



Investigating the Activity of Antioxidants Activities Content in *Apiaceae* and to Study Antimicrobial and Insecticidal Activity of Antioxidant by using SPME Fiber Assembly Carboxen/Polydimethylsiloxane (CAR/PDMS)

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Abstract

In present study, the antioxidant content in *Apiaceae* was investigated by two new methods such as hydraulic distillation (HD) and HS-SPME, and the composition of final product was recognized using gas chromatography and mass spectroscopy. So, in this research, the anti-oxidation property of the essence was examined. For this purpose, the antioxidant activity of *Apiaceae* and its stability was inspected in different concentrations (250, 500, 750 and 1000 ppm) using differential scanning calorimetry with oleic acid. The results showed the similarity between HD and HS-SPME, but the percentages of the compositions were different. Besides, the frequency of oxides of essence oils in HS-SPME method was higher than HD method. The experiments performed on the antioxidants activity of the herb, and the results showed that increasing the essence concentration prevents the oxidation of the oils.

Keywords: Antioxidants, Gas Chromatography, Mass spectroscopy, Scanning calorimetry, *Apiaceae*

1 Introduction

Herbs were used extensively in conventional medical profession for several diseases. Scientific studies confirmed the hopeful treatment ability of herbs for different diseases, including infectious diseases, cancer, atherosclerosis, scald and Neurological disease (1-4). Although side effects cannot be ignored totally for such herbs, fewer negative effects are conceivable for them in comparison with synthetic medicines, and in many cases they can reduce the toxicity of other medicines because of antioxidant properties. Nowadays, new recognized compositions between the herbs are applied as medicine and can be used as a key for cheaper disease diagnosis with fewer side effects (5-12). *Apiaceae* (*Prangos ferulacea*) is a

significant herb in traditional medical profession, which has been used as a treating medicine in many diseases, and several laboratorial studies have proved such treating property. *Prangos ferulacea* is distributed from Eastern Europe to Middle East and Central Asia (13). This herb has been used as carminative, laxative, stomach tonic, neuralgia relief, anti-inflammatory, antiviral, anti-parasite, antifungal and antibacterial (14-16). Phyto-chemical investigations lead to separation of Coumarins, alkaloids, Flavonoids, and Terpenoids from different species of *prangos*.

Further researches on aerial essences and seeds of *Prangos ferulacea* identified 33 types of oils. In the oil produced from the fruit, 39 compositions were determined which were mainly consisted of alpha-pinene. Monoterpenes, sesquiterpene, and Coumarins were the other essential compositions of the root. Antioxidants are the compounds protecting and decelerating the oxidation of other molecules and reducing oxidation speed with inhibition of free radicals, peroxide decomposition and chelating up the metals (17-22). The objective of this study

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was to elucidate the antioxidant content in *Prangos ferulacea* (Apiaceae) using different extraction techniques, e.g. hydraulic distillation and HS-SPME.

2 Materials and methods

2.1 Materials

All chemical materials were purchased from Merck and Fluka companies with desired purity like anhydrite sodium sulfate with 97% and normal hexane with 95% purity.

2.2 Instruments

The characterization of used instruments is shown in Table 1, and characterizations of the used fiber in the SPME method are also shown in Table 2. SPME fiber excluding solid phase and SPME fiber holder were made by SUPELCO (USA).

2.3 Experimental procedure

2.3.1 Hydro-distillation (HD) method

Hydro distillation is a method of extraction, which is sometimes used instead of steam distillation. This process of extraction is one of the most used traditional methods of extraction. In this work, 100 gram of dried herb was grinded, and the essence oil was extracted by Clevenger instrument for 3.5 hours. The oil was dried by anhydrous sodium-sulphate (Na_2SO_4) and kept in the dark and closed container till analysis.

2.3.2 Headspace Solid Phase Microextraction (HS-SPME) method

Solid-phase microextraction, or SPME, is a solid phase extraction sampling technique that involves the use of a fiber coated with an extracting phase, that can be a liquid (polymer) or a solid (sorbent), which extracts different kinds of analytes (including both volatile and non-volatile) from different kinds of media, that can be in liquid or gas phase. The quantity of analyte extracted by the fibre is proportional to its concentration in the sample as long as equilibrium is reached or, in case of short time, pre-equilibrium, with the help of convection or agitation (23, 24). The method carried out using minimum amount of herb powder (1 g) without using any solvent. The circulating bath was used in this experiment, which had the

ability of temperature control during extraction process. The fiber assembly was kept in a 10 ml glass container and the fiber holder attached to the container. The glass container was inside the circulating bath to reach the bath temperature. Then, the fiber assembly was in contact with upper section for 5 minutes, and then the fiber was pulled into the plunger. For heat desorption of pulled components on the fiber, the injection performed immediately into the GC-MS instrument.

2.3.3 Measurement of Antibacterial Activity of Apiaceae essential oil on microorganisms

The recommended medium for disk diffusion method is Nutrient agar, and we performed the test according to the guidelines of the Clinical and Laboratory Standards Institute (25-27). First the medium was prepared based on the proposed procedure then the mentioned medium was autoclaved and precisely placed in a 50 to 55°C water bath. Afterward, it was poured into round plastic petri and let to cool at room temperature. The stock bacterial culture was set at 0.5 McFarland standard with an optical density of 600 nm, and a sterile swab was applied to add this prepared culture to Nutrient agar plates. Then the disk blanks which had been saturated with the samples were located on the plates and incubated for hours at 37°C to determine the susceptibility of the samples. In this study, Ampicillin and sterile water were regarded as positive and negative control respectively. In final step, all the plates were assessed for the presence of inhibition zones. The microdilution broth assay was utilized to specify the minimum inhibitory concentrations (MICs) of the samples. Briefly, 2-fold serial dilutions of the samples and control groups were proposed with Nutrient broth in 96-well microplates. Then the bacterial stock (0.5 McFarland Standard) was added to previous suspension in a way that the first concentration was 6.25 mg/ml. To determine the minimum bactericidal concentrations (MBCs) of the samples, the obtained result of previous test could be useful. In a way, the lowest concentration of samples, which causes no growth of bacteria, was regarded (MBC). Components of Apiaceae After analysis, the components of essence oil of Apiaceae using HD and SPME-HS methods were determined and shown in Table 3.

Table 1: Characterization of used instruments

Apparatus	Applications	Model
GC-MS ²	To identify different substances within a test sample ¹⁵	GC-MS model HP-6890 supplied by Hewlett-Packard (USA)
DSC ³	is a physical characterization method used to study the thermal behavior of herb	
Electric mill grinder	this mill is needed for SPME method	M20 (IKA-WERKE, Germany)

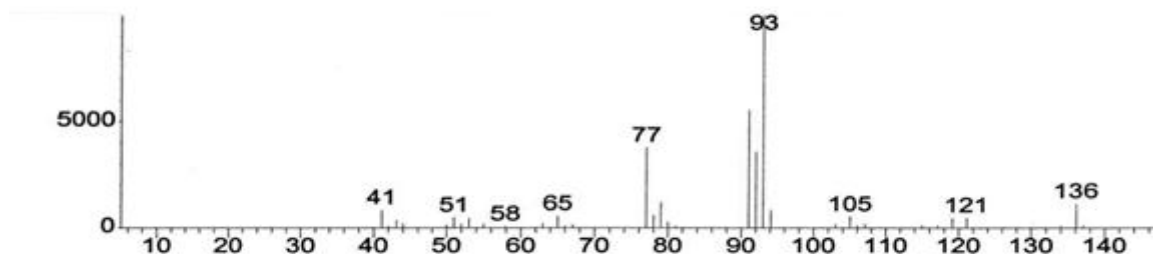
² Gas chromatography–mass spectrometry

³ Differential Scanning Calorimetry

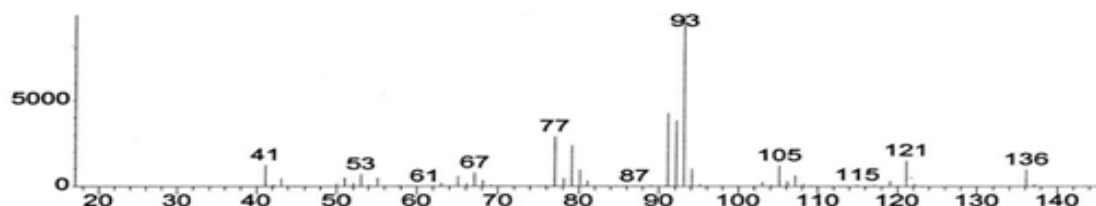
Table 2: Characterizations of the used fiber in the SPME method

Characterization of the used fiber in the SPME method
Type of fiber: (carboxen / poly dimethyl siloxane , CAR / PDMS)
Adsorbent thickness: 75 micrometer
Type of adsorbent connections: powerfully network
Color: Black

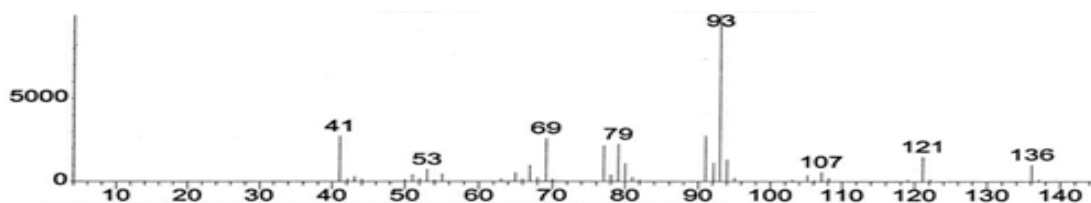
□-thujene		
I= 930	C ₁₀ H ₁₆	MW= 136



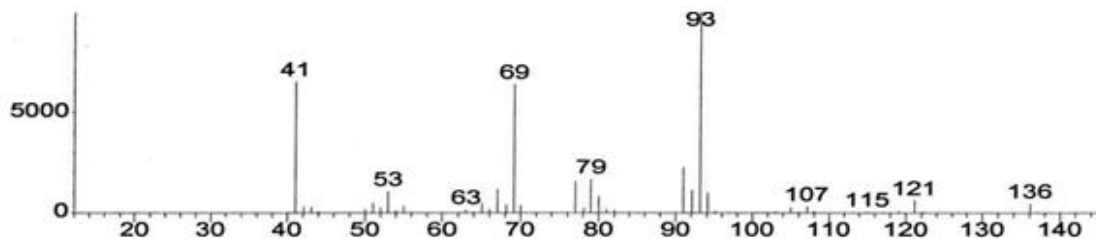
□-pinene		
I= 939	C ₁₀ H ₁₆	MW=136



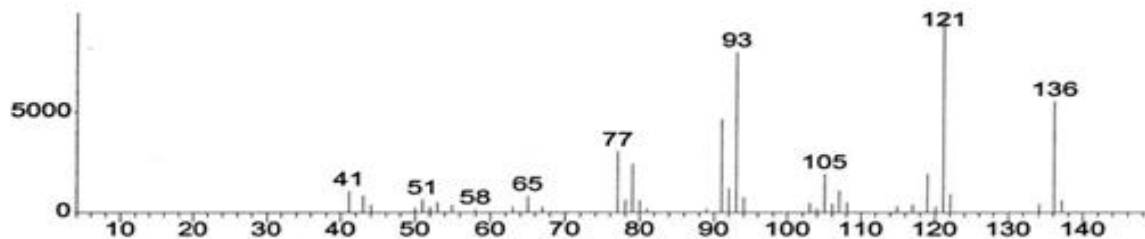
□-pinene		
I= 979	C ₁₀ H ₁₆	MW=136



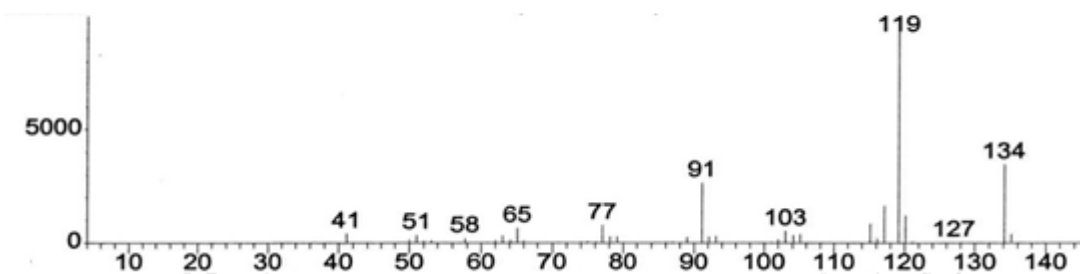
Myrcene		
I= 991	C ₁₀ H ₁₆	MW=136



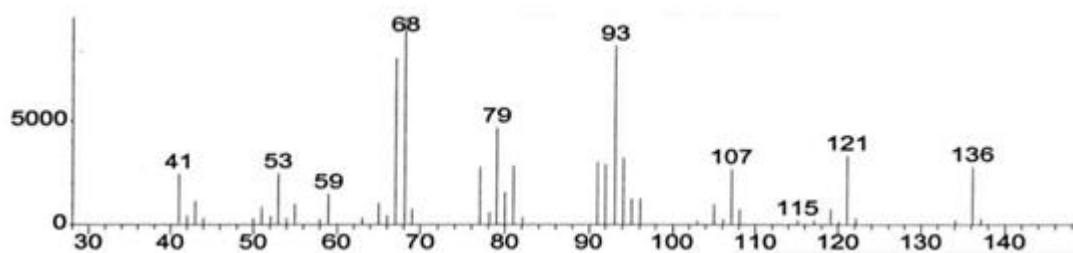
□-terpinene		
I= 1017	$C_{10}H_{16}$	MW=136



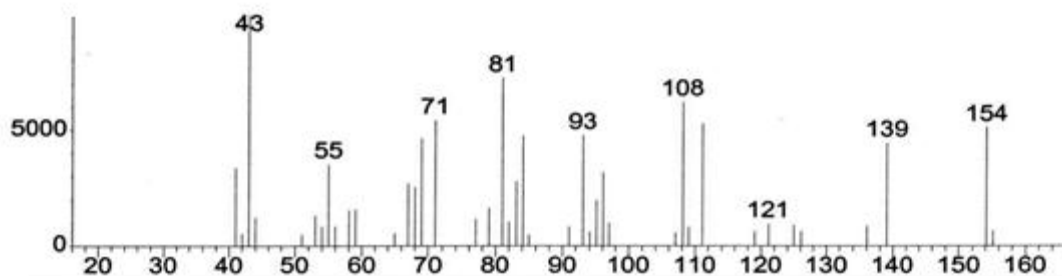
p-cymene		
I= 1025	$C_{10}H_{14}$	MW=134



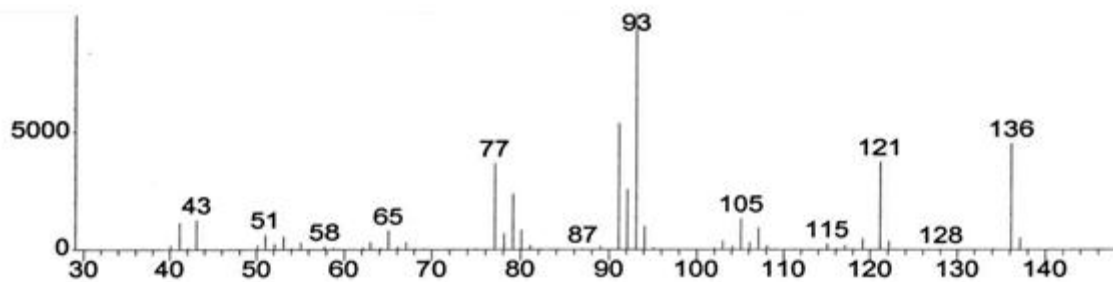
Limonene		
I=1029	$C_{10}H_{16}$	MW=136



1,8-cineole		
I= 1031	$C_{10}H_{18}O$	MW=154



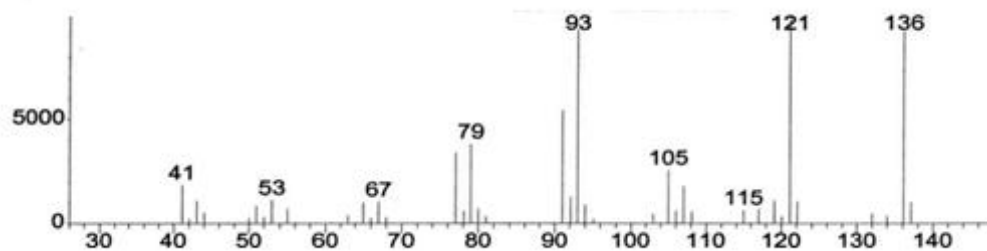
(Z)-□-ocimene		
I=1037	$C_{10}H_{16}$	MW=136

 α -terpinene

I= 1060

 $C_{10}H_{16}$

MW=136

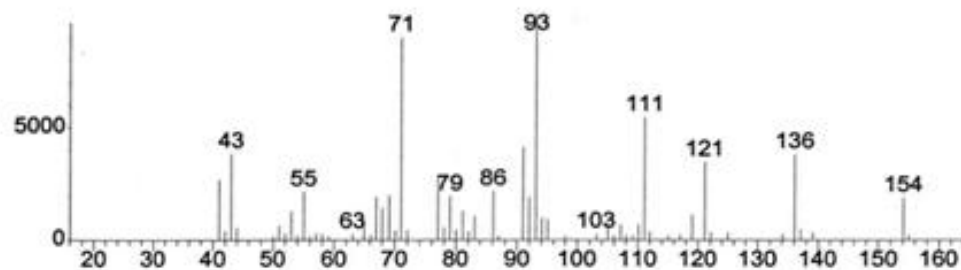


Artemisia triene

I= 1084

 $C_{10}H_{16}$

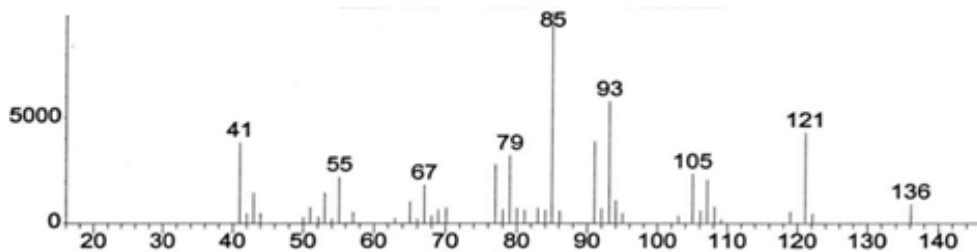
MW= 136

 α -terpinolene

I= 1089

 $C_{10}H_{16}$

MW=136

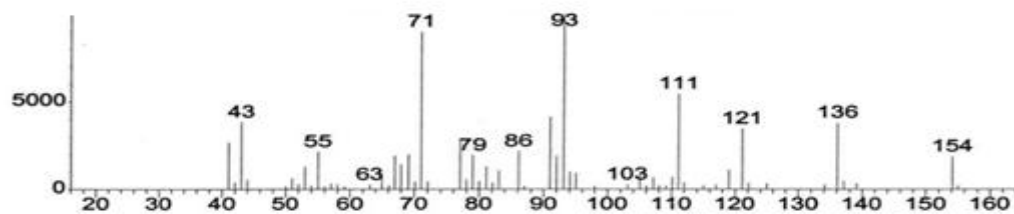


Gamma-terpinene

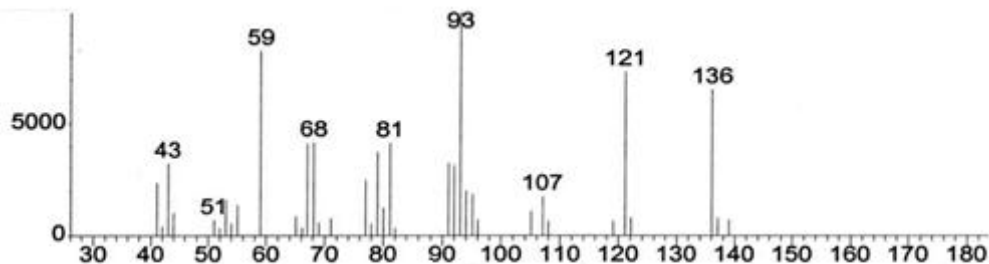
I= 1199

 $C_{10}H_{16}$

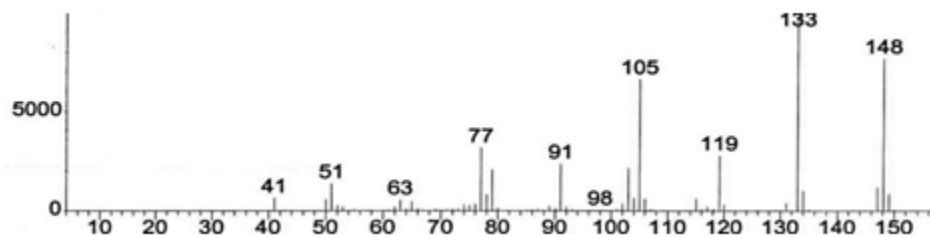
MW=136



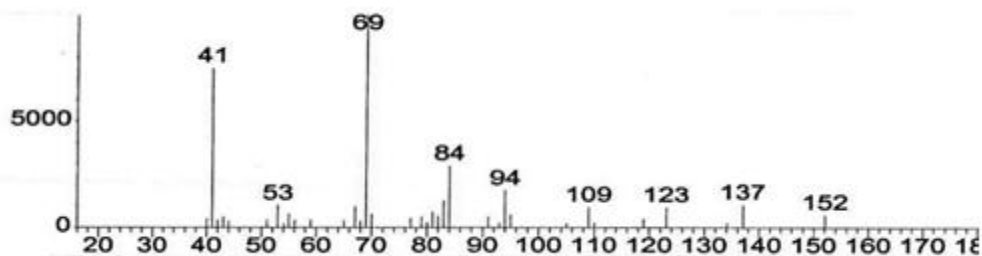
I=1220 $C_{12}H_{20}O_2$ Fenchyl acetate MW=196



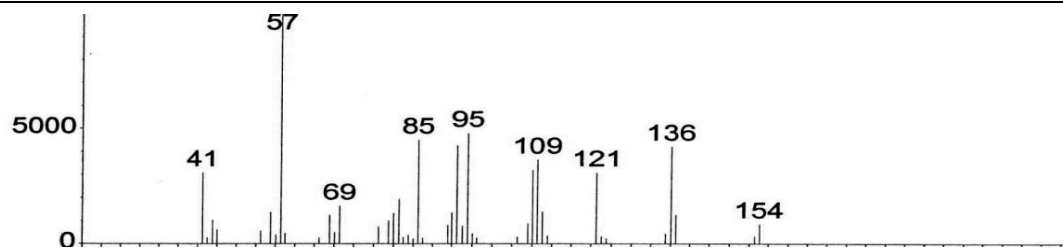
I= 1253 $C_{10}H_{12}O$ Anethole MW=148



I=1267 $C_{10}H_{16}O$ Geranial MW=152



I=1289 $C_{12}H_{20}O_2$ Bornyl acetate MW=196



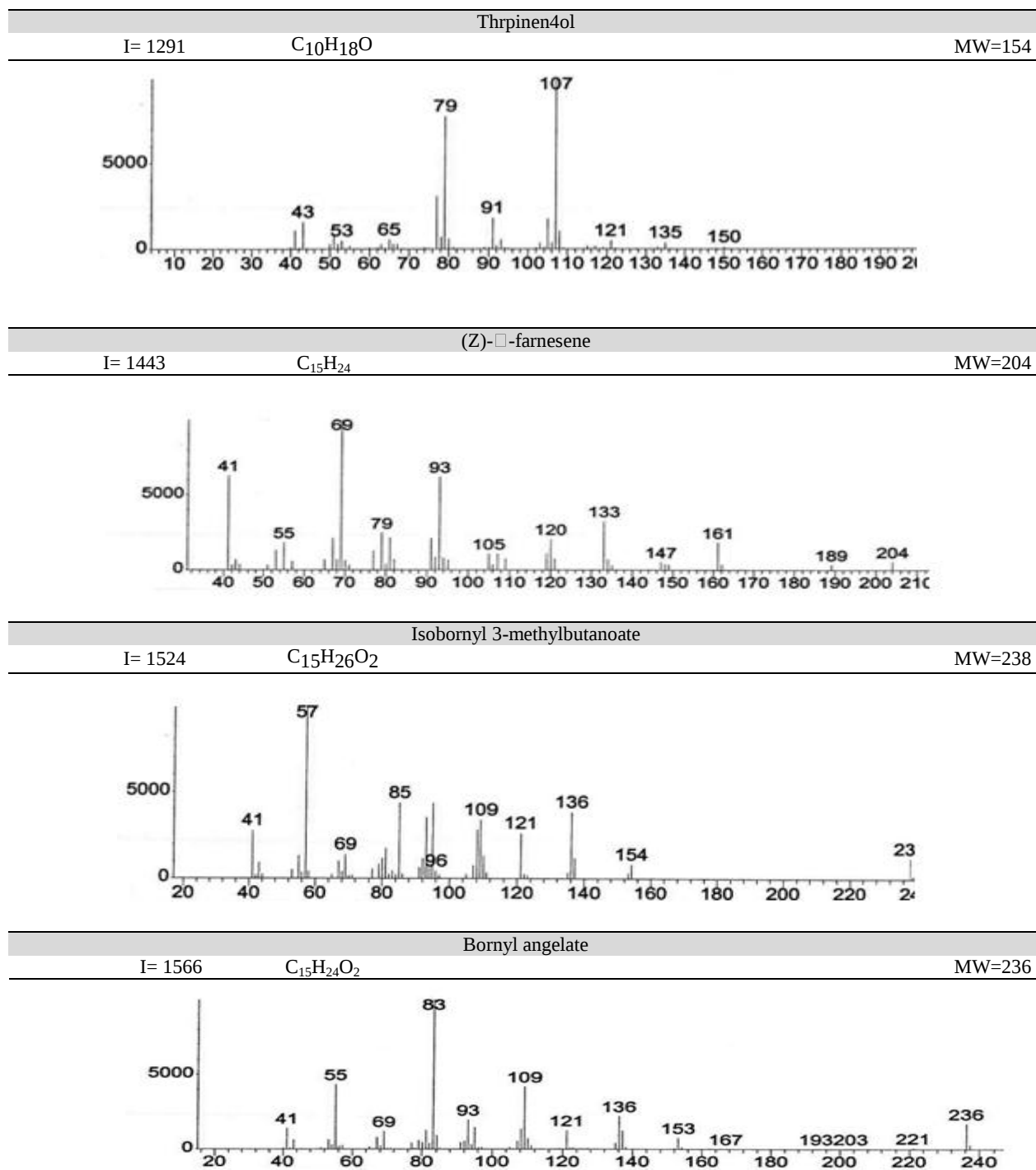


Figure 1: The mass spectra of identified compounds of Apiaceae

2.3.4 Test methods of measuring oxidation stability of essence oil using DSC

Measuring test by DSC carried out with 8 mg sample in the crucible without lid. The test completed with one atmosphere nitrogen pressure and 250°C heating. After

that, nitrogen gas removed, and oxygen flowed into the device, and heating continued up to 280°C. The oxygen flow was 50 ml/min, and thermal increase was 10°C/min. The empty crucible was used as control test. During the dynamic process, samples heated up from 25°C to 280°C

and oxidation stability of the samples determined from maximum temperature deflection curve.

Table 3: The components of essence oil of *Apiaceae* using HD and SPME-HS methods

Compound	I	HD (%)	HS-SPME (%) (60°C)
α - Thujene	930	0.66	0.88
α Pinene-	939	7.75	8.48
Pinene β -	979	0.72	0.87
Myrcene	991	1.62	2.33
α Terpinen-	1017	0.53	0.37
Para-cymene	1025	13.55	12.91
Limonen	1029	1.35	0.78
1,8-cineole	1031	0.34	-
Ocimene(z-beta)	1037	-	0.24
γ - Terpinene	1060	21.4	20.71
Artemisia alcohol	1084	0.48	-
α -terpineolene	1089	0.45	0.34
Thujene4ol	1169	1.59	-
γ - Therpineol	1199	0.72	-
Fenchy acetate(endo)	1220	0.33	-
Anethol(z)	1253	-	5.56
Geranial	1267	-	0.23
Bornyl acetate	1289	0.6	0.31
γ - Therpinental	1291	4.19	6.31
Acetanisol(meta)	1299	-	0.34
Geranyl acetate	1381	-	10.24
Fransense(z)beta	1443	-	0.23
Isobornyl-3-methyl	1524	0.54	0.74
Bornyl angelate	1566	9.42	10.2

2.3.5 Separation and identification of components in essence oils

As the components present in essence oils are known as volatile and semi-volatile oils, therefore GC-MS method was applied for separation and identification of the components. In order to confirm the identified components of standard mass spectrum, Quartz index was applied. Firstly, the Alkanes of C8-C25 were injected into GC-MS, and deterrence time for each Alkane was measured using $I=100n$ when 'n' is the number of carbons in related Alkane. Quartz index of essence oils was calculated using the following equation (28):

$$I=100n + 100((t_{ix}-t_{in}) / (t_{in}+1-t_{in}))$$

n: Carbon number of normal Alkan

$t_{in}+1$: Retention time of unknown compound

t_{in} : Retention time of normal alkane

After dewatering the produced oil, the oil was diluted using normal hexane (Merck) with the proportion of 1 to 10 and

then injected into GC-MS. Eventually, all components are determined using this apparatus.

The mass spectra of identified compounds of *Apiaceae* are showed in Figure 1. In this figure, I, MF and MW are Quartz Index, Molecular Formula, and Molecular Weight, respectively.

3 Results and discussions

3.1 Antioxidant activity of essence oil in *Apiaceae*

The antioxidant activity of essence in *Apiaceae* and its effect was measured in delaying the oxidation of fatty acid (oleic acid). Different concentrations of mentioned essence such as 250, 500, 750 and 1000 ppm (diluted with distilled water) were used in acetone as a solvent, and fatty acid without antioxidant (oleic acid) added to the mixture. The solution (1% v/w) injected into the oil container rotating with 1000 rpm speed. Oleic acid without antioxidants used as a control test. Figure 2 shows the oxidation stability of *Apiaceae* and control test according to dynamic process of DSC device.

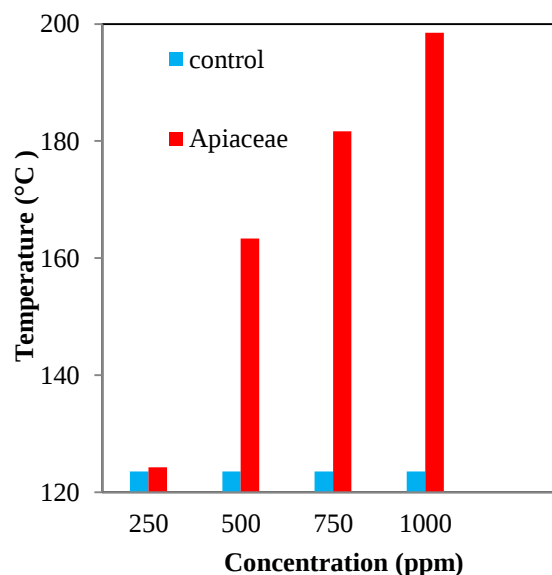


Figure 2: Measuring the oxidation stability of samples containing essence oil of *Apiaceae* and control test using a dynamic process of DSC device.

The vertical axis is temperature in °C, and horizontal axis is the concentration in ppm. The process was done under one atmosphere of nitrogen gas. Also, the oxygen flow rate and the heat rate were 50 ml /min and 10°C /min, respectively. As shown in Fig. 2, thermal stability of *Apiaceae* increased with increasing temperature. Also, the temperatures of oxidation stability of *Apiaceae* and control test are showed in Table 4. The effect of anti-oxidation stability of *Apiaceae* is depicted in Figure 3. For determining the effect of anti-oxidation, samples were analyzed at high temperatures. Calculate phenolic compounds of the concentration the measuring concentrations Acid gallic of different absorbance

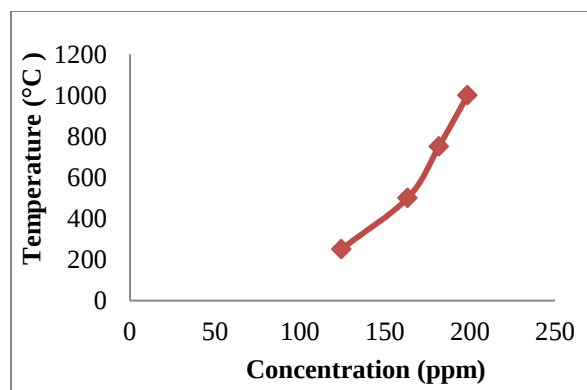


Figure 3: The temperature measurement of anti-oxidation stability of Apiaceae according to dynamic process of DSC device

Table 4: phenolic compounds of the concentration

Microliter Acid gallic	0	5	10	-	100
distilled water	500	495	490	-	400

calculated using the following equation (1):

$$\text{phenolic compounds of the concentration} = \frac{\text{Acid gallic}}{1000} \times \frac{20 \times 1}{1}$$

Amount of phenol in Apiaceae essential oil has been reported in Table 5 :

Table 5 : Amount of phenol in Apiaceae essential oil

Name plant	Essence	Mg/ Acid gallic
Apiaceae	Extraction	0.37

3.2 Antimicrobial effect of Apiaceae essential oil on microorganisms

Inhibitory effect of ethanolic extract and methanolic extract of *Apiaceae* against the eight mentioned bacterial strains and five different yeasts and fungus were evaluated through disk diffusion method, and the results are shown in figure 4. At the tested concentrations, ethanolic and methanolic extract showed the highest mean diameter of non-growth halo against *E. Coli* with the mean diameter of 22.8667 and 16.5067 mm respectively. Also, the lowest mean diameter of non-growth halo for both ethanolic and methanolic extract was for *Terichophyton verrucosum*.

In high concentrations both ethanolic and methanolic extracts of *Apiaceae* have significant antibacterial effects against mentioned microorganisms. It can be stated that, by increasing the concentration value, antibacterial effects grow in a concentration-dependent manner. Ethanolic and methanolic extracts of *Apiaceae* have the highest average of inhibitory effects against *E.coli* with the MIC values of 0.390 and 0.781 mg/ml respectively. These mentioned samples have more significant antibacterial activity against gram-negative strains, and MBC values verify the bacteriostatic performance of these extracts.

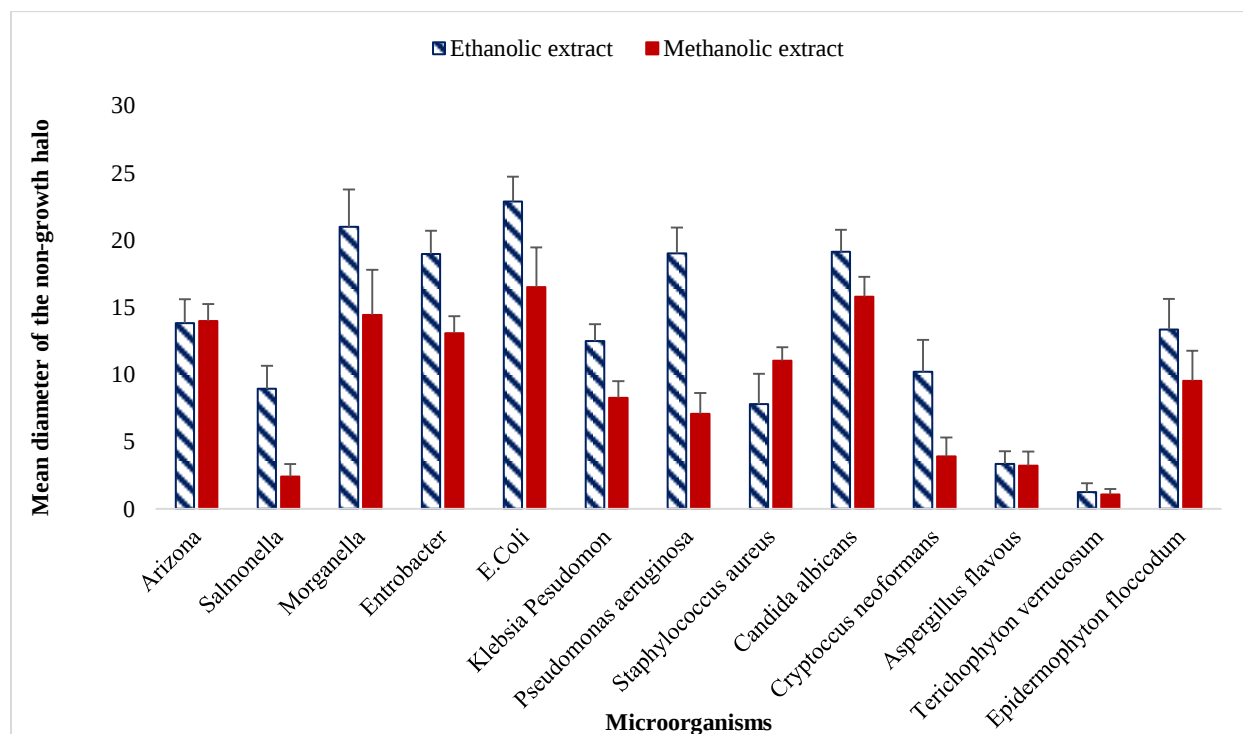


Figure.4: Inhibition zone (mm±SD) of Apiaceae essential oil on microorganisms

Table 6: MICs and MBC/MFC values of compounds against microorganisms

Microorganism	Ethanollic	Extract (mg/ml)	Methanolic	Exctract (mg/ml)
	MIC	MBC/MFC	MIC	MBC/MFC
Arizona	1.562	3.125	0.781	1.562
Salmonella	1.562	3.125	3.125	6.25
Morganella	0.781	1.562	3.125	6.25
Entrobacter	3.125	6.25	0.781	1.562
E.coli	0.390	0.781	0.781	1.562
Klebsia Pesudomon	3.125	6.25	0.781	1.562
Pseudomonas aeruginosa	3.125	6.25	1.562	3.125
staphylococcus aureus	3.125	6.25	0.781	1.562
Candida albicans	0.781	1.562	0.781	1.562
Cryptococcus neoformans	1.562	6.125	6.25	12.5
Aspergillus flavous	3.125	6.25	3.125	6.25
Terichophyton verrucosum	6.125	6.25	6.25	12.5
Epidermophyton floccodum	1.562	3.125	1.562	3.125

Also, the inhibition performances of both ethanolic and methanolic extracts of Apiaceae against five different yeasts and fungus are confirmed by MBC tests (Table 6). Finally it can be concluded that the antibacterial performance of ethanolic and methanolic extracts of Apiaceae is acceptable.

4 Conclusion

In this study, the antioxidant effect of Apiaceae essence oil was investigated using oleic acid and Differential Scanning Calorimetry. By increasing the concentration of essence oil, thermal stability of oleic acid increases. The Differential Scanning calorimetry method is practical in industry for measuring antioxidant properties of oils and fats because of simplicity, no toxicity, and high speed. The required time is short and small amount of the sample is enough. While there is no need for solvent and chemical reagents, the applied method is also helpful to qualify the oil and its oxidation stability. The scanning calorimetry tests on Apiaceae essence oil reveal that antioxidant activity depends on several parameters including substrate, oil type, available fatty acids, presence of other materials like Tocopherols, phenolic compounds, applied concentration of essences, stability measurement method and antioxidant activity. Also, following results are obtained: 1. The antioxidants of Apiaceae essence increase the stability of oil oxidation. 2. Increasing the concentration of antioxidants leads to increase in antioxidant activity and oxidation prevention. 3. Essences prevent oxidation improvement during primary steps and diffusion stage. 4. The conventional application of Apiaceae can play a significant role in clinical trials and treatment of many diseases as it contains many herbal compounds like antibiotics, palliatives, and diabetes reagents. Although this ability was not restricted to one order or division within the macro Apiaceae essential oil, but all of them offered opportunities to produce new types of bioactive compounds. Minimum inhibitory concentration (MIC) of investigated Apiaceae essential oil against bacterial isolates was tested in Mueller Hinton broth by Broth macro dilution method. The control tube contained only organisms and

devoid of investigated Apiaceae essential oil. The lowest concentration, which did not show any growth of tested organisms after macroscopic evaluation, was determined as Minimum inhibitory concentration (MIC). The activity was determined by measuring the diameter of the inhibition zone (in mm). The antimicrobial activities were expressed as zone of inhibition and minimum inhibitory concentration (MIC). Identification of compounds from a crude extraction of ferulacea essential oil was carried out by GC/MS technique. Finally, Apiaceae essential oil could serve as a useful source of new antimicrobial agents.

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