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THE IMPACT OF THE HARVEST DATES IN THE CHEMICAL COMPOSITION AND PHYSICAL PROPERTIES OF VOLATILE OILS AND FATTY ACIDS OF *ROSMARINUS OFFICINALIS* VAR. *TEMIFOLIUS*

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Abstract

In this research, the aim was to determine the impact of five harvesting time instances at the chemical composition of essential oils and fatty acids from aerial parts (leaves) of *Rosmarinus officinalis* var. *temifolius* cultivated in Mosul.10th July gave the highest percentage of oil, density, specific gravity and refractive index. The quantitative of five groups were determined using gas liquid Chromatography, ten standards of Volatile oil and seven standards of fatty acids was available, Results showed that the harvest time (10th march) was recorded the highest concentrations of limonene which scored about (4.60%). Methyl ester of all fatty acids (heptanoic, Lauric, palmitic, Linoleic, Linolenic and oleic acids were shown in harvest time (15th march and 15th may). Linoleic acid appeared at the highest concentration on 10th July (8.90%).

***Keywords*:** *Rosmarinus officinalis*, harvest date, essential oils, fatty acids, GLC.

Introduction

Rosmarinus officinalis (Rosemary) is an aromatic flowering plant belongs to the family Lamiaceae, cultivated in southern Europe, east and west the Arabian peninsula, and Mediterranean basin (Abdullah *et al*., 2010). The name of rosemary had been derived from two Latin word rose and marinus which means dew of the sea (Valtcho *et al*., 2015). Rosemary is timber evergreen, perennial plant, fragrant as flavored, leaves like-needle and flowers are white, pink, crimson or blue (Harvathova *et al*., 2010). It grows up to 2m high, with densely-leafy erect branches, a well-known valuable therapeutic benefit herb this is extensively used in medicines products (Okoh *et al*., 2010). Many components in rosemary oil such as α-pinene, camphene, Limonene, Camphor, 1,8 – cineole, borneol, cymene, and thymol are anticipated to make a contribution to its antimicrobial properties and antioxidative (Noui *et al*., 2019); (luma *et al*., 2020). Other compounds are oil α-thujene, sabinene, β-pinene myrcene, α-Treponeme, linalool, Camphor, Eugenol, Methyl Eugenol and bornyl acetate (Iram *et al*., 2018). Commonly volatile and aromatic oils acquired via the steam or hydro-distillation of many plants (*Majda et al.,* 2019). Various parts had been use to gain essentials oils, these encompass the flowers, Steam, leaves, Seeds, bark, roots and timber through Section elements (Azfali *et al*., 2009). Numerous studies had been reported on the chemical composition of the volatile oil of *R*osmarinus *officials* belonging to extraordinary regions in the world (Khorshidi *et al*., 2009). Fatty acid composition is commonly decided by (GLC) of the corresponding methyl esters organized using both acid or reaction of base catalyzed (Sana *et al*., 2016). Rosemary is used to treat extraordinary illnesses including: depression, in somniac and arthritic pain (Zargari, 1995) Moreover, oils compounds received from plants, have recognized antimicrobial, antifungal and insecticidal effectiveness. Volatile oils have many treatment and they useful resource the distribution of medicine and antiseptics (Oka *et al*., 2000; Ozcon & Chalchat, 2008). The Target of my observe is diagnosed the Installation of rosemary oil at five dates of harvesting time and to identify of many volatile oils and fatty acids using GLC analysis (Clark and Menary, 1984); (Gul, 1994); (Noguchi and Ichimura, 2004).

Materials and Methods

Vegetal material

My research has been done in the nursery of flowers colors, under heated plastic house in 2017 and 2018. Plants selected at the age of one year maintained in pots were evaluated at diameter 35cm containing 10 kg of mixture (1 soil: 1 peatmoss: 1 perlite).

Temperature and humidity are recorded during the research period, but the cover of plastic house was removed at the beginning of April/2018 and replace it with the green network Table (1). The harvest was done at two-month intervals between November 2017 and July 2018. All the plants were cutting at a high of 40 cm before starting the experiment in August 2017, as well as this plant height was measured before every harvest. Plants were fertilized with Nano NPK (20:20:20) at 2g/L spraying on vegetative growth once every month.

The experiment was arranged according to Randomized Complete Block Design consisting of 5 harvests time in three replicates, every harvest includes four plants and the averages were compared according to Duncan test by probability level of 5%.

Table 1 : Average of temperature and relative humidity of the months during the research

Harvest time	Temperature (C°)		Humidity %	
	great	small	great	small
November 2017	22 .90	8.41	52.3	45.4
January 2018	14.83	5.50	65.6	55.2
March 2018	19.40	8.50	60.4	52.2
May 2018	35.60	18.20	39.1	29.6
July 2018	42.20	27.20	28.0	16.0

* Temperature and Humidity was measured using wireless Hygro-thermometer

The leaves of rosemary were harvested in five date as explained above and dried in an open air and stored to perform extraction.

Essential oil extraction

For the purpose of conducting an extraction of essential oils, shade dried leaf parts of rosemary had been subjected to hydrodistillation using a Clevenger-kind equipment for 3.5 hours mixing 150g of leaves in 500mL of distilled water.

The volatile oils collected have been dried over anhydrous sodium sulphate and preservated at 4°C a while the evaluation was carried out (Clevenger, 1928). The physical properties of 60 volatile oil samples was measured such as percentage ratio, density, specific gravity, refractive index was studied too (Guenther, 1977).

Fatty acids extraction

Leaves of *R. officinalis* about 150g were extracted with 1000 mL of petroleum ether by the use of Soxhlet device for a period of 72 hours at 65°C. The mixture became evaporated under vacuum in a rotary evaporator at 65°C until 20mL, then the raw extract(crude)after evaporating became subjected to saponification with 5%(potassium hydroxide) by use of reflux for3 hours to eliminate the long chain fatty acid from the esters (Vogel, 1973). The extract became expressed as raw fat and using for the trans-methylation of the fatty acids. Fatty acids methyl esters in ether had been injection into gas chromatograph.

Gas liquid chromatographic analysis

Analysis of the essential oils and fatty acids was carried out on model 7769 by use of Flame Ionization Detector (FID) and stationary phase Cn-Wax 55 CB (30m × 0.32mm × 0.25µm) film thickness melt silica capillary column. Helium become the carrier fuel at 20 mL/min. Programming of temperature changed into carried out from 70°C-250°C at 30°C/min. The temperatures of injector and detector had been 210°C and 230°C. The injection volume turned into 10µL.

Identification of compounds

Analysis of essential oils and fatty acids have been quantified by using assessment with calibration curves generated through injecting increasing amounts of well known solutions(standard) received with the aid of dilution of a sample. Each height was diagnosed on the basis of retention time, the relative quantities of individual compounds have been calculated primarily based on GLC peak height without use of correction factor.

Results and Discussion

Characteristics of vegetative growth

Plant height, Number of branches, herb dry weight and dry leaf weight is given in Table (2). The harvesting time had affected on vegetative growth, there is significantly differences between the harvests. The third harvest (10^{th} march) was exceeded significantly in plant height, number of branches, herb dry weight and dry leaf weight which reached 78.67 cm, 5.20, 135.17g and 89.92g respectively compared with the harvest date (10^{th} November) which about 53.16 cm, 2.76, 92.34g and 32.21g. This result agreed with the report of Zewdinesh (2010) and Belay (2007) that showed on increment of plant height with harvest age, was also reported by Beata and Annal (2015) who indicated plant height, yield of dry herb and dry leaves effected by harvest times in August, September and October.

Chauhan *et al*. (2011) in various climatical condition, the growth was exceeded significantly in most vegetative traits such as height, fresh and dry herb while thyme changed into harvested 115 day after sowing seeds. Saliha and Memet (2001) also mentioned that the highest plant height of rosemary was obtained on March but the lowest height plant of rosemary was obtained on December.

Table 2 : Plant height, number of branches, herb dry weight and dry leaf weight affected by harvesting harvests

Harvesting time	Plant height	Number of branches	Herb dry weight (g)	dry leaf weight (g)
10^{th} November 2017	53.16 e	2.76 e	92.34 d	32.21 e
10^{th} January 2018	67.89 d	3.11 d	121.51 b	73.11 b
10^{th} March 2018	78.67 a	5.20 a	135.17 a	89.92 a
10^{th} May 2018	74.84 b	4.92 b	101.21 c	51.19 c
10^{th} July 2018	70.33 c	3.55 e	90.42 e	43.25 d

Essential oil properties

Table (3), shows that the highest oil content was 1.81%, density and specific gravity reached 0.9390 g/cm^3 and 0.9380 respectively in the harvesting time (10^{th} July) and the refractive index was 1.3590, while (10^{th} January) gave the lowest recorded values (0.36%, 0.9030 g/cm^3, 0.9100 and 1.3420) respectively.

Table 3 : Physical properties of *Rosmarinus officinalis*

Harvesting time	Oil %	Oil g/150 g	Density g/cm^3	Specific gravity	Refractive index
10^{th} November 2017	0.46 d	0.64 d	0.9060 d	0.9140 e	1.3480 d
10^{th} January 2018	0.36 e	0.55 e	0.9030 e	0.9100 d	1.3420 e
10^{th} March 2018	0.86 c	1.30 c	0.9170 c	0.9210 c	1.3500 c
10^{th} May 2018	1.08 b	1.63 b	0.9300 b	0.9250 b	1.3570 b
10^{th} July 2018	1.81 a	2.72 a	0.9390 a	0.9380 a	1.3590 a

Different letters are significant at 5% level. The results indicated that essential oil Percentage and the other physical properties was affected by harvesting time, the important factor that influenced on the production of essential oil was seasonal, climatic variations, and temperature (Jalal *et al*., 2009). Tulok *et al*. (1997) were investigated of the rosemary volatile oil production and plants growth affected by many Locations, plant age, harvesting time, CO_2 concentrations, methods of distillation and soil characters (Guillen and Cabo, 1996; Boelens, 1985; Tewari and Virmani, 1987; Liusia *et al*., 1996).

The correlation between volatile oil content and average temperature were shown in table (1, 3). Similar consequences were reported by Zewdinesh *et al*. (2012) who explained that oil content (1.63 – 2.77 %) Bensebia and Allia, (2015) found oil content (0.2-1.3) %.

Height value of volatile oil percentage was received in July and May (1.81, 1.08%) respectively. On the alternative hand, the lowest percentage of oil was determined in January 2018(0.36%) and November 2017(0.46%). Saliha and Memet (2011) showed that the highest percentage of oil was appeared in July (0.6.67%) and the lowest percentage of oil was determined in January (0.200%) and in March (0.233%). Also, Singh *et al*. (2000) showed that the harvest in September content and yield of oil become reduced, that is duo to the shorter photoperiod at some point of autumn.

The high density, specific gravity, refractive index of oil in July is attributed to the high temperature at July (42.2°C) that explained in Table(1) causes decrease the concentration of non-solid compounds which accompanies it increase the concentration of solid compounds (oxygenated) that leads to increase the Physical properties.

Essential oil composition

The essential oil of five harvested time from rosemary was analyzed by GLC with Flame Ionization Detector (FID). The qualitative and quantitative results of individual components are shown in figure (1, 2, 3); Table (4) major constituents in the essential oil of *R. officinalis* were limonene, Eugenol, Linalool and B-pinene in the harvesting time (10th March). Their content of all components at different growth phases varied in a rather wide range. For instance, the percentage of myrcene from November 2017 to July 2018 steadily decreased from 1.320% to 0.237% in March. Composition of essential oil is strongly dependent on developmental stage of the plant (ontogeny), and therefore harvesting time is one of the most important factors influencing in oil (Cerven & Zheljazkov, 2009). The effect of harvest dates on chemical content of essential oil in different members of family Lamiaceae has additionally been reported by way of these variations indicated that the dynamics of volatile composition in aromatic plant is possibly related to the expression of different genes indifferent harvesting time, this conclusion is supported by (Ghada *et al*., 2020)

Table 4 : Volatile oil content of *Rosmarinus officinalis* harvested at various growth stages by GLC

Chemical constituents	Standard Rt (min)	Harvest time				
		10th November Con.%	10th January Con.%	10th March Con.%	10th May Con.%	10th July Con.%
α- Pinene	3.11	0.070	**	0.072	0.001	**
β- Pinene	3.45	0.012	**	0.166	0.002	**
Camphor	4.62	0.031	**	0.237	**	**
Sabinene	5.59	0.602	**	0.024	4.223	0.003
Myrcene	6.014	1.320	0.021	0.237	3.130	**
Terpinene	6.73	**	0.029	0.108	1.932	**
Limonene	8.005	0.007	0.030	4.609	0.812	0.059
Linalool	9.125	**	0.011	0.172	0.203	0.029
Eugenol	9.917	0.333	0.007	0.466	0.013	0.616
Camphene	12.73	**	0.006	**	**	0.085

**** The compound was not found**

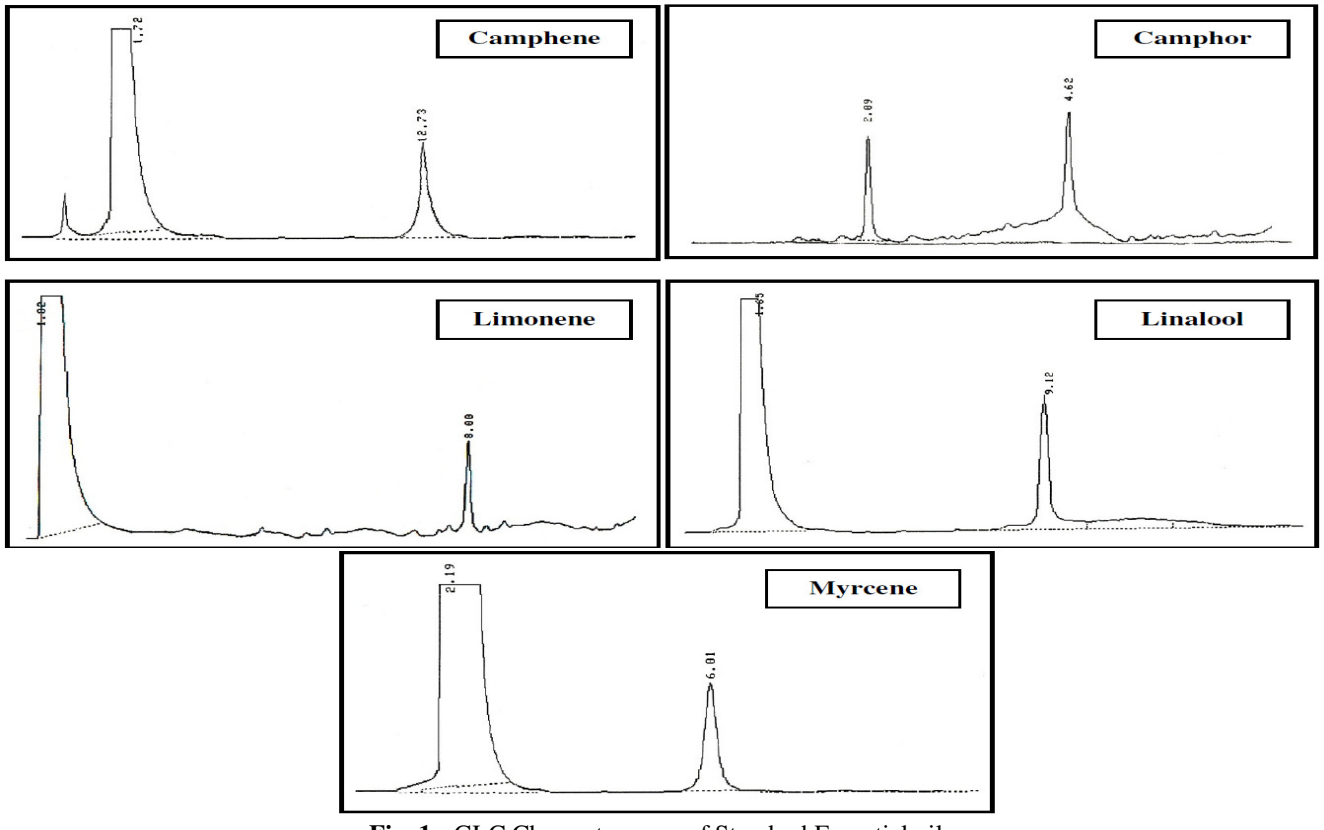

Fig. 1 : GLC Chromatograms of Standard Essential oils.

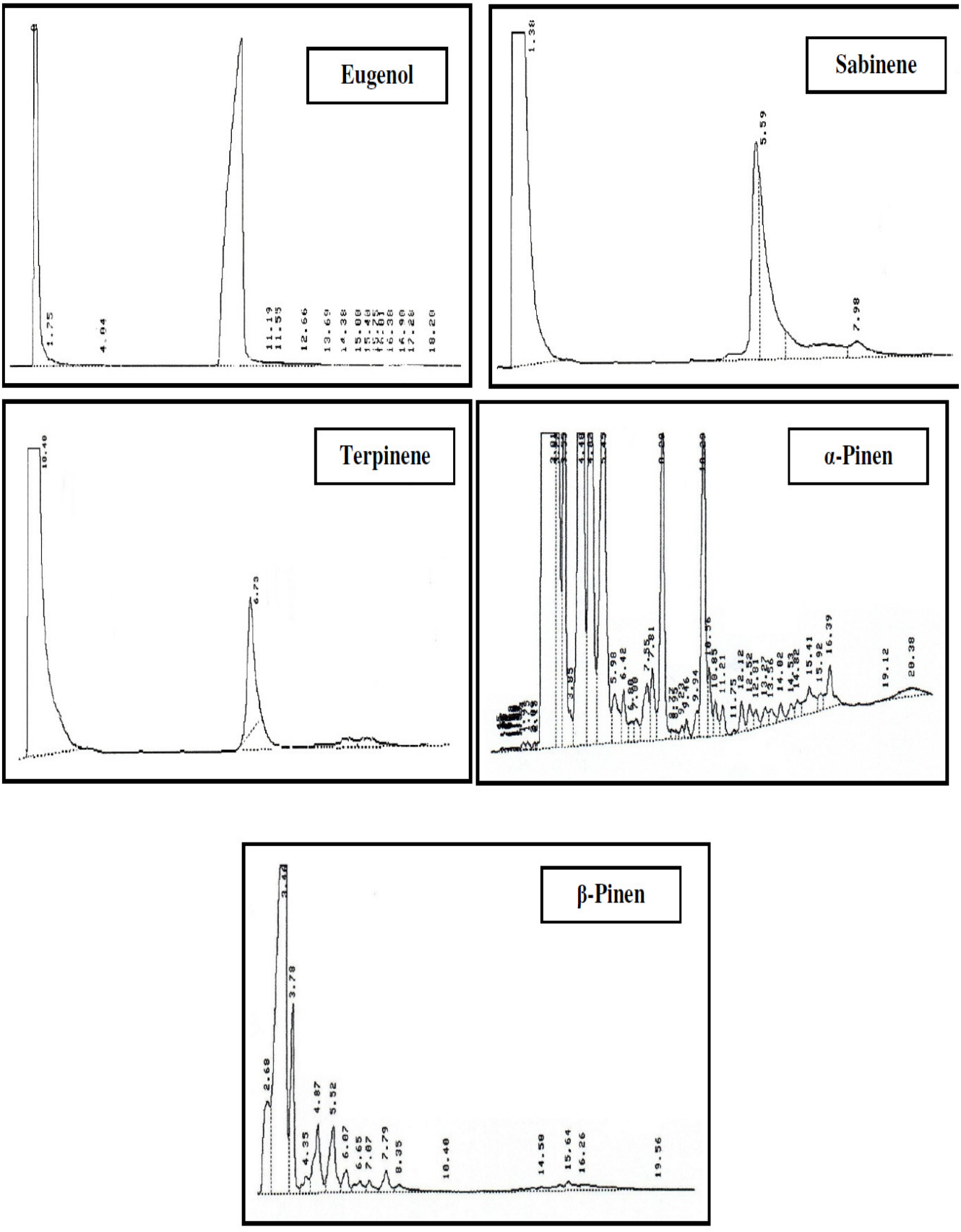

Fig. 2 : GLC Chromatograms of standard Essential oils.

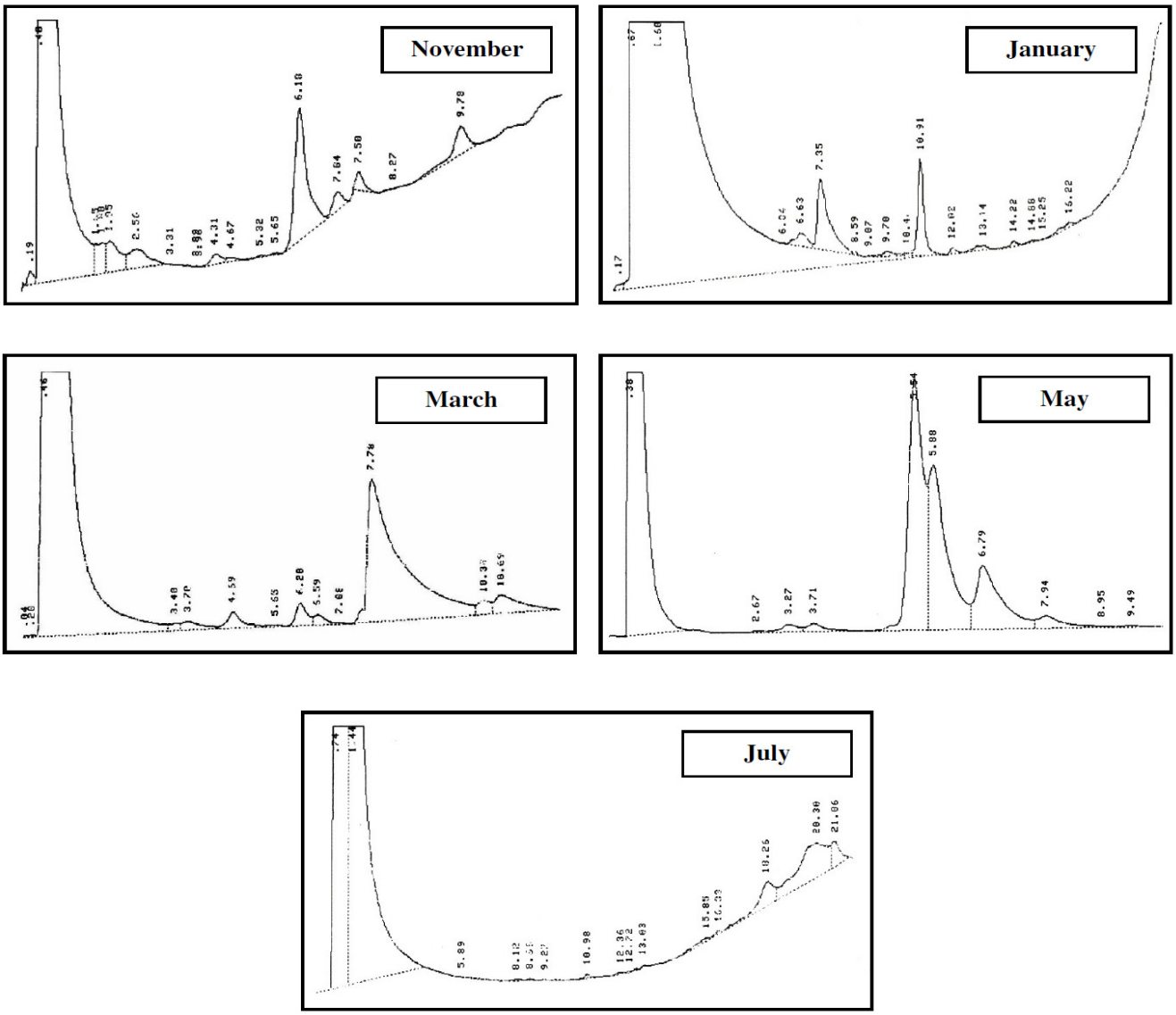

Fig. 3 : GLC Chromatograms of Essential oil.

Fatty acids composition

The results of the of the gas chromatographic analysis of the methyl esters of fatty acids of rosemary leaves revealed that seven standard was appeared at the harvest (10^{th} March and 10^{th} May), while three compounds of fatty acids (Luric, Palmatic, Lenolic) were identified in the harvest (10^{th} July); Table (5) and Fig. (4, 5).

Mostafa *et al*. (2005) found that the fatty acids composition of crude karkade seed oil (*Hibiscus sabdariffa*) contained Palmatic, Oleic and Linoleic acids were identified in higher amounts than other fatty acids. And a result of Who verified that the quantities evaluation confirmed the presence of fatty acids of rosemary leaves (Peiretti *et al*., 2012). A close result was indicated by Sana *et al*. (2016) that fixed oil of rosemary plant contains fatty acids such as myristic, palmatic, oleic, linoleic, linolenic.

Table 5 : Fatty acids composition of *Rosmarinus officinalis* harvested at various growth stages by GLC

Fatty acids	Standard Rt (min)	Harvest time				
		10^{th} November Con.%	10^{th} January Con.%	10^{th} March Con.%	10^{th} May Con.%	10^{th} July Con.%
Heptanoic acids	6.92	0.09	0.083	0.0104	0.267	**
Octanoic acids	8.38	0.179	0.03	0.0622	0.01132	**
Luric acid	12.57	0.028	0.9	0.03	0.532	0.160
Palmatic acid	15.99	0.033	**	0.099	0.948	0.181
Lenolic acid	16.99	0.019	0.01	0.0201	0.214	8.906
Lenolenlc acid	17.314	0.0339	0.02	0.0237	0.784	**
Oleic acid	18.612	**	**	0.0139	0.1592	**

**** The compound was not found**

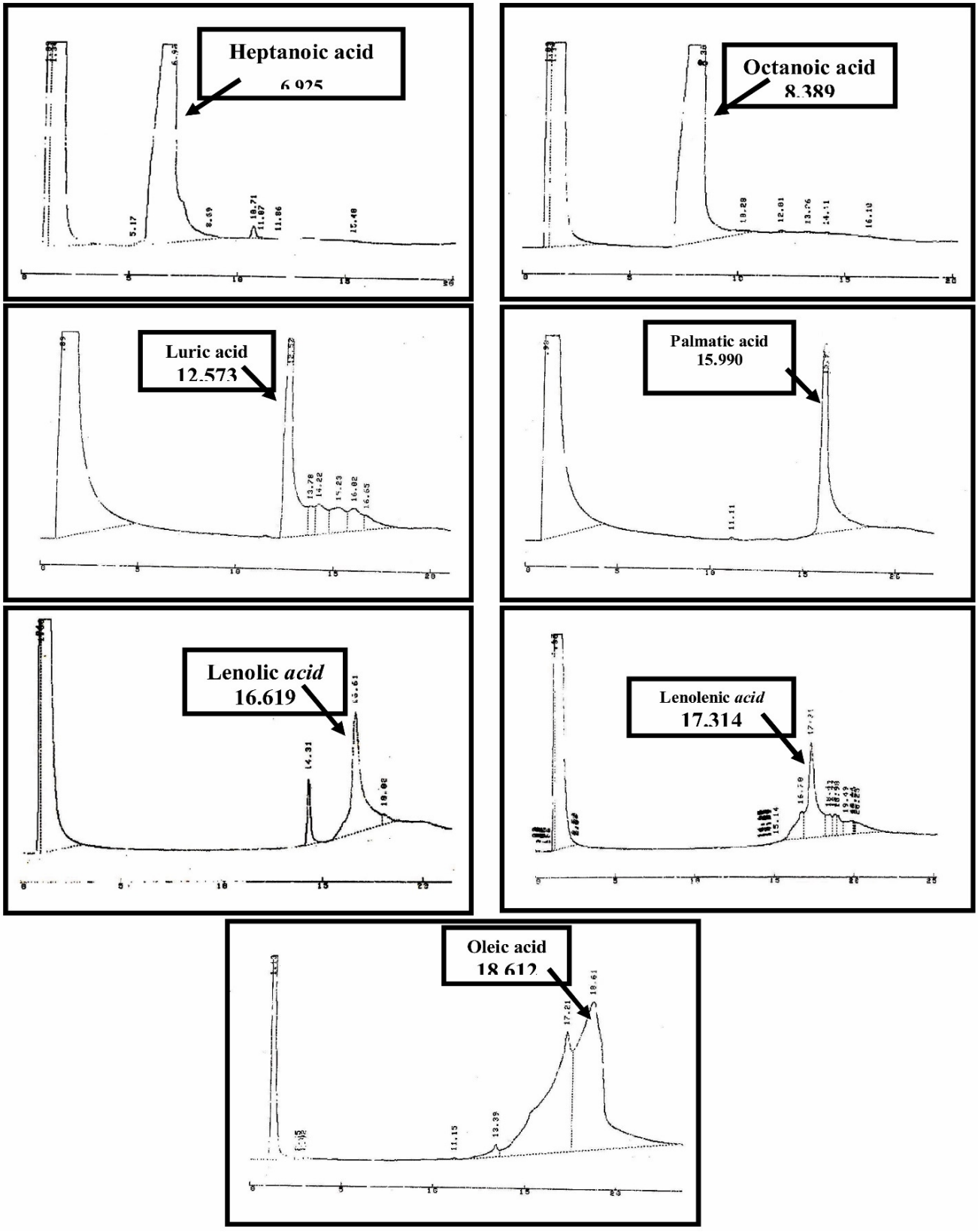

Fig. 4 : GLC Chromatograms of Standard Fatty acids.

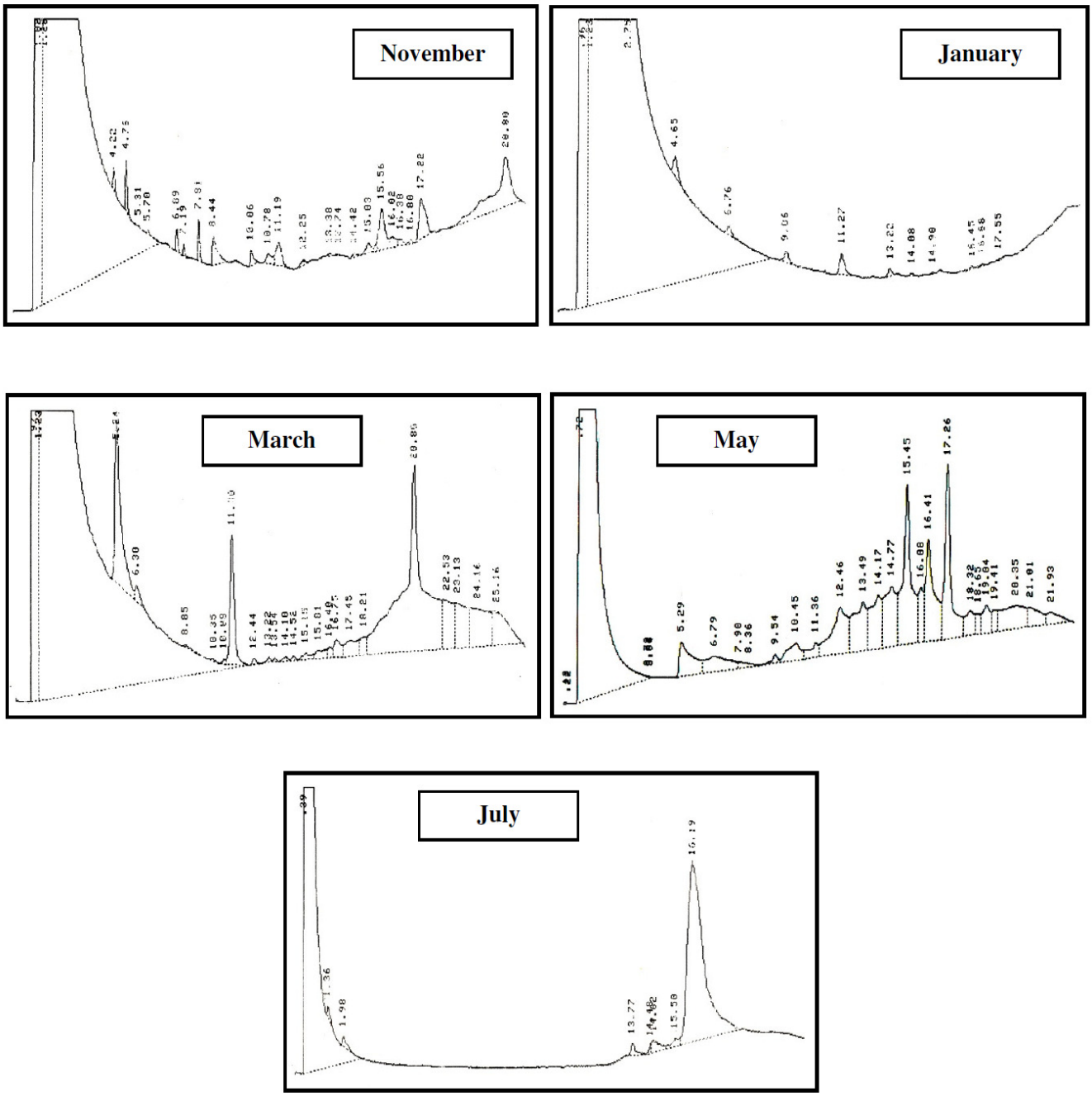

Fig. 5 : GLC Chromatograms of Fatty acids.

Discussion

Harvest date is one of the most essential element effecting of the good quality of volatile oils. It is a herb early or overdue led to a low yield of leaves in addition to the volatile oil installation. The stage of plant development are both immature herb or over immature resulting in a poor yield of herb and oil installation , as well as the timing of harvest, the number of harvests in step with year greatly affects the yield and the installation of essential oil.

Main effect of harvesting time had effected significantly on all oil parameters such as essential oil content (%), density, specific gravity and refractive index. As well as Absence of fatty acids were effected too by harvesting time. Comparing these dates of harvesting time, many of oil and fatty acid compounds was obtained in all agricultural indicators for the duration of the harvest (10^{th} March and 10^{th} May).

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