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Chemical Composition of Rosemary Essential Oil Extracted With Microwave Assisted and Hydrodistillation Techniques by Gas Chromatography-Mass Spectrometer

Reem Mohammad Ayad Alqarni

B.Sc. in Chemistry, Chemistry Department, Faculty of Science, Beshah University, KSA

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ABSTRACT

Background: Rosmarinus officinalis L.essential oil is of high economic value; it is widely used several industries. Variation in essential oils yield and chemical composition is affected by the geographical region and the extraction method. Therefore, there is a growing interest in developing more efficient extraction methods that maintain the activity of chemical components of the oil. Microwave-assisted hydrodistillation extraction is a promising technique for extraction of essential oils.

Aim: The purpose of this study is to study the effect of hydrdistillation and microwave-assisted hydrodistillation extraction methods on the yield and chemical structure of Rosmarinus officinalis L. essential oil. Rosmarinus officinalis L. were collected from five different geographical regions is Kingdom of Saudi Arabia (KSA); Abha, Asir, Al-Baha, Taif-Al-Hada, and Taif-Al-Haweya. The obtained oils were analyzed qualitatively and quantitatively using Gas chromatography-Mass Spectrometer to identify the components of the oils obtained using each method.

Results: Both method identified a total of 44 compounds, the major compounds were Eucalyptol (P-Cineol) (44.68-25.70%), α-Pinene (22.68-7.48%),Camphor (13.74-3.48%), Bornyl Acetate (13.57-1.09%), Borenol (10.39-5.54%), Camphen (7.32-3.26%).Results also indicated that the geographic location and the environment influenced in chemical composition of Rosmarinus officinalis L. essential oil.

Conclusion: This study compared the chemical composition and yield of rosemary essential oils from five regions in the KSA using hydrodistillation and microwave extraction methods. Results showed variations in strains and extraction methods, with microwave extraction achieving similar yields but limitations in energy consumption, extraction time, and environmental impact. Further research is needed to optimize methods, consider soil and climate effects, and determine suitable harvest time and season.

Keyword: Rosmarinus officinalis, Gas chromatography-Mass spectrometer, Essential oils analysis

INTRODUCTION

Essential oils are liquids Extracted from various plant parts and around 3000 type of essential oils have been identified so far (Van de Braak and Leijten, 1999). Essential oils are used in several industries that are heavily used by consumers such as cosmetics and pharmaceutical industries (detergents, perfumes, and soaps), food industry (soft drinks, food products), chemical industry (insecticides). Therefore, there is an increased demand on essential oils, which increases essential oils production and consumption. In turn, this demands improving the traditional methods of extracting essential oils and developing innovative extraction methods that are with improved oils quality, quantity, economic and environment friendly (Cuvelieret al., 1996).

Essential oils are extracted by various traditional methods including hydrodistillation, mechanical extraction, organic solvent extraction, etc. Generally, these methods require long extraction time which may eventually lead to loss active compounds of the oils (Han et al., 1995). Moreover, these methods are not flexible in terms of changing its factors to select certain compounds (Han et al., 1995).There is a growing interest in developing more efficient extraction methods, referred to as advanced extraction methods, these include microwave and assisted extraction, etc. Hydrodistillation is one of the most widely used extraction methods since eighty's and until now. It owes its popularity to its low cost and its green approach compared to the advanced methods or solvent extraction (Masango, 2005).Hydrodistillation exhibit some drawbacks such as the produced oil quality, and it is time consuming. While microwave assisted extraction depend on generating the heat inside the plant/plant part itself leading to internal heating, resulting in reduced heating time, and improving the oil yield (Goullieux and Pain, 2005). Despite that there is an increased interest in extracting essential oils using microwave, few studies have addressed the variation in essential oil components and yield in plants. Consequently, optimizing the extraction method is of utmost importance to preserve the essential oils activity,

quality, reduce production costs and time, and cost effective in terms of energy consumption, while maintaining the precious active compounds in the oils (Hinneburg et al., 2005). As well, it is important to separate these compounds. The compounds can be volatile (a mass unit up to 220) and semi-volatile (a mass unit up to 400). Such range is adequate for the gas chromatography analysis. Hence, the gas chromatography is the basis of many techniques. Overall, GC-MS and GC-FID are used to identify chemical compounds in essential oils (Marriott et al., 2002).

Rosemary has been used for its antiseptic and food preservative properties for years all over the world. It is also a known kitchen herb with antimicrobial, anti-inflammatory, anti-oxidant and anti- carcinogenic activity (Cuvelieret al., 1996).

It is apparent the rosemary essential oil is of economic importance and it is also natural and save alternative to many of the used chemicals. Chemical composition of rosemary essential oils is affected by geographical region and the extraction method, which affects its yield, quality, economic value and activity. Although several studies were carried-out to investigate the chemical composition of R. officinalis L. essential oil, from different geographical locations, there is no study of the chemical composition of essential oil from rosemary grown in KSA, to assess the effect of traditional and advanced extraction methods, hydrodistillation and microwave assisted extraction. Therefore, the main of this research is to study the effect of hydrdistillation and microwave-assisted hydrodistillation extraction methods on the yield and chemical structure of Rosmarinusofficinalis L. essential oil. Rosmarinusofficinalis L. were collected from five different geographical regions is Kingdom of Saudi Arabia (KSA); Abha, Asir, Al-Baha, Taif-Al-Hada, and Taif-Al-Haweya.

METHODS

1. Chemicals

All Standards and reagents were GC grade Sigma-Aldrich Chemicals, USA.

2. Plant material

Rosemary leaves (Rosmarinusofficinalis L., Labiatae)were collected in February 2015from different regions in Kingdom of Saudi Arabia such as Abha, Asir, Al-Baha, Taif-Al-Hada and Taif-Al-H aweya as shown in the map below (Figure 1), Where people frequently use this plant in traditional medicine. The collection plant was taxonomically kindly identified at the department of Biology, faculty of Sciences, Taif University by Professor Yassin Al-Sodany.

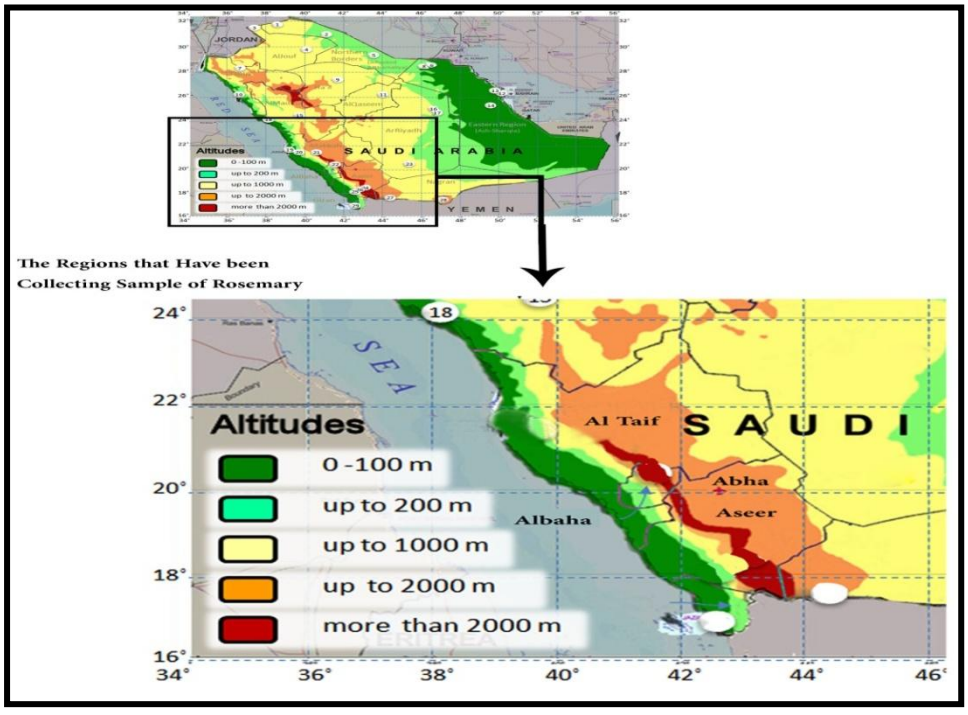

Figure 1: The Regions that have been Collecting Samples of Rosmarinusofficinalis L.

3. Essential Oil Extraction

a) Hydrodistillation Method

Typically 300 grams of the fresh leaves were cut into smaller pieces and hydro distilled for 3h using Clevenger type apparatus, producing a less colored essential oils at a yield of 0.5-0.9 %. Oils were dried over 2 grams of

anhydrous sodium sulfate and filtered. The obtained oils were collected in a sealed vial and stored at -5 ºC.

b) Microwave-Assisted Hydrodistillation Apparatus and Procedure Microwave distillation (MD) was performed at atmospheric pressure with a Milestone NEOS &GR microwave apparatus, using a fixed power of 650 W for 30 min. Temperature was monitored by an external infrared sensor.
The fresh plant materials (300 g, each) were ground into small pieces, then placed in a flat bottom flask (5 L) with 700 mL water, and subjected to microwave distillation (MD) using a Clevenger-type apparatus with cooling bath (-5 ◦ C) system (30 min) (yield (v/w):between 0.6- 1.0%.

4. Essential Oil Analysis

4.1 Sample Preparation for GC/MS Analysis

A quantity of 10 µl from the essential oil was mixed with 1 ml of GC grade n-hexane. The new mixture was agitated for one min, and 1 µl was injected into the GC–MS by using the auto sampler injector.

4.2 Gas Chromatography-Mass Spectrometry System

The aim of the selection and definition of chromatographic condition is to achieve a proper separation of the components of the oil, both for the qualitative analysis, and for the proper quantification.
The analysis of the samples was performed using gas chromatograph (GC, Model CP-3800, Varian, and Walnut Creek, CA, USA) coupled with a mass spectrometer (MS, Model Saturn 2200, Varian) and auto sampler (Model Combi Pal, Varian) system. The separation was done using a VF-5ms fused silica capillary column (5% phenyl-dimetheylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 µm, Varian). For MS detector, electron impact (EI) ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant low rate of 1 ml/min. Injector and mass transfer line temperature were set at 250 and 300 ^{o}C, respectively. The Optimization condition for oven temperature was programmed for 1 min at 60 ^{o}C, 60-120 ^{o}C at 1.5 ^{o}C/min then hold for 1 min and 150-260 ^{o}C at 10 ^{o}C/min then hold for 1 min at 260 ^{o}C, solvent delay time 3 min. The injection of the samples was carried out with the auto-sampler for 1 µl with a split ratio 1/20. The conditions of analysis and specification of the instrument were optimized for a better separation and resolution. Identification of components was based on matching with mixed standard and Wiley and NIST electronic library.

RESULTS

A total of Forty-four compounds were identified in the essential oils extracted from different Rosmarinusoficinalis L. plants collected in Kingdom of Saudi Arabia and extracted by hydrodistillation (HD) and microwave-assisted hydrodistillation (MAHD). Analysis of the essential oils using GC/MS technique.The retention time composition and the percentage of different oil.

1.1 Chemical Composition of Essential Oils Extracted from Abha Samples

In Abha sample, (Figure 2) and (Table 1), Thirty-eight compounds extracted with hydrodistillation technique were identified. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (P-Cineol) (36.98%), α-Pinene (20.09 %), Camphor (8.92%), Borenol (6.91%), Camphen (5.56%), β-Pinene (3.87%), Verbenone (3.11%), D-Limonene (2.81%), β-linalool (2.55%), α-Terpineol (2.15%), Bornyl Acetate (1.62%), Myrcene (1.36%), α-Phellandrene (1.33%), 4-Terpineol (1.22%), Geraniol (1.19%). Also, In Abha sample, Thirty-eight compounds extracted with microwave technique were identified. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (P-Cineol) (26.98%), α-Pinene (20.60%), Camphor (10.05%), Camphen (6.03%), Borenol (5.54%), β-Pinene (3.62%),D-Limonene (3.60%), Verbenone (2.91%), β-Caryophyllene (2.60%), α-Phellandrene (1.96%), α-Terpineol (1.94%), Bornyl Acetate (1.66%), Beta-linalool (1.94%), Myrcene (1.90%), Gamma-Terpinene (1.75%), Para-Cymene (1.51%), 4-Terpineol (1.39%), Alpha-Terpinene (1.29%), β-linalool (1.48%), Geraniol (1.45%), γ-Terpinene (1.34%), P-Cymene (1.15%).

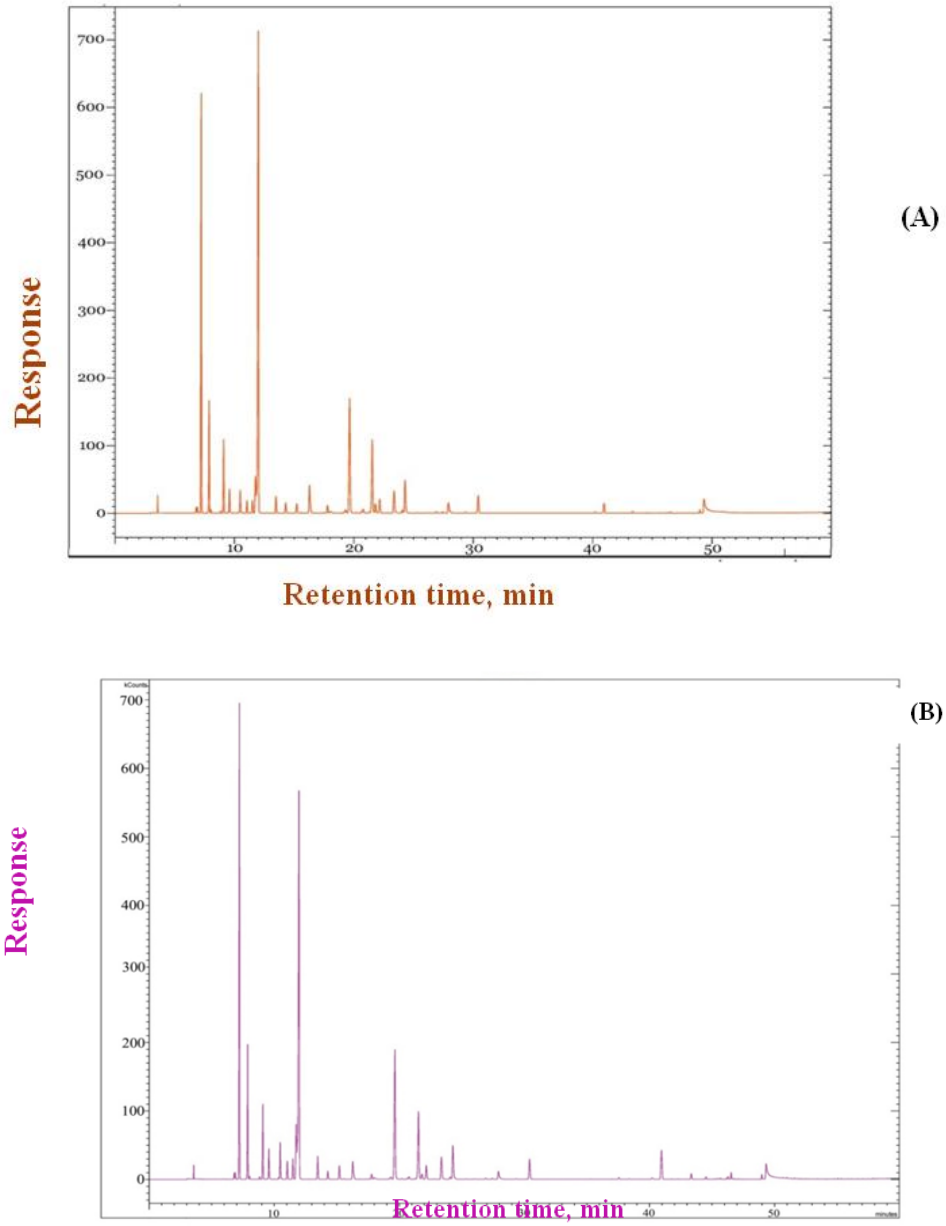

Figure 2: Typical GC-MS chromatogram for rosemary essential oil extracted by hydrodistillation (A) and microwave assisted technique (B) collected from Abha, KSA.

Table 1: Retantion time, chemical composition and relative percent of Abha, extracted by HD and MWHD rosemary essential oil analysed by GC-MS.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
1	3.56	Octane (C8)	44512	0.44	43084	0.38
2	6.80	Tricyclene	23388	0.23	27561	0.24
3	6.88	α-Thujene	29644	0.29	28671	0.25
4	7.21	α-Pinene	2.027e+6	20.09	2.315e+6	20.60
5	7.87	Camphen	561666	5.56	677616	6.03
6	8.02	Verbenene	15401	0.15	13908	0.12
7	8.83	Sabinene	10575	0.10	13187	0.11
8	9.09	β-Pinene	390708	3.87	407523	3.62
9	9.57	Myrcene	137320	1.36	163124	1.45
10	10.48	α-Phellandrene	134865	1.33	220469	1.96
11	11.04	α-Terpinene	76908	0.76	-	-

12	11.50	P-Cymene	81527	0.80	130107	1.15
13	11.76	D-Limonene	284238	2.81	404893	3.60
14	12.00	Eucalyptol (P-Cineol)	3.730e+6	36.98	3.032e+6	26.98
15	13.48	γ-Terpinene	111459	1.10	150980	1.34
16	14.29	Trans-Sabinene hydrate	73887	0.73	55691	0.49
17	15.21	α- Terpinolene	71023	0.70	99762	0.88
18	16.29	β-linalool	257702	2.55	166542	1.48
19	17.79	Chrysathenone	59865	0.59	37442	0.33
20	19.30	Verbenol	32935	0.32	22202	0.19
21	19.64	Camphor	1.018e+6	8.92	1.130e+6	10.05
22	20.63	Pinocamphone	10759	0.10	-	-
23	20.77	Pinocarvone	35990	0.35	23638	0.21
24	21.54	Borenol	696957	6.91	622570	5.54
25	21.81	Cis-Pinocamphone	69132	0.68	39963	0.35
26	22.16	4-Terpineol	123624	1.22	119848	1.06
27	23.37	α-Terpineol	217628	2.15	217977	1.94
28	24.06	Isobornylformate	28029	0.27	20787	0.18
29	24.29	Verbenone	314443	3.11	328019	2.91
30	26.89	Cis-Citral	-	-	10304	0.09
31	27.92	Geraniol	120089	1.19	90437	0.80
32	30.41	Bornyl Acetate	163889	1.62	187389	1.66
33	37.55	α-Copaene	2615	0.02	15268	0.13
34	38.38	Linolyl acetate	4530	0.04	4561	0.04
35	40.21	Methyl Eugenol	10988	0.10	14862	0.13

Table 1: Cont.

NO	**Retantion time**	**Compound Name**	**HD**		**MWHD**	
			Area	**% Percentage**	**Area**	**% Percentage**
36	40.95	β-Caryophyllene	96125	0.95	292733	2.60
37	43.34	α-Humulene	12193	0.12	41017	0.36
38	46.53	Δ-Cadinene	6279	0.06	42010	0.37
39	48.98	Caryophyllene oxide	17209	0.17	23966	0.21
	Total		10085102	100	11235111	100

1.2 Chemical Composition of Essential Oils Extracted from Asir Samples

In Asir sample, (Figure 3) and (Table 2), Thirty-six compounds extracted with hydrodistillation technique were identified from the oil. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (Para-Cineol) (35.81%), α-Pinene (19.92%), Borenol (7.79%), Verbenone (5.67%), β-linalool (3.74%), Camphor (3.63%), Camphen (3.26%), β-Pinene (2.89%), α-Terpineol (2.51%), Geraniol (2.41%), D-Limonene (2.24%), Myrcene (1.34%), 4-Terpineol (1.17%), Bornyl Acetate (1.09%), Chrysathenone (1.01%), Cis-Pinocamphone (1.01%). In the same region, Thirty-four compounds extracted with microwave technique were identified from the oil. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (P-Cineol) (28.02%), α-Pinene (22.68%), Borenol (7.51%), Verbenone (6.58%), Camphen (3.53%), Camphor (3.48%), β-Pinene (3.22%), β-linalool (3.19%), D-Limonene (2.55%), α-Terpineol (2.54%), Geraniol (2.48%), β-Caryophyllene (1.55%), Myrcene (1.41%), Bornyl Acetate (1.16%), 4-Terpineol (1.11%), Chrysathenone (1.10%), γ-Terpinene (1.05%).

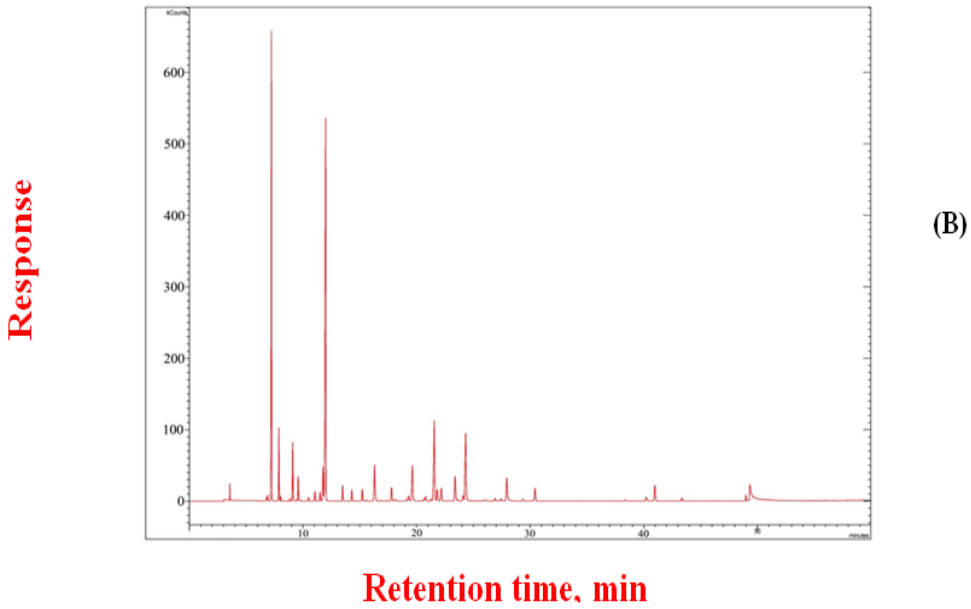

Figure 3: Typical GC-MS chromatogram for rosemary essential oil extracted by hydrodistillation (A) and microwave assisted technique (B) collected from Asir, KSA.

Table 2: Retantion time, chemical composition and relative percent of Asir, extracted by HD and MWHD rosemary essential oil analysed by GC-MS.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
1	3.56	Octane (C8)	59042	0.37	39594	0.42
2	6.80	Tricyclene	20204	0.12	13368	0.14
3	6.88	α-Thujene	34034	0.21	24262	0.25
4	7.22	α-Pinene	3.099e+6	19.92	2.136e+6	22.68
5	7.87	Camphene	508659	3.26	333074	3.53
6	8.02	Verbenene	33625	0.21	20694	0.21
7	8.83	Sabinene	17488	0.11	11068	0.11
8	9.09	β-Pinene	450009	2.89	303850	3.22
9	9.57	Myrcene	208506	1.34	133038	1.41
10	10.48	α-Phellandrene	28077	0.18	18632	0.19
11	11.04	α-Terpinene	79996	0.51	-	-
12	11.50	P-Cymene	90495	0.58	52065	0.55
13	11.76	D-Limonene	349413	2.24	240971	2.55
14	12.02	Eucalyptol (P-Cineol)	5.571e+6	35.81	2.639e+6	28.02
15	13.48	γ-Terpinene	133320	0.85	99089	1.05
16	14.28	Trans-Sabinene hydrate	143670	0.92	71204	0.75
17	15.21	α- Terpinolene	111357	0.71	78096	0.82
18	16.30	β-linalool	582024	3.74	300450	3.19
19	17.79	Chrysathenone	157542	1.01	104429	1.10
20	19.28	Verbenol	80000	0.51	40746	0.43
21	19.64	Camphor	600243	3.63	328309	3.48
22	20.62	Pinocamphone	24756	0.15	-	-
23	20.77	Pinocarvone	77450	0.49	35679	0.37
24	21.57	Borenol	1.213e+6	7.79	707285	7.51
25	21.82	Cis-Pinocamphone	158436	1.01	85543	0.90
26	22.16	4-Terpineol	183323	1.17	104995	1.11
27	23.38	α-Terpineol	391864	2.51	239430	2.54
28	24.10	Isobornylformate	77034	0.49	44291	0.47
29	24.33	Verbenone	883445	5.67	619862	6.58
30	26.89	Cis-Citral	-	-	24939	0.26

31	27.93	Geraniol	376205	2.41	234151	2.48
32	30.42	Bornyl Acetate	170040	1.09	109249	1.16
33	37.55	α-Copaene	-	-	-	-
34	38.36	Linolyl acetate	17051	0.10	-	-
35	40.19	Methyl Eugenol	64831	0.41	32047	0.34

Table 2: Cont.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
36	40.96	β-Caryophyllene	93788	0.60	146170	1.55
37	43.34	α-Humulene	15816	0.10	16206	0.17
38	46.53	Δ-Cadinene	-	-	-	-
39	48.98	Caryophyllene oxide	52546	0.33	29785	0.31
	Total		15557046	100	9417571	100

1.3 Chemical Composition of Essential Oils Extracted from Al-Baha Samples

From Al-Baha sample, (Figure 4) and (Table 3), Thirty-six compounds extracted with hydrodistillation technique were identified from the oil (Table 4.4). The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (P-Cineol) (32.79%), α-Pinene (18.45%), Borenol (9.78%), Verbenone (5.41%), Camphor (4.95%), Camphen (3.71%), β-linalool (3.68%), β-Pinene (3.20 %), α-Terpineol (2.61%), Geraniol (2.38%), D-Limonene (2.33%), Bornyl Acetate (2.18 %), 4-Terpineol (1.47%), Myrcene (1.24%), Chrysathenone (1.09%), Cis-Pinocamphone (1.01%). In the same region, Thirty-five compounds extracted with microwave technique were identified from the oil. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (Para-Cineol) (25.70%), α-Pinene (18.09%), Borenol (9.42%),Verbenone (7.41%), Camphor (4.78%), β-linalool (3.32%), Camphene (3.31%), β-Pinene (3.13%), α-Terpineol (2.82%), Geraniol (2.77%), D-Limonene (2.39%),Bornyl Acetate (2.36%),β-Caryophyllene (2.24%), 4-Terpineol (1.42%), Chrysathenone (1.29%), Myrcene (1.28%).

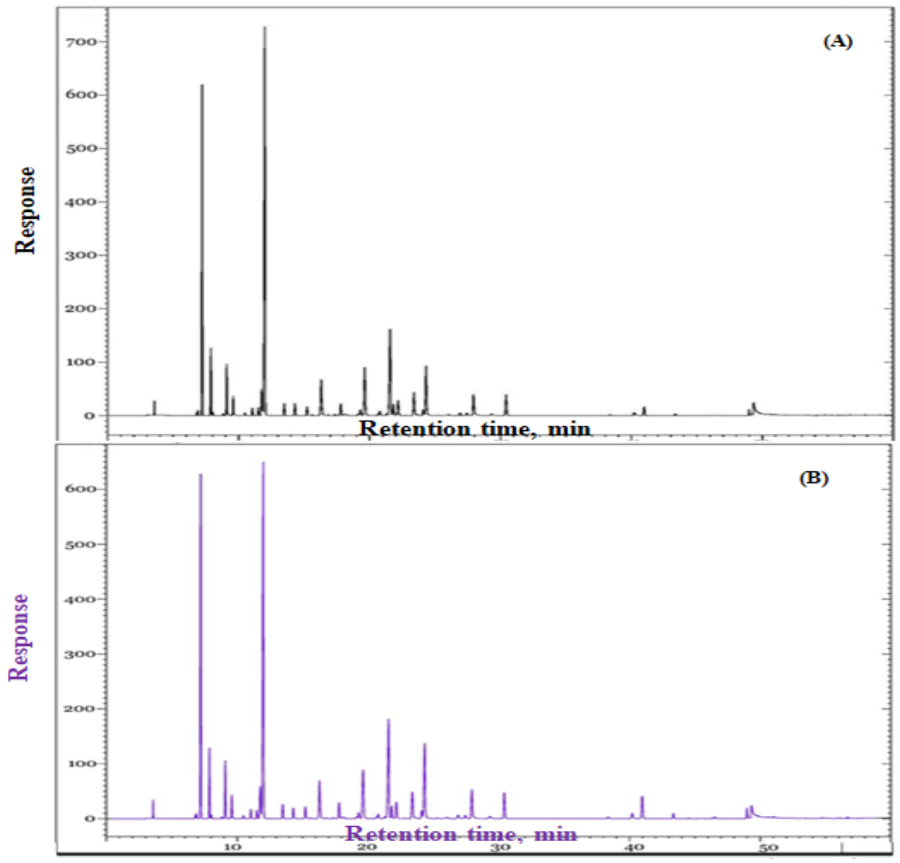

Table 3: Retantion time, chemical composition and relative percent of Al-Baha, extracted by HD and MWHD rosemary essential oil analysed by GC-MS.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
1	3.56	Octane (C8)	48814	0.43	56494	0.44
2	6.80	Tricyclene	15957	0.14	17371	0.13
3	6.88	α-Thujene	27219	0.24	26727	0.21
4	7.22	α-Pinene	2.049e+6	18.45	2.299e+6	18.09
5	7.87	Camphene	412601	3.71	420851	3.31
6	8.02	Verbenene	20233	0.18	21059	0.16
7	8.83	Sabinene	13660	0.12	13452	0.10
8	9.09	β-Pinene	355681	3.20	398267	3.13
9	9.57	Myrcene	138260	1.24	163582	1.28
10	10.48	α-Phellandrene	17921	0.16	22783	0.17
11	11.04	α-Terpinene	58881	0.53	-	-
12	11.50	P-Cymene	64498	0.58	67524	0.53
13	11.76	D-Limonene	259623	2.33	304069	2.39
14	12.02	Eucalyptol (P-Cineol)	3.641e+6	32.79	3.265e+6	25.70
15	13.48	γ-Terpinene	99192	0.89	120395	0.94
16	14.28	Trans-Sabinene hydrate	104795	0.94	91763	0.72
17	15.21	α- Terpinolene	79804	0.71	104930	0.82
18	16.30	β-linalool	409027	3.68	422365	3.32
19	17.79	Chrysathenone	121953	1.09	164214	1.29
20	19.28	Verbenol	64059	0.57	64553	0.50
21	19.64	Camphor	597940	4.95	608409	4.78
22	20.62	Pinocamphone	12756	0.11	-	-
23	20.77	Pinocarvone	50240	0.45	49737	0.39
24	21.57	Borenol	1.086e+6	9.78	1.197e+6	9.42
25	21.82	Cis-Pinocamphone	112993	1.01	115330	0.90
26	22.16	4-Terpineol	163989	1.47	181232	1.42
27	23.38	α-Terpineol	290816	2.61	359260	2.82
28	24.10	Isobornylformate	62480	0.56	-	-
29	24.33	Verbenone	601461	5.41	941593	7.41
30	26.89	Cis-Citral	-	-	51218	0.40
31	27.93	Geraniol	264422	2.38	352905	2.77
32	30.42	Bornyl Acetate	242741	2.18	300186	2.36
33	37.55	α-Copaene	-	-	-	-
34	38.36	Linolyl acetate	12054	0.10	17734	0.13
35	40.19	Methyl Eugenol	41548	0.37	71359	0.56

Table 3: Cont.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
36	40.96	β-Caryophyllene	102010	0.91	285295	2.24
37	43.34	α-Humulene	14730	0.13	47387	0.37
38	46.53	Δ-Cadinene	1484	0.01	9832	0.07
39	48.98	Caryophyllene oxide	40624	0.36	69020	0.54
	Total		11102526	100	12701896	100

1.4 Chemical Composition of Essential Oils Extracted from Al-Taif Samples

From Al-Taif, two samples were collected from two different regions, Al-Hada and Al-Haweyah. Forty compounds extracted with hydrodistillation technique were identified from the oil of Al-Hada (Figure 5) and (Table 4),. The most abundant constituents (≥ 1%) in the essential oil to be Eucalyptol (Para-Cineol) (36.65%), Bornyl Acetate (13.57%), Camphor (11.65 %), Borenol (10.39%), α-Pinene (7.89%), Camphen (7.32%), β-Caryophyllene (3.40%), D-Limonene (3.27%), α-Terpineol (2.81%), β-Pinene (2.77%), Myrcene (1.37%), 4-Terpineol (1.37%), P-Cymene (1.20%). Thirty-six compounds extracted with microwave technique were identified from the oil of Al-Hada. The most abundant constituents (≥ 1%) in the essential oil to be Eucalyptol

(Para-Cineol) (33.52%), Camphor (12.06%), Bornyl Acetate (9.91%), Borenol (8.03%), α-Pinene (7.48%), Camphen (5.79%), β-Caryophyllene (4.69%), β-Pinene (3.98%), D-Limonene (2.73%), α-Terpineol (2.15%), Myrcene (1.51%).

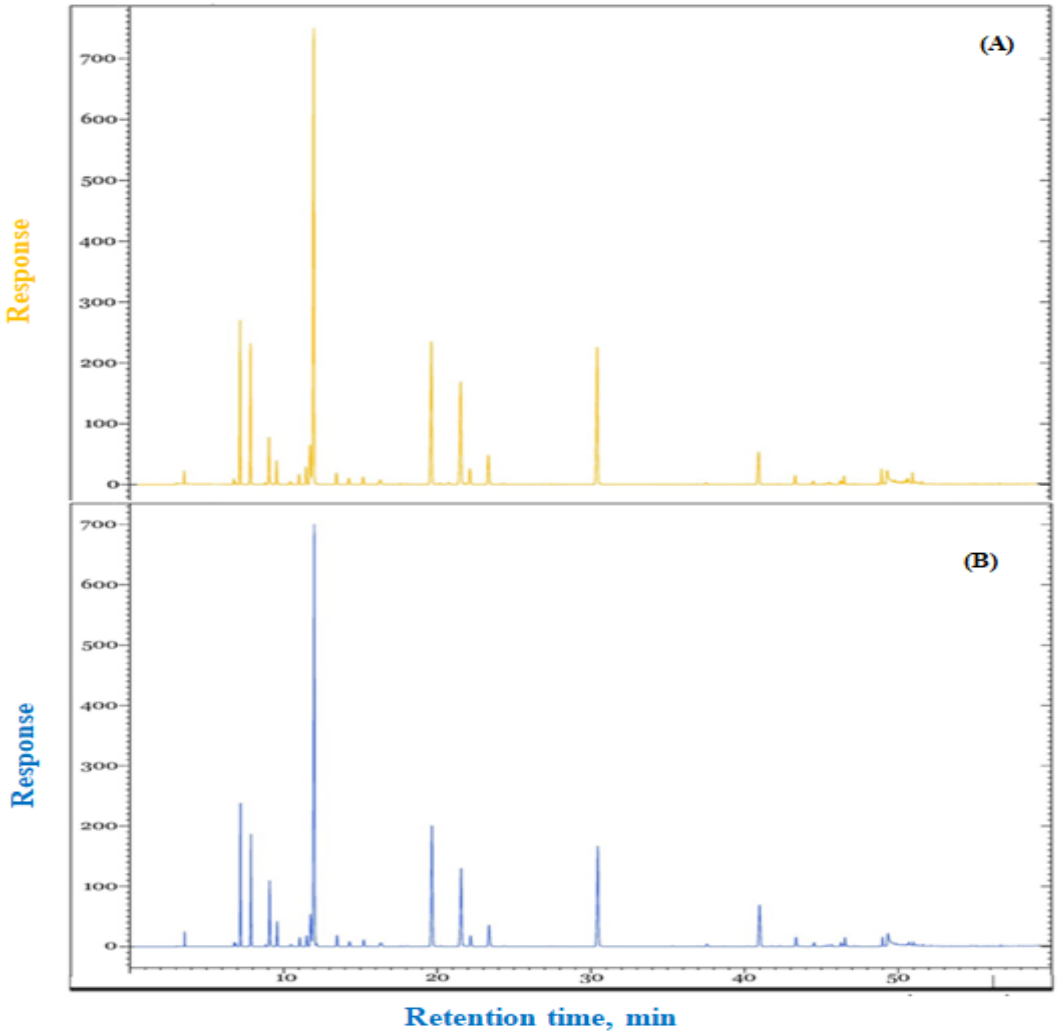

Figure 5: Typical GC-MS chromatogram for rosemary essential oil extracted by hydrodistillation (A) and microwave assisted technique (B) collected from Al-Hada, KSA.

Table 4: Retantion time, chemical composition and relative percent of Al-Hada, extracted by HD and MWHD rosemary essential oil analysed by GC-MS.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
1	3.56	Octane (C8)	44588	0.41	45237	0.44
2	6.80	Tricyclene	26657	0.24	20002	0.19
3	6.88	α-Thujene	10612	0.09	16353	0.15
4	7.22	α-Pinene	843345	7.89	767913	7.48
5	7.87	Camphene	782879	7.32	593675	5.79
6	8.02	Verbenene	-	-	-	-
7	8.83	Sabinene	10442	0.09	10736	0.10
8	9.09	β-Pinene	296160	2.77	408300	3.98
9	9.57	Myrcene	146679	1.37	155598	1.51
10	10.48	α-Phellandrene	18333	0.17	12627	0.12
11	11.04	α-Terpinene	68121	0.63	-	-
12	11.50	P-Cymene	128767	1.20	79325	0.77
13	11.76	D-Limonene	350287	3.27	280899	2.73

14	12.02	Eucalyptol (P-Cineol)	3.917e+6	36.65	3.437e+6	33.52
15	13.48	γ-Terpinene	84441	0.79	83183	0.81
16	14.28	Trans-Sabinene hydrate	47192	0.44	39889	0.38
17	15.21	α- Terpinolene	56733	0.53	53240	0.51
18	16.30	β-linalool	21171	0.19	21560	0.21
19	17.79	Chrysathenone	2871	0.02	1889	0.01
20	19.28	Verbenol	3360	0.03	3605	0.03
21	19.64	Camphor	1.429e+6	11.65	1.237e+6	12.06
22	20.62	Pinocamphone	-	-	-	-
23	20.77	Pinocarvone	22420	0.20	-	-
24	21.57	Borenol	1.111e+6	10.39	824154	8.03
25	21.82	Cis-Pinocamphone	6744	0.06	2128	0.02
26	22.16	4-Terpineol	147124	1.37	100816	0.98
27	23.38	α-Terpineol	300801	2.81	220499	2.15
28	24.10	Isobornylformate	3823	0.03	-	-
29	24.33	Verbenone	12329	0.11	5073	0.04
30	26.89	Cis-Citral	-	-	-	-
31	27.93	Geraniol	-	-	-	-
32	30.42	Bornyl Acetate	1.451e+6	13.57	1.017e+6	9.91
33	37.55	α-Copaene	18367	0.17	24206	0.23
34	38.36	Linolyl acetate	-	-	-	-
35	40.19	Methyl Eugenol	3569	0.03	4668	0.04

Table 4: Cont.

NO	**Retantion time**	**Compound Name**	**HD**		**MWHD**	
			Area	**% Percentage**	**Area**	**% Percentage**
36	40.96	β-Caryophyllene	364327	3.40	481578	4.69
37	43.34	α-Humulene	71377	0.66	79807	0.77
38	46.53	Δ-Cadinene	25218	0.23	62133	0.60
39	48.98	Caryophyllene oxide	59588	0.55	62445	0.60
40	50.32	Cubenol	101772	0.95	20846	0.20
41	50.56	Aromadendrene oxide	24400	0.22	16263	0.15
42	50.67	α-Cadinol	26772	0.25	16759	0.16
43	50.73	Tau-Muurolol	28026	0.26	35421	0.34
44	51.59	-Bisabololα	36265	0.33	10814	0.10
	Total		10685814	100	10252641	100

Forty compounds extracted with hydrodistillation technique were identified from the oil of Al-Haweyah (Figure 6) and (Table 5),. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (Para-Cineol) (44.68%), Camphor (12.01%), Bornyl Acetate (9.29%), Borenol (9.01%), α-Pinene (8.07%), Camphen (6.38%), β-Pinene (4.19%), β-Caryophyllene (3.58%), α-Terpineol (2.65%), D-Limonene (2.55%), Myrcene (1.45%), 4-Terpineol (1.29%). Thirty-six compounds extracted with microwave technique were identified from the oil of Al-Haweyah. The most abundant constituents (≥ 1%) in the essential oil were found to be Eucalyptol (Para-Cineol) (32.58 %), Camphor (13.74 %), Bornyl Acetate (8.50 %), Aα-Pinene (7.68%), β-Caryophyllene (7.06%), Borenol (6.65 %), Camphen (5.26 %), D-Limonene (3.05%), α-Terpineol (2.74%), β-Pinene (1.47%), Myrcene (1.44%), α-Humulene (1.19%), 4-Terpineol (1.10%), Δ-Cadinene (1.05%),γ-Terpinene (1.00%).

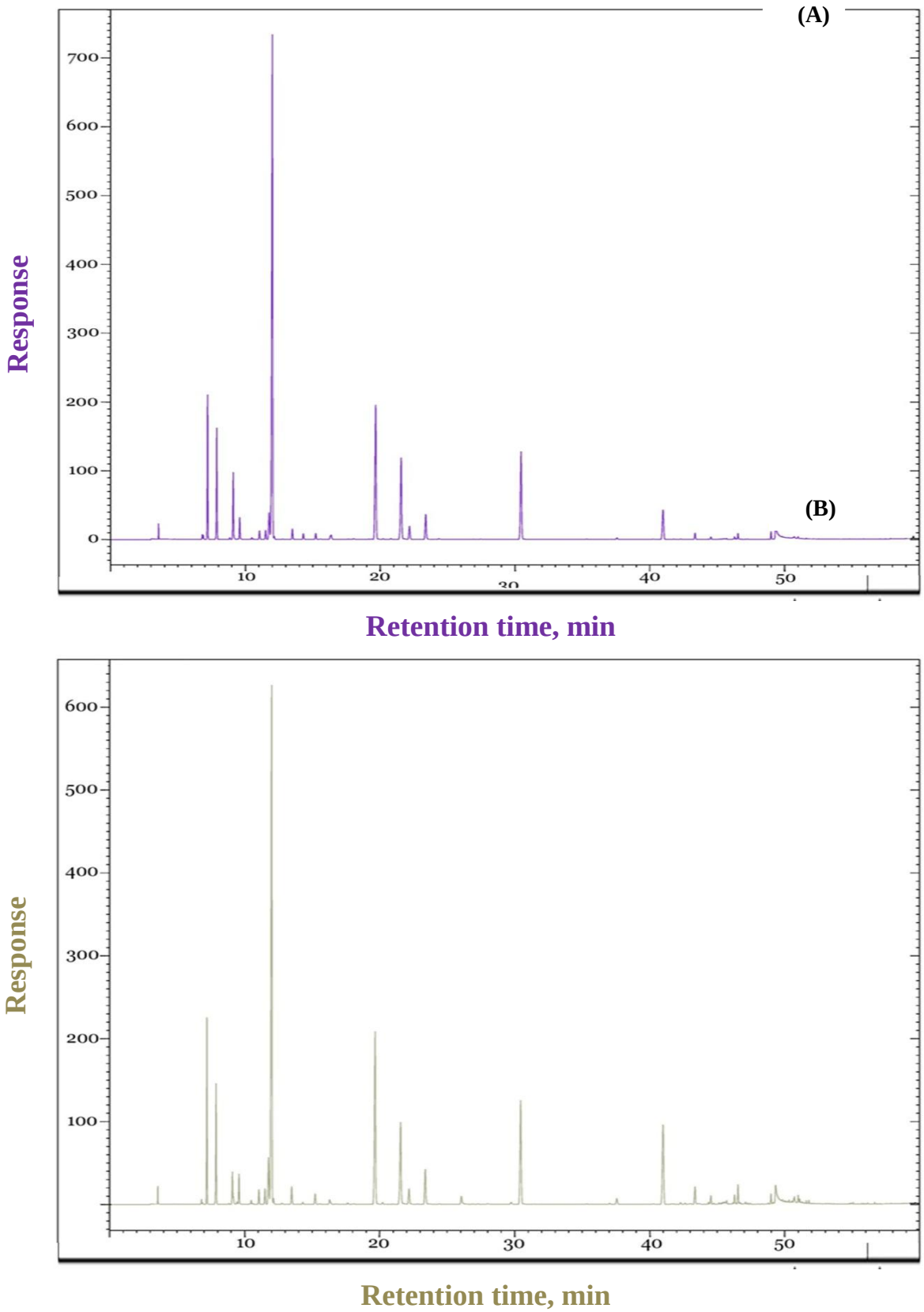

Figure 6: Typical GC-MS chromatogram for rosemary essential oil extracted by hydrodistillation (A) and microwave assisted technique (B) collected from, KSA.

Table 5: Retantion time, chemical composition and relative percent of Al-Haweya, extracted by HD and MWHD rosemary essential oil analysed by GC-MS.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
1	3.56	Octane (C8)	37414	0.44	40736	0.43
2	6.80	Tricyclene	20284	0.24	15964	0.17
3	6.88	α-Thujene	15989	0.18	4371	0.04
4	7.19	α-Pinene	679299	8.07	715547	7.68
5	7.88	Camphen	537225	6.38	490030	5.26
6	8.83	Sabinene	8450	0.10	2829	0.03
7	9.09	β-Pinene	352750	4.19	137761	1.47
8	9.58	Myrcene	122622	1.45	134794	1.44
9	10.48	α-Phellandrene	9700	0.11	19239	0.20
10	11.05	α-Terpinene	50273	0.59	-	-
11	11.50	P-Cymene	59421	0.70	81595	0.87
12	11.76	D-Limonene	214744	2.55	284841	3.05
13	12.01	Eucalyptol (P-Cineol)	3.761e+6	44.68	3.034e+6	32.58
14	13.48	γ-Terpinene	66765	0.79	93885	1.00
15	14.29	Trans-Sabinene hydrate	39089	0.46	9982	0.10
16	15.22	α- Terpinolene	40119	0.47	60021	0.64
17	16.35	β-linalool	51168	0.60	35684	0.38
18	17.81	Chrysathenone	1690	0.02	7107	0.07
19	19.36	Verbenol	3842	0.04	1726	0.01
20	19.64	Camphor	1.179e+6	12.01	1.280e+6	13.74
21	20.78	Pinocarveol	6949	0.08	1935	0.02
22	21.55	Borenol	758959	9.01	619968	6.65
23	21.84	Cis-Pinocamphone	2612	0.03	-	-
24	22.17	4-Terpineol	108717	1.29	103241	1.10
25	23.37	α-Terpineol	223235	2.65	255910	2.74
26	24.08	Isobornylformate	1168	0.01	-	-
27	24.34	Verbenone	6477	0.07	-	-
28	26.89	Cis-Citral	-	-	-	-
29	27.93	Geraniol	4831	0.05	4831	0.05
30	30.44	Bornyl Acetate	782487	9.29	791844	8.50
31	37.56	α-Copaene	13863	0.16	42170	0.45
32	38.36	Linolyl acetate	-	-	-	-
33	40.24	Methyl Eugenol	1385	0.01	1679	0.01
34	40.98	β-Caryophyllene	301682	3.58	657797	7.06
35	43.35	α-Humulene	45031	0.53	111457	1.19

Table 5: Cont.

NO	Retantion time	Compound Name	HD		MWHD	
			Area	% Percentage	Area	% Percentage
36	46.54	Δ-Cadinene	35093	0.41	98451	1.05
37	48.99	Caryophyllene oxide	36557	0.43	54286	0.58
38	50.32	Cubenol	3330	0.03	22576	0.24
39	50.56	Aromadendrene oxide	2211	0.02	18795	0.20
40	50.67	α-Cadinol	4876	0.05	20220	0.21
41	50.73	Tau-Muurolol	8328	0.09	42274	0.45
42	51.59	α-Bisabolol	2109	0.02	12112	0.13
	Total		8416913	100	9309658	100

Table 6 lists the composition together with the percentage and retention time of Rosemarinuseofficinialis L essential oils from different localities in KSA that were extracted by HD and MAHD. The most abundant components found in HD essential oils were Eucalyptol (P-Cineol) (44.68-25.70%), α-Pinene (22.68-7.480%),Camphor (13.74-3.48%), Bornyl Acetate (13.57-1.09%), Borenol (10.39-5.54%), and Camphen (7.32-3.26%). Furthermore, the main components of MAHD essential oils were Eucalyptol (P-Cineol) (33.52-

25.70%), α-Pinene (22.68-7.480%),Camphor (13.74-3.48%), Bornyl Acetate (9.91-1.16%), Borenol (9.42-5.54%), Camphen (6.03-3.31%), and β-Caryophyllene (7.06-1.55%). (Figure 26), shown Dominated compounds in the different Rosmarinusoficinalis L. chemotypes.

(Table 10) and (Figure 28), shown chemical classes of Rosemarinuseofficinialis L essential oils extracted by HD and MAHD. The essential oils which extracted by HD, contained monoterpene hydrocarbons(40.47-21.85%),oxygenated monoterpenes (72.96-52.09%),sesquiterpene hydrocarbons (10.53-1.03%), oxygenated sesquiterpenes (1.79-0.8%), and other components (14.06-0.42%). While the essential oils which extracted by MAHD, contained monoterpenes (40.47-21.85%),oxygenatedmonoterpenes (66.15-52.09%),sesquiterpenes (10.53-2.03%), oxygenatedsesquiterpenes (1.03-0.8%), and other components (10.4-0.42%).

Table 6: Chemical composition for rosemary essential oils from different locations in KSA,extracted by HD and MWHD technique.

NO	Oil constituent	% of oil from sample of										
		Retantion time	Abha		Asir		Al-Baha		Al-Taif Al-Hada		Al-Taif Al-Haweyah	
			HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD
			%	%	%	%	%	%	%	%	%	%
1	Octane (C8)	3.56	0.44	0.38	0.37	0.42	0.43	0.44	0.41	0.44	0.44	0.43
2	Tricyclene	6.80	0.23	0.24	0.12	0.14	0.14	0.13	0.24	0.19	0.24	0.17
3	α-Thujene	6.88	0.29	0.25	0.21	0.25	0.24	0.21	0.09	0.15	0.18	0.04
4	α-Pinene	7.22	20.09	20.60	19.92	22.68	18.45	18.09	7.89	7.48	8.07	7.68
5	Camphene	7.87	5.56	6.03	3.26	3.53	3.71	3.31	7.32	5.79	6.38	5.26
6	Verbenene	8.02	0.15	0.12	0.21	0.21	0.18	0.16	-	-	-	-
7	Sabinene	8.83	0.10	0.11	0.11	0.11	0.12	0.10	0.09	0.10	0.10	0.03
8	β-Pinene	9.09	3.87	3.62	2.89	3.22	3.20	3.13	2.77	3.98	4.19	1.47
9	Myrcene	9.57	1.36	1.45	1.34	1.41	1.24	1.28	1.37	1.51	1.45	1.44
10	α-Phellandrene	10.48	1.33	1.96	0.18	0.19	0.16	0.17	0.17	0.12	0.11	0.20
11	α-Terpinene	11.04	0.76	-	0.51	-	0.53	-	0.63	-	0.59	-
12	P-Cymene	11.50	0.80	1.15	0.58	0.55	0.58	0.53	1.20	0.77	0.70	0.87
13	D-Limonene	11.76	2.81	3.60	2.24	2.55	2.33	2.39	3.27	2.73	2.55	3.05
14	Eucalyptol (P-Cineol)	12.0	36.98	26.98	35.81	28.02	32.79	25.70	36.65	33.52	44.68	32.58
15	γ-Terpinene	13.48	1.10	1.34	0.85	1.05	0.89	0.94	0.79	0.81	0.79	1.00
16	Trans-Sabinene hydrate	14.28	0.73	0.49	0.92	0.75	0.94	0.72	0.44	0.38	0.46	0.10
17	α- Terpinolene	15.21	0.70	0.88	0.71	0.82	0.71	0.82	0.53	0.51	0.47	0.64

Table 9:cont.

NO	Oil constituent	% of oil from sample of										
		Retantion time	Abha		Asir		Al-Baha		Al-Taif Al-Hada		Al-Taif Al-Haweyah	
			HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD
			%	%	%	%	%	%	%	%	%	%
18	β-linalool	16.30	2.55	1.48	3.74	3.19	3.68	3.32	0.19	0.21	0.60	0.38
19	Chrysathenone	17.79	0.59	0.33	1.01	1.10	1.09	1.29	0.02	0.01	0.02	0.07
20	Verbenol	19.28	0.32	0.19	0.51	0.43	0.57	0.50	0.03	0.03	0.04	0.01
21	Camphor	19.64	8.92	10.05	3.63	3.48	4.95	4.78	11.65	12.06	12.01	13.74
22	Pinocamphone	20.62	0.10	-	0.15	-	0.11	-	-	-	-	-
23	Pinocarvone	20.77	0.35	0.21	0.49	0.37	0.45	0.39	0.20	-	0.08	0.02
24	Borenol	21.57	6.91	5.54	7.79	7.51	9.78	9.42	10.39	8.03	9.01	6.65
25	Cis-	21.82	0.68	0.35	1.01	0.90	1.01	0.90	0.06	0.02	0.03	-

	Pinocamphone											
26	4-Terpineol	22.16	1.22	1.06	1.17	1.11	1.47	1.42	1.37	0.98	1.29	1.10
27	α-Terpineol	23.38	2.15	1.94	2.51	2.54	2.61	2.82	2.81	2.15	2.65	2.74
28	Isobornylformate	24.10	0.27	0.18	0.49	0.47	0.56	-	0.03	-	0.01	-
29	Verbenone	24.33	3.11	2.91	5.67	6.58	5.41	7.41	0.11	0.04	0.07	-
30	Cis-Citral	26.89	-	0.09	-	0.26	-	0.40	-	-	-	-
31	Geraniol	27.93	1.19	0.80	2.41	2.48	2.38	2.77	-	-	0.05	0.05
32	Bornyl Acetate	30.42	1.62	1.66	1.09	1.16	2.18	2.36	13.57	9.91	9.29	8.50
33	α-Copaene	37.55	0.02	0.13	-	-	-	-	0.17	0.23	0.16	0.45
34	Linolyl acetate	38.36	0.04	0.04	0.10	-	0.10	0.13	-	-	-	-
35	Methyl Eugenol	40.19	0.10	0.13	0.41	0.34	0.37	0.56	0.03	0.04	0.01	0.01

Table 6:cont.

NO	Oil constituent	% of oil from sample of									
		Retantion time	Abha		Asir		Al-Baha		Al-Taif Al-Hada		Al-Taif Al-Haweyah	
			HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD	HD	MWHD
			%	%	%	%	%	%	%	%	%	%
36	β-Caryophyllene	40.96	0.95	2.60	0.60	1.55	0.91	2.24	3.40	4.69	3.58	7.06
37	α-Humulene	43.34	0.12	0.36	0.10	0.17	0.13	0.37	0.66	0.77	0.53	1.19
38	Δ-Cadinene	46.53	0.06	0.37	-	-	0.01	0.07	0.23	0.60	0.41	1.05
39	Caryophyllene oxide	48.98	0.17	0.21	0.33	0.31	0.36	0.54	0.55	0.60	0.43	0.58
40	Cubenol	50.32	-	-	-	-	-	-	0.95	0.20	0.03	0.24
41	Aromadendrene oxide	50.56	-	-	-	-	-	-	0.22	0.15	0.02	0.20
42	α-Cadinol	50.67	-	-	-	-	-	-	0.25	0.16	0.05	0.21
43	Tau-Muurolol	50.73	-	-	-	-	-	-	0.26	0.34	0.09	0.45
44	α-Bisabolol	51.59	-	-	-	-	-	-	0.33	0.10	0.02	0.13

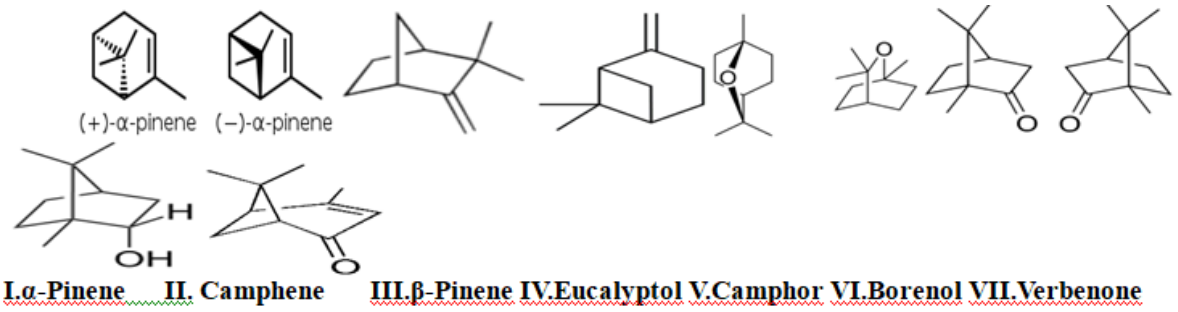

Figure 7: Dominated compounds in the different Rosmarinusoficinalis L. chemotypes.

Table 7: Chemical Classes of rosemary essential oils extracted by HD and MWHD technique.

Classes (Grouped compounds)	**% of oil from sample of**											
	Abha		**Asir**		**Al-Baha**		**Al-Taif Al-Hada**		**Al-Taif Al-Haweyah**		**Total**	
	HD	**MWHD**	**HD**	**MWHD**	**HD**	**MWHD**	**HD**	**MWHD**	**HD**	**MWHD**	**HD**	**MWHD**
	%	%	%	%	%	%	%	%	%	%	%	%
Monoterpene Hydrocarbons	39.15	40.47	33.13	36.71	32.48	31.266	26.361	24.14	25.82	21.85	31.35	30.88

Oxygenated Monoterpenes	65.21	52.09	65.81	57.62	66.15	60.55	63.9	57.42	70.51	57.37	72.96	63.13
Sesquiterpene Hydrocarbons	1.32	3.67	1.03	2.03	1.41	3.22	5.23	7.04	5.13	10.53	2.82	5.29
Oxygenated sesquiterpene	-	-	-	-	-	-	1.79	0.8	0.19	1.03	0.46	0.36
others	3.06	2.72	3.47	3.49	4.73	4.78	14.06	10.4	9.77	9.01	0.41	0.42

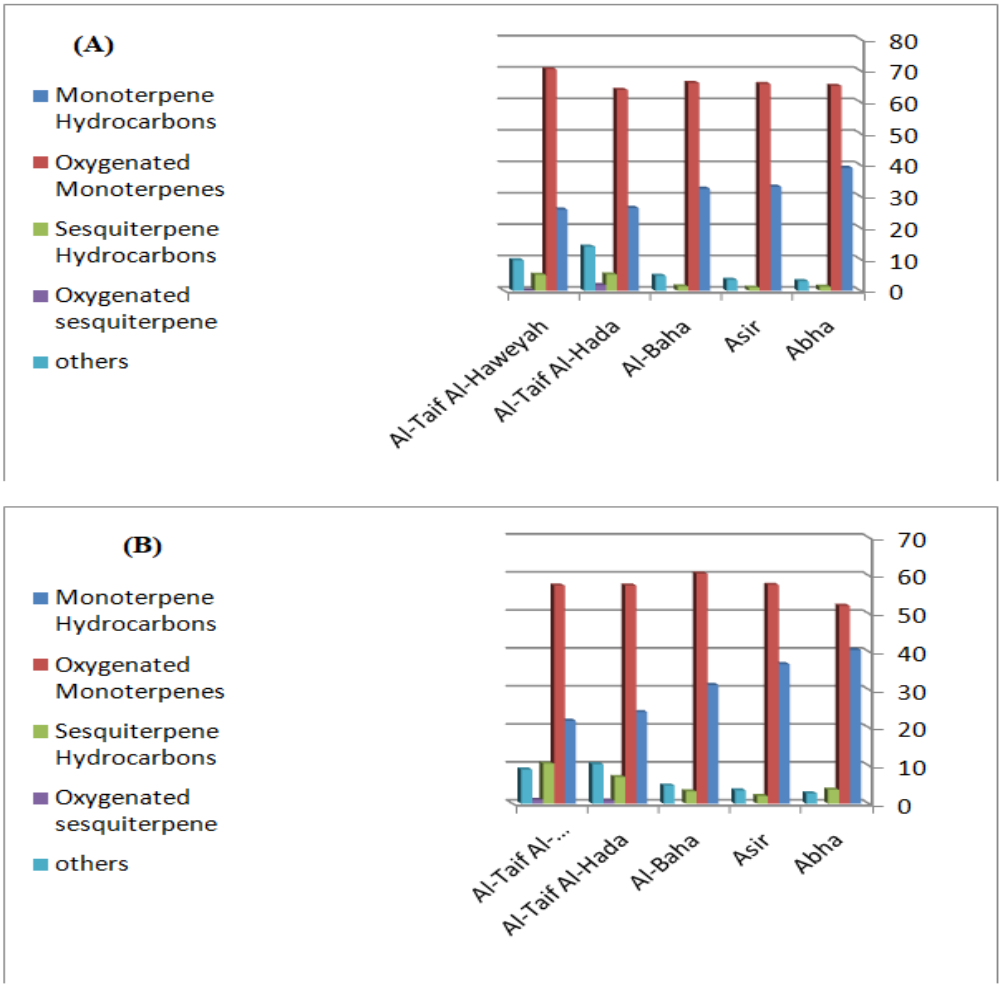

Figure 8: Histograms of chemical classes of rosemary essential oils extracted by HD (A), and MAHD (B).

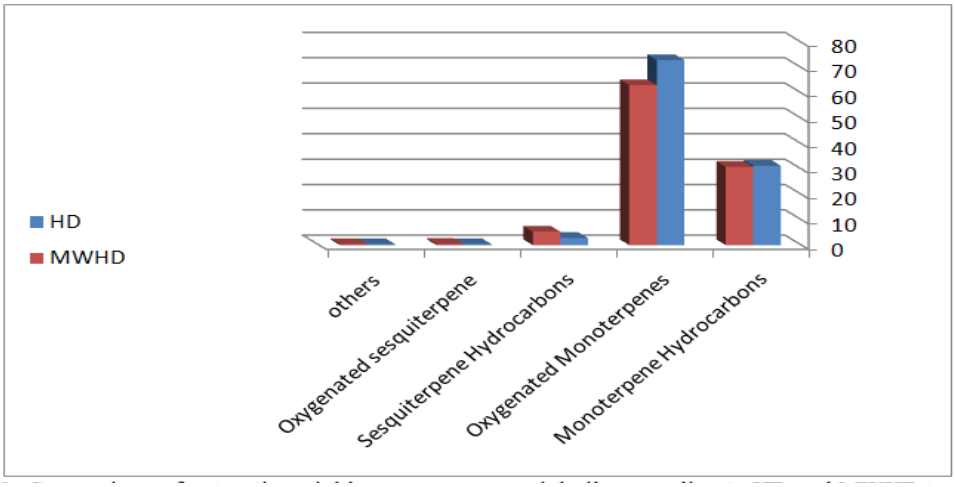

Figure 9: Comparison of extraction yields rosemary essential oils according to HD and MWHD technique.

DISCUSSION

The study analyzed the yields of essential oils extracted from rosemary using hydrodistillation and microwave assisted extraction methods. The hydrodistillation yielded an average of 0.5-0.9%, while the microwave assisted extraction yielded 0.6-1.0%. The extraction time was 3 hours at boiling temperature for hydrodistillation and 650 W for 30 minutes for microwave heating. The major components identified were Eucalyptol (P-Cineol), α-Pinene, Camphor, Bornyl Acetate, Borenol, and Camphen. The oils were grouped into five classes: Monoterpene Hydrocarbons, Oxygenated Monoterpenes, Sesquiterpene Hydrocarbons, Oxygenated Sesquiterpene, and Other compounds. The highest percentage of compounds were oxygenated monoterpenes.

Another study found 63 components of rosemary oil in Camel and Range Research Center of Al-Jouf(Guetat et al., 2014). Chemical analysis revealed Eucalyptol (P-Cineol) (40%), α-Pinene (20%), Camphor (8%), Bornyl Acetate (6%), Borenol (5%), and Camphene (3%) as the main components in KSA. In Al-Jouf, 1, 8-Cineole (23.16%), verbinone (13.45%), Borneol (4.51%), Camphor (4.10%), Camphene (3.30%), α-Terpineol (2.43%), and Bornyl acetate (1.66%) were identified.This assures that the geographical region significantly influences the chemical composition of rosemary essential oils, as demonstrated in a study examining oils from Tarabah, Raniah, Al-Hada, and Al-Haweyah locations in Taif Governorate, KSA.

In addition, another study also found that geographical region not only affects the essential oil's chemical composition but also impacts the yield using hydrodistillation method (Bazaid et al., 2013). This study found that rosemary essential oil, containing α-pinene, Camphine, 1,8-cineole, Verbinone, and Borneol, has different concentrations in different locations. In the Alhada region of El-Taif governorate, α-pinene (34.78%), Camphine (4.13%), 1,8-cineole (27.42%), Verbinone (11.25%), and Borneol (8.34%) were the main components (Bazaid et al., 2013). Different from this study, our study found that α-pinene and camphen were abundant compounds in the same location, while Eucalyptol had a similar concentration (36.65%), Borneol had a higher concentration (10.39%), and Verbinone had a lower concentration (less than 1%).

Additionally, Bazaid et al., (2013) found that rosemary essential oil contains almost 70% α-pinene, Camphine, 1,8-cineole, Verbinone, and Borneol. In comparison, our study indicated that the most dominant compounds were α-pinene (8.07%), Camphene (6.38%), Eucalyptol (44.68%), and Borenol (9.01%) in Alhaweiah location, while Verbinone was found in less concentration (0.07%). This highlights the influence of geographical region on the heterogeneity in rosemary essential oil chemical composition. In Taif-Al-Haweyah and Taif-Al-Hada region, high levels of Eucalyptol, Camphor, Bornyl Acetate, and Camphene were found in the oil. Asir and Abha had high rates of α-pinene, while Al-Baha and Taif-Al-Hada region had high Borenol levels. The oil also contained other components of a lower perertye.

The heterogeneity in the chemical composition of rosemary essential oil varies by region, with 23 compounds identified in Morocco from Sefrou city, including camphor, 1.8-cineole, camphene, β-pinene, bornylacetate, limonene, borneol, and cymene , according to Derwich et al. (2011).Chahboun, et al. (2014) identified dimenthol (38.83%), campholène aldehyde (16.02%), α-pinene (11.05%), camphene (5.31%), Terpenyl acetate (4.92%), and borneol (10%) as the main components of rosemary oil in Morocco, according to a study. The richness of Morocco's essential oils in 1.8-Cineole, α and β-Pinene, Camphor and Camphene was also reported by Fechtal (Fechtal et al., 2001), and was further confirmed by Khia (Khia, et al., 2014). In Tafersoust region of Morocco α-Pinene, Camphor were said to be the main components of rosemary essential oil in this region of Morocco Derwichet al., (2011). It was further reported that Moroccan rosemary oil is of high content of one of the three following compounds α-Pinene (for plants collected from Rabat), Cineole (for plants found in El Ateuf), Camphor (for plants collected from Taforhalt) Elamrani(Elamrani et al., 2000). This variation in chemical composition of rosemary essential oils could be attributed to several factors including, environmental factors (Bernath, 1991), geographical conditions of the collection area, the climatic conditions of the region (Fellah et al., 2006).Similarly, a study in Iran found that rosemary oil's chemical composition varies based on its location. In the Lalehzar region, 49 compounds were found, while in the Kerman region, 31 compounds were found. The hydrodistillation method revealed that the main components of the oil in the Lalehzar region were pinene, 1, 8-cineole, camphene, -myrcene, broneol, camphor, and Verbenol. In contrast, the Kerman region showed a higher concentration of these compounds (Jamshidi et al., 2009).

The effect of geographical distribution on the chemical composition of rosemary essential oil was further reported in several other studies, including studies in Algeria (Lograda et al., 2007), and Sudan (Elhassan et al., 2014).The chemical composition of rosemary essential oils are also known to be directly affected by the extraction method. In our study, both hydrodistillation and microwave assisted extraction methods have successfully identified almost the same number of compounds. However, in the Abha region, the microwave extraction method failed to identify α-Terpinene and Pinocamphone, while hydrodistillation methods identified them at 0.7% and 0.1%, respectively. The hydrodistillation method also failed to identify Cis-Citral compound, which was identified by microwave at 0.09% concentration. In the Al-Baha region, microwave extraction failed to identify α-TerpinenePinocamphone and Cis-Citral, while hydrodistillation methods identified all components of rosemary essential oil. In the Al-Haweya region, microwave extraction failed to identify α-Terpinene, Isobornylformate, and Verbenone, despite hydrodistillation's successful identification of these compounds. The

study also found that α-pinene isolation from rosemary was affected by the extraction time, with 15 minutes being the optimum extraction time for this compound. It was also reported rosemary essential oil yield is maximized at conditions of heating at 120°C, for 15 min (Tarum, 2015).Essential oils from aromatic plants contain oxygenated compounds in equivalent amounts, with monoterpene hydrocarbons present in equivalent amounts in both HD and MWHD. HD oil is slightly more concentrated in oxygenated compounds, but both methods maintain a similar concentration of dominant components. Microwave extraction yields are less time-consuming and energy-efficient, while hydrodistillation, using water as a polar solvent, accelerates reactions and forms intermediates. Both methods show an equivalent yield, with HD demonstrating a slightly higher concentration of oxygenated compounds (Ferhat et al., 2007).

We performed the microwave extraction at 60 degrees, which lead to a similar yield as that of the hydrodistillation. It was reported that performing the extraction at 70 degrees and even higher to 125 and 150 degrees, increases the extraction yield. The extraction yield is more successful in maintaining rosemary antioxidant properties compared to ultrasound methods, with higher temperatures resulting in a higher extraction yield. Microwave extraction is also reported to be more effective in achieving similar yields (Jacotet-Navarro et al., 2015). High temperatures and heating conditions significantly impact the chemical composition of essential oils. Some compounds are fragile to thermal fluctuations, leading to the loss of specific compounds or variations in their abundance. Microwave assisted extraction failed to reproduce these components, except for limonene and α-terpinene, as per Alitonou et al. (2012).

Microwave assisted extraction is a quicker method for obtaining essential oils, with 30 minutes of microwave heating providing an equivalent yield to 180 minutes of hydrodistillation. This method yields higher concentrations of oxygenated compounds, which are more valuable and valuable due to their stronger odor. Monoterpenes hydrocarbons, which have less economic value and contribute less to cosmetics, are more abundant in essential oils obtained through hydrodistillation. It was also showed that microwave extraction successfully extracts the oils while leaving the cell intact but, hydrodistillation causes disruption in the cellular material and induces damage to the external layer of the fruit. This clearly states that hydrodistillation have some undesirable effects such as that it causes violent vaporization and subsequently changes causing the cell wall to rupture (Ferhat et al., 2005). A similar effect was reported in microwave assisted extraction when roses were subjected to extensive microwave heating, the pressure formed in the roses glands causing them to rupture quicker than that in hydrodistillation(Par´e and Belanger 1997, and Chen and Spiro 1995). In our experience and the studies by others, microwave extraction is simple and easy to perform, yet still it withholds serious hazards if to be carried out by inexperienced hands. So, for industry scale microwave extraction application a strict safety measures must be planned and followed. And those dealing with microwave must be properly informed trained and must receive close guidance throughout the extraction process.

CONCLUSION

This study compared the chemical composition and yield of rosemary essential oils from five regions in the KSA using hydrodistillation and microwave extraction methods. The results showed that the rosemary strains differed between governorates and plants collected from different locations. The microwave extraction method achieved similar yields to hydrodistillation extraction. However, it had limitations in energy consumption, extraction time, and environmental impact. Further research is needed to optimize the method, consider soil and climate effects, and determine the most suitable harvest time and season. The study also suggests that the essential oil of Rosmarinusoficinalis L can be used as flavor agents, antioxidants, antimicrobials, antifungals, and antiseptics in various applications. Further research is needed to further elaborate on the biological activities of rosemary essential oils and evaluate their safety before human use.

REFERNCES

1. Alitonou, G., Koudoro, A. Y., Sossou D. J., Yehouenou, B., Avlessi, F., Adeoti, S., Menut, C., & Sohounhloue, D., 2012. Volatile constituents and biological activities of essential oil from Securidaca longepedunculata Fers. growing in Benin.St Cerc St CICBIA, 13:33-42.
2. Bazaid, A., El-Amoudi, S., Ali, F., & Abdel-Hameed, S. 2013. Volatile oil studies of some aromatic plants in Taif region. Journal of Medicinal Plants, 1(5):119-128.
3. Bernath, J., Danos, B., & Hethelyi, E. 1991.Variation in essential oil spectrum on Salvia species affected by environment. Herba Hungarica, 30(1-2):35-48.
4. Chahboun, N., Esmail, A., Rhaiem, N., Abed, H., Amiyare, R., Barrahi, M., Berrabeh, M., Oudda, H **,. &**Ouhssine, M. 2014. Extraction and study of the essential oil Rosmarinus officinalis cuellie in the region of Taza, Morocco. Pharma Chemica, 6: 367-372.
5. Chen, S. S., & Spiro, M. 1995. Kinetics of microwave extraction of rosemary leaves in hexane, ethanol and a hexane + ethanol mixture. Flavour and Fragrance Journal, 10: 101–112.
6. Cuvelier, M., Richard, H., & Berset, C. 1996. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. Journal of the American Oil Chemists' Society,

73(5):645-652.
7. Derwich, E., Benziane, Z., & Chabir, R. 2011. Aromatic and medicinal plants of Morocco: chemical composition of essential oils of Rosmarinus officinalis andJuniperus phoenicea. International Journal of Applied Biology and Pharmaceutical Technology, 2(1): 145-153.
8. Elamrani, A., Zrira, S., Benjilali, B., & Berrada, M. 2000. A study of Moroccan rosemary oils. Journal of Essential Oil Research, 12(4):487-495.
9. Elhassan, I. A., & Nisreen M., O. 2014. New Chemotype Rosmarinus officinalis L. (Rosemary) "R. officinalis ct. bornyl acetate". American Journal of Research Communication, 2(4): 232-240.
10. Fechtal, M., Chaouch, A., & Talbi, M. 2001. Chemical composition, antibacterial and antifongic activities of essential oils from the leaves of Eucalyptus camaldulensis and its natural hybrid (clone 583). Acta Botanica Gallica, 148(3):183-190.
11. Fellah, S., Diouf, P., Petrissans, M.,Perrin, D., Romdhane, M., & Abderrabba, M. 2006.Chemical composition and antioxidant properties of Salvia officinalis L. oil from two culture sites in Tunisia. Journal of Essential Oil Research, 18(5):553-556.
12. Goullieux, A., Pain, J. 2005.Ohmic Heating 18.Emerging technologies for food processing:469.
13. Guetat, A., Al-Ghamdi, F., & Osman, A. 2014. 1, 8-Cineole, α-Pinene and Verbenone chemotype of essential oil of species Rosmarinus officinalis L. from Saudi Arabia. International Journal of Herbal Medicine, 2(2 Part C): 137-141.
14. Han, J., Lawson, L., Han, G., & Han, P. 1995. A spectrophotometric method for quantitative determination of allicin and total garlic
15. Hinneburg, I., Neubert, R. 2005.Influence of extraction parameters on the phytochemical characteristics of extracts from buckwheat (Fagopyrum esculentum) herb.Journal of agricultural and food chemistry, 53(1):3-7.
16. Jamshidi, R., Afzali, Z., & Afzali, D. 2009. Chemical composition of hydrodistillation essential oil of rosemary in different origins in Iran and comparison with other countries. American-Eurasian J of Agric Environ Sc, 5: 78-81.
17. Khia, A., Ghanmi, M., Aafi, A., Satrani, B., Aberchane, M., & Yakhlef, B. 2014. Aromatic and Medicinal Plants in North Africa: Opportunities, Constraints and Prospects.Novel Plant Bioresources: Applications in Food, Medicine and Cosmetics, 409-423.
18. Lograda, T., Ramdani, M., Chalard, P., & Figueredo, G. 2014. Antibacterial activity of essential oils of Rosmarinus officinalis from Eastern Algeria. Global J Res Med Plants Indigenous Med, 3: 232-242.
19. Marriott, P., Mondello, L., Shellie, R., Dugo, G. 2002. Characterisation of lavender essential oils by using gas chromatography–mass spectrometry with correlation of linear retention indices and comparison with comprehensive two-dimensional gas chromatography.Journal of Chromatography A,970(1):225-234.
20. Masango, P. 2005. Cleaner production of essential oils by steam distillation. Journal of Cleaner Production, 13(8): 833-839.
21. Pare, J. R. J., & Belanger, J. M. R. 1997 . Instrumental methods in food analysis. Amsterdam: Elsevier.
22. Tarum, A., 2015. The determination of three terpens in rosemary by GC, using two extraction methods, microwave assisted extraction and ultrasonic extraction, with isooctane as solvent, thiosulfinates. Annals of Biochemistry, 225: 157–160.
23. Van de Braak, S., & Leijten, G. 1999. Essential oils and oleoresins.CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam,