acid were present in the seeds of *Helianthus annuus L.* (Table 4; Figure 5). *Cuminum cyminum* L. seeds had rutin concentrations of 0.272 mg/mL, cinnamic acid concentrations of 0.012 mg/mL, and quercetin concentrations of 0.086 mg/mL. (Table 4; Figure 6).

B. Gas chromatography-mass spectrometry

The extracts from all 5 plant species were analyzed by GC-MS. Thirteen chemical compounds were observed during GC-MS analysis of *Anethum graveolens* extract (Table 5; Figure 7). GC-MS analysis of *Cinnamomum cassia* extract resulted in the identification of 20 compounds (Table 6; Figure 8). Nineteen chemical compounds were detected in *Lepidium sativum* extract during GC-MS analysis (Table 7; Figure 9). Two chemical compounds were identified in *Helianthus annuus* extract (Table 8; Figure 10). Twentynine

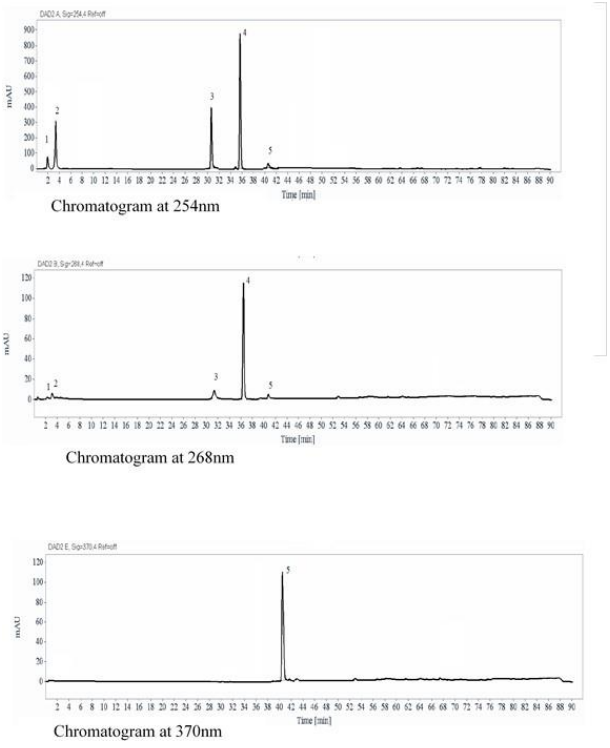

Figure 1: Chromatogram of five standards including (1) gallic acid; (2) caffeic acid; (3) rutin; (4) cinnamic acid; (5) quercetin at wavelength of (254, 268 and 370nm)

chemical compounds were observed during GC-MS analysis of *Cuminum cyminum* extract (Table 8; Figure 11).

4. Discussion

Here and for the first time, the chemical reasons behind the use of 5 medicinal plants in folk medicine in Jeddah to treat respiratory diseases were highlighted. The HPLC and GCMS analysis revealed the chemical composition of these 5 medicinal plants. *Cuminum cyminum* was frequently cited in Jeddah's traditional medical literature (72 citations [2], and the findings presented here indicate that it contains rutin, cinnamic acid, and quercetin [16]. Other unidentified peaks were also revealed in *Cuminum cyminum* extract, which could be coumarin, apigenin, luteolin, salicylic acid, or gallic acid. Additionally, the outcomes here demonstrated that *Cinnamomum cassia* included rutin and quercetin (64 citations) [2]. According to Prasad et al. [17], *Cinnamomum cassia*

Sample number	Plant species	Rutin	Cinnamic acid	Gallic acid	Quercetin	Caffeic acid
3	*Anethum graveolens*	0.105 mg/mL	NF	NF	NF	NF
5	*Cinnamomum cassia*	0.121 mg/mL	NF	NF	0.022 mg/mL	NF
12	*Lepidium sativum*	NF	0.309 mg/mL	0.304 mg/mL	NF	NF
14	*Helianthus annuus*	0.030 mg/mL	NF	0.112 mg/mL	NF	0.017 mg/mL
26	*Cuminum cyminum*	0.272 mg/mL	0.012 mg/mL	NF	0.086 mg/mL	NF

Table 3: Standards quantitative in 6 separate samples

NAME	M W	RT, min	AREA	%, RELATIVE
Camphor	152	6.69	1380163	0.271
(+)Dihydrocarvone	152	7.45	4048917	0.796
(TRANS)-Dihydrocarvone	152	7.55	2877497	0.566
Dihydrocarveol	154	7.83	1133477	0.223
Carvone	150	8.13	156379472	30.740
Piperitone	152	8.28	17950612	3.529
Thymol	150	8.91	941040	0.185
2,3-Pinanediol	170	9.19	953363	0.187
4-Isopropenyl-1-methyl-1,2-cyclohexanediol	170	9.45	1520012	0.299
Myristicin	192	11.68	1345733	0.265
Elemicin	208	12.02	802586	0.158
Resacetophenone dimethyl ether	180	12.14	2788075	0.548
Apioline	222	13.00	307385472	60.423

Table 4: GC-MS analysis results for the methanolic *Anethum graveolens* extract

NAME	M W	RT, min	AREA	%, RELATIVE
.(-)-Borneol	154	7.14	563292	0.051
trans-Cinnamaldehyde	132	7.67	1474687	0.132
Coumaran	120	7.96	11451253	1.027
p-Cumic aldehyde	148	8.08	8801058	0.790
(E)-Cinnamaldehyde	132	8.45	764745536	68.616
Phenyl glycol	138	8.81	2293413	0.206
.p-Vinylguaiacol	150	9.12	12429150	1.115
Cinnamic acid, methyl ester	162	9.98	3726971	0.334
cis-o-Methoxycinnamic acid	178	10.42	1653058	0.148
trans-2-Hydroxycinnamic acid, methyl ester	178	10.53	4342059	0.390
γ-Muurolene	204	11.44	7184384	0.645
o-Methoxycinnamaldehyde	162	11.68	40135360	3.601
.(+)-δ-Cadinene	204	11.97	8202288	0.736
α-Calacorene	200	12.15	12317391	1.105
tau.-Muurolol	222	13.48	15793299	1.417
α-Cadinol	222	13.66	2163671	0.194
α-Bisabolol	222	14.16	4748533	0.426
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	180	14.70	12457665	1.118
1-Hexen-3-ol, 5-nitro-1-phenyl-, (R*,R*)-	221	15.70	40057788	3.594
Corymbolone	236	16.57	3011198	0.270

Table 5: GC-MS analysis results for the methanolic *Cinnamomum cassia* extract

contains quercetin and kaempferol. Additionally, *Lepidium sativum* was found to contain gallic acid and cinnamic acid (40 citations) [2], while Agarwal and Verma [18] noted the presence of quercetin and kaempferol in it. Because of this, the chemical ingredients are present to promote the effectiveness of using these medicinal plants in Jeddah's ethnomedical practice. Although *Helianthus annuus* and *Anethum graveolens* were only referenced twice each in Jeddah's traditional medical literature [2], the findings indicated that both species contain antioxidants. *Helianthus annuus* included rutin, gallic acid, and caffeic acid in their entirety. Additionally, *Helianthus annuus* extract had other unidentified peaks that could be heliannone, quercetin, kaempferol, luteolin, or apigenin, as found by Guo et al. [19]. Additionally, the outcomes demonstrated that *Anethum graveolens* contains rutin. *Anethum graveolens* extract also showed other unidentified peaks that might contain quercetin or isorhamnetin [20]. The significance of *Helianthus annuus* and *Anethum graveolens* in medicine can now be emphasized. *Helianthus annuus* seeds include phenolic compounds, flavonoids, vitamins, and polyunsaturated fatty acids, which have antioxidant, antihypertensive, anti-inflammatory, antibacterial, and cardiovascular effects [19], [21]. In ethnomedicine, they are used to cure a variety of illnesses including heart disease, laryngeal and lung infections, bronchial, whooping cough, coughs, and colds [19], [22]. *Anethum graveolens* is said to have pharmacological properties, including antibacterial and antihypercholesterolemic action. It is typically used to treat colic, indigestion, and gas. On the gastrointestinal smooth muscles, it exerts an antispasmodic action [20].

NAME	M W	RT, min	AREA	%, RELATIVE
Linalol	154	6.10	520978	0.538
Benzyl nitrile	117	6.26	6207478	6.416
.(+)-Carvone	150	8.12	664822	0.687
Linalyl anthranilate	273	8.43	1291771	1.335
Estragole	148	8.76	553495	0.572
Thymol	150	8.91	13013517	13.451
Cyclohexene, 2-ethenyl-1,3,3-trimethyl-	150	9.03	369677	0.382
Benzyl Isothiocyanate	149	9.60	1155248	1.194
Eugenol	164	9.69	4955997	5.122
α-Copaene	204	10.27	401736	0.415
β-Caryophyllene	204	10.80	7618720	7.875
α-Humulene	204	11.21	617447	0.632
cis-(-)-2,4a,5,6,9a-Hexahydro-3,5,5,9-tetramethyl(1H)benzocycloheptene	204	11.50	210070	0.217
α-Selinene	204	11.59	398830	0.412
δ-Cadinene	204	11.96	974733	1.007
2,4-Dimethoxyacetophenone	180	12.13	10665928	11.024
Caryophylene oxide	220	12.67	2382862	2.463
Apiole	204	12.96	19246332	19.893
γ-Tocopherol	416	35.944	14448837	14.934

Table 6: GC-MS analysis results for the methanolic *Lepidium sativum* extract

NAME	M W	RT, min	AREA	%, RELATIVE
2,4-Decadienal, (E,E)-		8.86	25299036	40.60
2,4-Decadienal, (E,E)-		9.15	37007664	59.40

Table 7: GC-MS analysis results for the methanolic *Helianthus annuus* extract

NAME	M W	RT, min	AREA	%, RELATIVE
.(Z)-2-Heptenal	112	3.65	6656313	0.552
o-Cymol	134	4.93	13408253	1.112
γ-Terpinen	136	5.53	15986144	1.325
α,p-Dimethylstyrene	132	5.94	813890	0.067
Terpineol, cis-β-	154	6.11	1662216	0.138
Undecane	156	6.30	10085599	0.836
L-pinocarveol	152	6.74	1332552	0.110
1-(1-Ethyl-2,3-dimethyl-cyclopent-2-enyl)-ethanone	166	6.87	770108	0.064
1,3-Cyclohexadiene-1-methanol, 4-(1-methylethyl)-	152	7.45	17458060	1.447
Myrtenol	152	7.60	2075721	0.172
(-)-cis-Sabinol	152	7.78	1852926	0.154
o-Isopropylphenol	136	8.02	738756	0.061
Cumaldehyde	148	8.10	151885824	12.592
(4-Isopropyl-2-cyclohexen-1-yl)methanol #	154	8.58	5152651	0.427
2-Caren-10-al	150	8.73	29693390	2.462
3-Caren-10-al	150	8.81	75838128	6.287
Cumic alcohol	150	8.95	1544098	0.128
Ethanone, 1-(6-methyl-7-oxabicyclo[4.1.0]hept-1-yl)-	154	9.02	4928331	0.409
Silane, (4-ethylphenyl)trimethyl-	178	9.90	40756708	3.379
(Z)-β-Farnesene	204	11.14	16571838	1.374
β-Cubebene	204	11.39	12796313	1.061
Ethanone, 1-(2-hydroxy-4,5-dimethylphenyl)-	164	11.97	2549467	0.211
2-Tridecenal, (E)-	196	12.34	14783201	1.226
Carotol	222	12.92	4108275	0.341
Corymbolone	236	16.60	12647698	1.049
14,15,16-Trinor-8.xi.-labdan-6β-ol, 8,13-epoxy-	266	29.25	325730048	27.005
Bicyclo[4.1.0]heptan-2-ol, 1β-(3-methyl-1,3-butadienyl)-2α,6β-dimethyl-3β-acetoxy-	264	33.16	57751644	4.788
3-Methyl-but-2-enoic acid, 2,2-dimethyl-8-oxo-3,4-dihydro-2H,8H-pyrano[3,2-g]chromen-3-yl ester	328	33.55	5734812	0.475
Stigmasterol	412	38.49	17011470	1.410

Table 8: GC-MS analysis results for the methanolic *Cuminum cyminum* extract

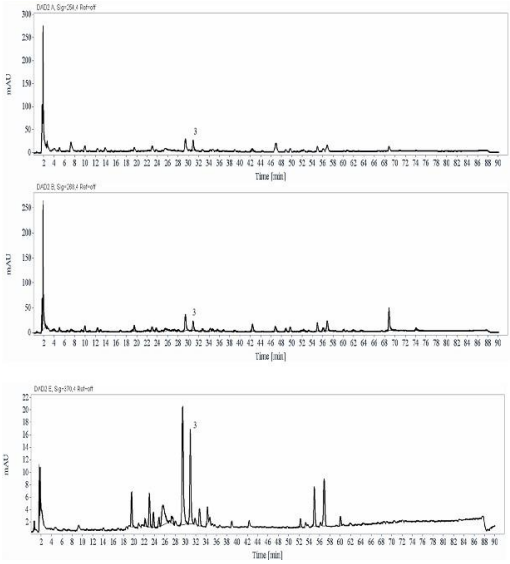

Figure 2: *Anethum graveolens* seeds' HPLC chromatograms detected at (254, 268 and 370nm). Peaks: 3= rutin

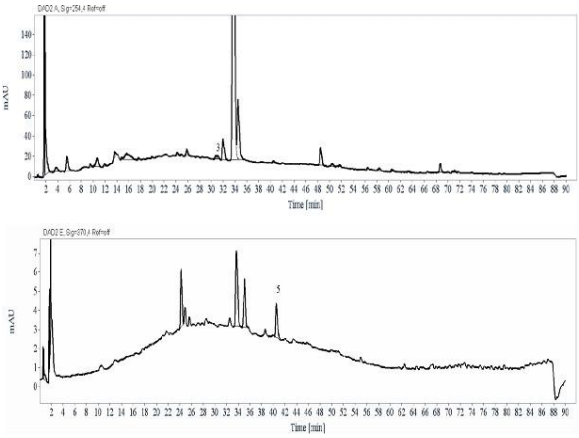

Figure 3: *Cinnamomum cassia* barks' HPLC chromatograms detected at (254 and 370nm). Peaks: 3 = rutin; 5 = quercetin

5. Conclusion

The HPLC and GCMS analysis revealed the chemical composition of these 5 medicinal plants which were used in folk medicine in Jeddah to treat respiratory diseases. The results revealed that all these 5 medicinal plants contained antioxidants. Therefore, it can be concluded that the chemical composition of these therapeutic plants and their ethnomedicinal significance are consistent. From this point on, the many metabolites can be separated to conduct pharmacological testing and develop into a secure medication for a variety of ailments. Additionally, ethnobotanical investigations must be the primary focus of phytochemical screening to finish traditional medicine research and find novel medications.

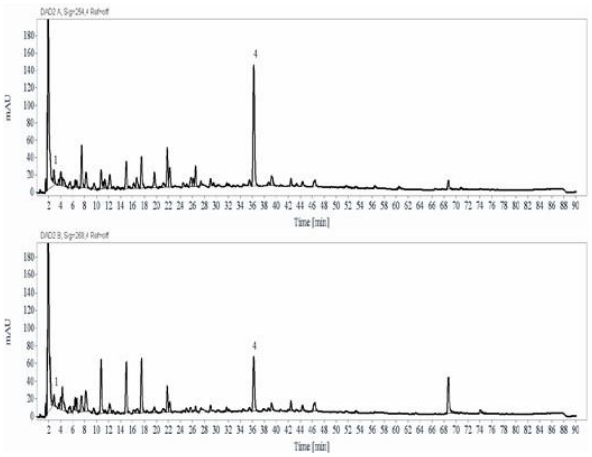

Figure 4: *Lepidium sativum* seeds' HPLC chromatograms detected at (254 and 268nm). Peaks: 1 = gallic acid; 4 = cinnamic acid

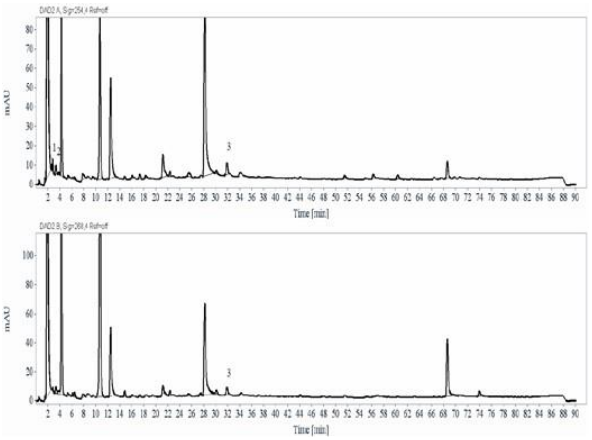

Figure 5: *Helianthus annuus* seeds' HPLC chromatograms detected at (254nm and 268nm). Peaks: 1 = gallic acid; 2 = caffeic acid; 3 = rutin

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Ethics Approval Statement

Ethics approval was received from King Abdulaziz University (KAU), Unit of Biomedical Ethics Research Committee, Ethics Committee (Reference No 671-19).

Conflict of interest

The authors declare no conflict of interests. All authors read and approved final version of the paper.

Authors Contribution

All authors contributed equally in this paper.

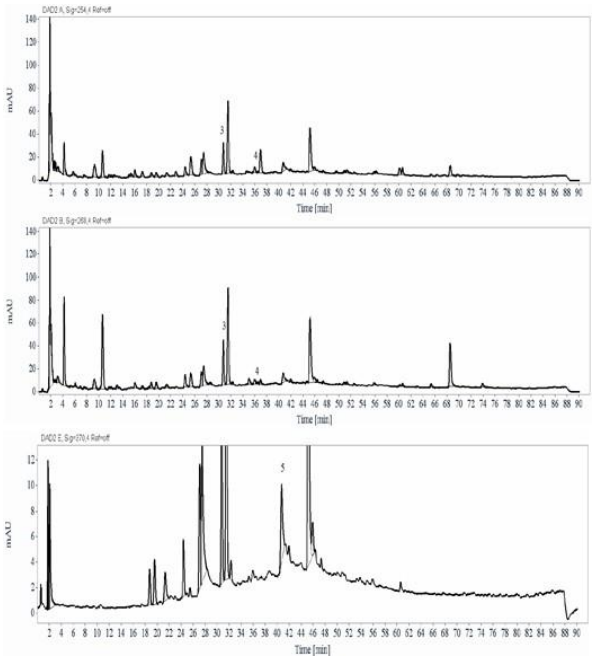

Figure 6: *Cuminum cyminum* seeds' HPLC chromatograms detected at (254, 268, and 370nm). Peaks: 3 = rutin; 4 = cinnamic acid; 5 = quercetin

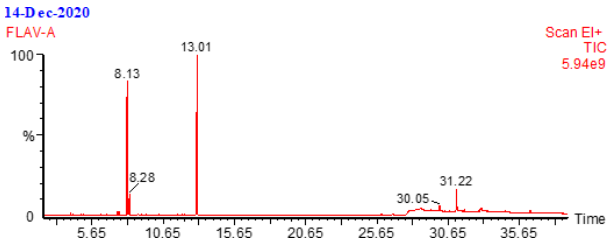

Figure 7: GC-MS chromatograph for methanolic extract of *Anethum graveolens*

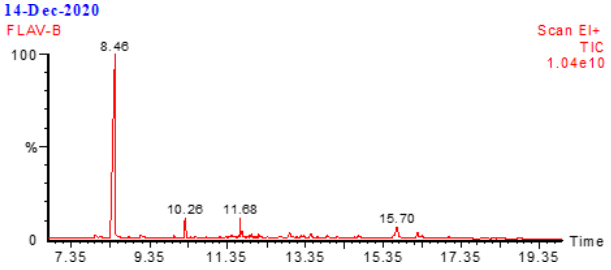

Figure 8: GC-MS chromatograph for methanolic extract of *Cinnamomum cassia*

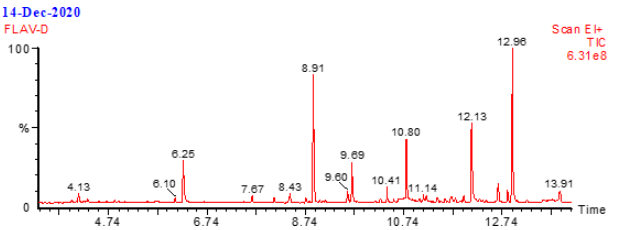

Figure 9: GC-MS chromatograph for methanolic extract of *Lepidium sativum*

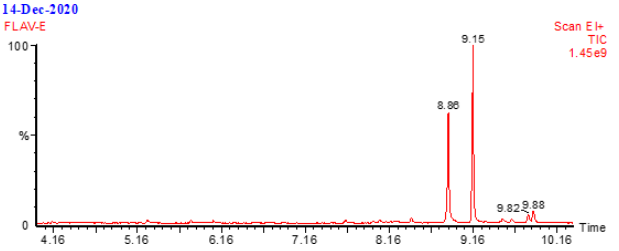

Figure 10: GC-MS chromatograph for methanolic extract of *Helianthus annuus*

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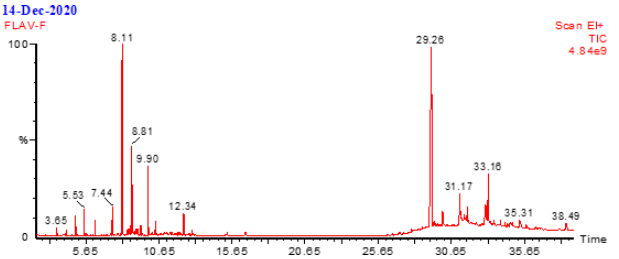

Figure 11: GC-MS chromatograph for methanolic extract of *Cuminum cyminum*

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