Essential Oil Composition of Laurus nobilis L. from Bethlehem Area

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Abstract:

Palestine, as part of the Mediterranean area, is famous in several aromatic plants rich in several essential oils with medical applications such as Laurus nobilis L. known as Bay tree. The dried leaves are used widely in cooking; the essential oil is generally used for flavors and scents. As a therapeutic plant, bay leaves have been used as a treatment for skin rashes andrheumatism. In addition, Laurel essential oil is important to the cosmetic industry; it is also used for the preparation of hair lotion due to its antidandruff activity and for the therapy of psoriasis. Laurus nobilis L. fruits are rich in fatty acid content and generally developed for the production of perfumed soaps and candles. The essential oil of leaves has antibacterial and antimicrobial properties too . The main chemical constituents of the essential oil include 1-8 Cineole, Pinene, Sabinene, Linalool, Eugenol, Terpinylacetate, MethylEugenol, as well as other esters and terponoids. Fruits contain fixed oil which is grease dark-green aromatic fluid. The research is concerned to check for the percentage of the important chemical ingredients between the leaves and fruits cultivated from different regions in Bethlehem Governorate and its variety in the Wet and Dry season for at least one year. The isolation of essential oils from bay leaves and fruits is done by hydro distillation followed by fractioning of the extractives by HPLC. The research will encourage farmers and the Palestinian community to plant such trees as a source for natural medical applications and flavors.

Keywords: Bay, Laurus Nobilis l., Essential Oil, Dry season, Wet Season

Introduction:

Essential oils are concentrated volatile aromatic compounds produced by plants that give their pleasant scents. It is extracted from a particular species of plant life from oil sacs in flowers, leaves, stems, roots, seeds, bark, resin or fruit rinds. The amount of essential oils is present in small percentages; it can be from 0.01% to 10 % of the total weight. It is widely used in foods, medicines, and cosmetics for their flavor and their remedial or odoriferous properties. (Choudharyetal. 2013). These oils have powerful antimicrobial properties with wide range of therapeutic constituents. (Patrakar et al. 1999). It contains hormone-like compounds, initiate cellular regeneration, and work as chemical defense against fungal, viral, and animal foes. The essential oils have a similar structure to some compounds found in blood and tissues, allowing them to be compatible with human physiology. (Dataset, 2007), (Marzoukia et al.2009).

Bethlehem, as part of the Mediterranean area, is famous in several aromatic

plants rich in several essential oils with medical applications such as Laurus nobilis L. known as Bay tree. The botanical name is Laurus nobilis L. from Lauraceae family; it has synonyms as Lauruspersea ,Bay laurel, Sweet bay, True bay, Grecian laurel. Its common Arabic name is Ghar. The bay tree is either a shrub or small tree with 20m high; its leaves are shiny, leathery and green. The fruits are cherry like purple to black in color. Bay is a plant of great industrial importance in foods, drugs, and cosmetics. The dried leaves and essential oils are used extensively in the food industry for seasoning of meat products, soups and fishes. As a medicinal plant, bay leaves are used as a treatment for rheumatism, dermatitis, skin rashes, earaches and for the cure of psoriasis. (Moghtader, et al. 2012), (Hammer et al. 1999).

In addition, Laurel essential oil is important to the cosmetic industry; it is also used for the preparation of hair lotion due to its antidandruff activity. Laurus nobilis L. fruits contain both fixed and volatile oils. The fixed oil is rich in fatty acid content which is grease dark-green aromatic fluid and generally developed for the production of traditional Palestinian perfumed soaps and candles. The essential oil of leaves has antibacterial and antimicrobial properties too. The main chemical constituents of the essential oil include: 1,8-Cineole, Pinene, Sabinene, Linalool, Eugenol, Terpinylacetate, MethylEugenol, as well as other esters and terponoids. (Moghtader et al. 2012), (Dataset, 2007).

The isolation of essential oils from bay leaves and fruits is done by steam distillation followed by fractioning of the extractives by HPLC for its qualitative and quantitative chemical components. (Zoran, 2009). (Macid, 2005). The research will encourage farmers and local communities to plant such trees as a source for natural medical applications and flavors and produce traditional Palestinian soaps.

The research is concerned to check for the percentage of the important chemical ingredients between the leaves and fruits cultivated from different regions in Bethlehem governorate and its variety in the Wet and Dry season for at least one year. The Wet season in Palestine starts in October and ends in May. The research started by making a survey on the presence of bay trees in Bethlehem governorate. The study is nearly limited to a range of eleven different bay tree locations and the evaluation of its chemical components starts to check the variety percentage of chemical components according to the Wet and the Dry season. The eleven samples give good and precise correlation for the percentage chemical constituents. The isolation of essential oils from bay leaves and fruits is done by Hydrodistillation technique with chloroform solvent.

The research is the first study on the essential oil of bay leaves and fruits in Bethlehem region. This research is the first study on the determination of the percentage of the chemical components of essential oil of bay leaves and fruits in Bethlehem region in addition to the first study in the West Bank too. The research will encourage farmers and local communities to plant such trees as a source for natural medical applications and flavors and produce traditional Palestinian soaps. The research will evaluate the major percentages of the chemical constituents of the bay essential oil. This new evaluation is a good indicator for future applications either in the pharmaceutical or industrial users as a Palestinian origin.

Study Area:

The study area is Bethlehem region which lies on the Eastern Basin of the Mountain Aquifer. Conferring to Palestinian Meteorological Department, the average annual rainfall for Bethlehem governorate is 518.4 mm/yr. The average annual rainfall for Bethlehem during 1900 to 2006 from Cremisan Monastery of coordinates 166400E to 126000N and at an elevation of 820 m is 574.7 mm/yr. More than 80% of the rainfall is received during the winter season which is from December to May. (Zeitoun, 2011). Bethlehem contributes to arid and semi-arid climate with an increase in aridity towards the Eastern Slopes in the Jerusalem desert. (Rofe and Raffety, 1963). The soil is mainly limestone type occupying the maximum area. Bethlehem has five major soil types which are: Bare Rocks and Desert Lithosols, Brown Lithosols and Loessial Arid Brown Soils, Brown Rendzinas and Pale Rendzinas, Terra Rossas, Brown Rendzinas and Pale Rendzinas and Brown Lithosols and LoessialSerozems The study area has mainly two types of soil: Brown Lithosols and Loessial Arid Brown Soils and Brown Rendzinas and pale Rendzinas. (ARIJ, 1997), (ARIJ, 1995). Figure (1) shows the five soil types of Bethlehem governorate. According to Rofe and Raffety 1963, the lithology is mainly limestone, dolomite, dolomitic limestone, marl, chalky marl, chert and chalky limestone. These soil types may effect on the chemical constituents of the essential oil.

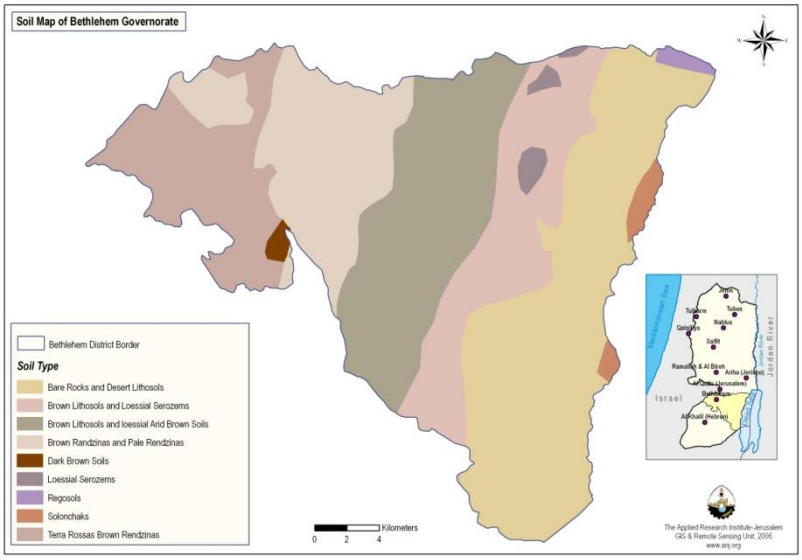

Figure (1): Soil map of Bethlehem governorate. (ARIJ, 2007)

Methodology:

1. The research is concerned to extract the essential oil from fresh leaves, dry leaves and fruits cultivated from different regions in Bethlehem governorate in the Wet and Dry season for 2017-2018. The number of samples is aroundeleven from different locations in Bethlehem governorate taking into consideration the developmental stage of the leaves and fruits and soil type.
2. The isolation of essential oils from bay leaves and fruits is done by steam distillation techniquewith chloroform for four hours followed by fractioning of the extractives by HPLCtechnique. Steam distillation is a special type of distillation for temperature sensitive materials like oils. The temperature of the steam must be high enough to vaporize the oil present without destroying the plants or burns the essential oils which will lose its therapeutic value.

3. HPLC Analysis:

The purity of the compounds is identified by analytical HPLC using a diode array detector and comparing the results with HPLC Primary Referenced standards.The components identification is achieved by the HPLC analysisusing Prominence-i-LC-2030C equipped with Rb-18 column of25 cm. The flow rate is 1µml/min, and the wavelength is 218nm. The mobile phase is 85% Acetonitrile with 15% of doubled distilled water.
The relative percentage of the essential oil constituents is expressed as a percentage by peak area normalization in HPLC. Identifications is made by comparing the total area of each peak in the sample with that of HPLC Primary Referenced standards such as: 1,8-Cineol, Sabinene, Alpha Terpineol, Alpha Pinene, Beta Pinene and Terpinal Acetate.The components of essential oil were identified on the basis ofcomparison oftheir retention time and area with the area of the referenced samples.

The following Figure (2) shows the process flow chart for extracting the essential oil from cleaned and dried bay leaves.

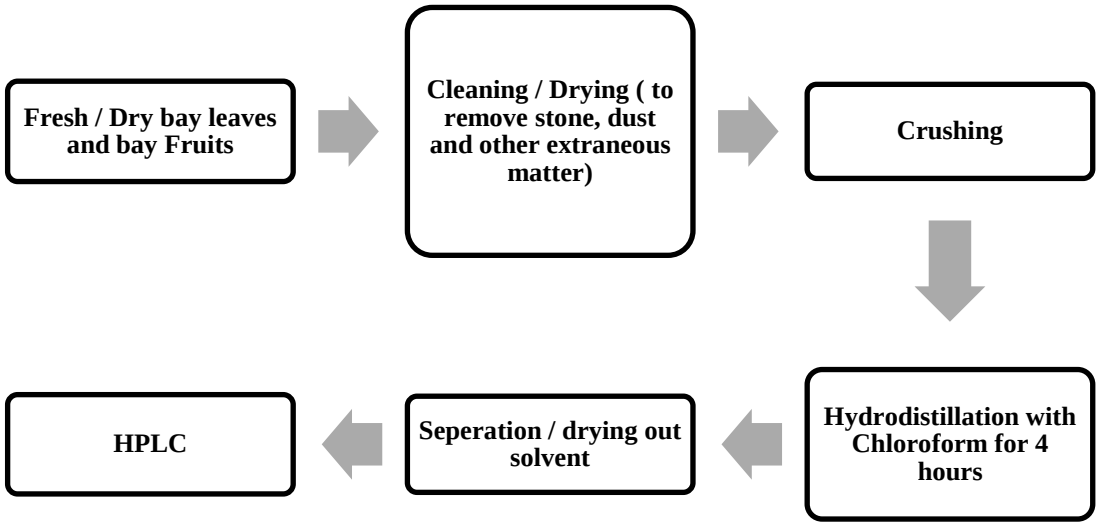

Figure (2): Process flow chart for the extraction of essential oil.

Field samples

The samples are collected from several locations in Bethlehem area. I faced a difficulty in getting samples from the eastern part of Bethlehem area because the local inhabitants are not used to plant such trees. The total number of samples is limited to eleven different locations in Bethlehem area in addition to duplicates from neighboring sites for some samples.

Table (1): Field samples code and location.

Sample #	**# samples of Dry season**			**# samples of Wet season**			**Location**
	Wet Leaves	Dry Leaves	Fruits	Wet Leaves	Dry Leaves	Fruits	
1	1A	1C		1AA	1CC		Khader 1
2	2A	2C		2AA	2CC		Nahalin
3	3A	3C		3AA	3CC		Deheishe
4	4A	4C		4AA	4CC		Beit Jala
5	5A	5C	5B	5AA	5CC	5BB	Bethlehem
6	6A	6C		6AA	6CC		Irtas 1
7	7A	7C		7AA	7CC		Battir 1
8	8A	8C		8AA	8CC		Doha
9	9A	9C		9AA	9CC		Battir 2
10	10A	10C		10AA	10CC		Irtas 2
11	11A	11C		11AA	11CC		Khader 2

Resultand Discussion:

The research is concerned to extract the essential oil from fresh leaves, dry leaves and fruits cultivated from different regions in Bethlehem governorate in the Wet season which is from October till May and Dry season from June till September for the year 2017-2018.A survey is done first to check on the presence of bay trees in different locations in Bethlehem Area. Unfortunately, the eastern part of Bethlehem has almost no bay trees. It is maybe because of dry weather and the villagers are not used to plant such trees. The total number of samples is around eleven samples for the study. I also tested some more samples as duplicate samples for the analysis. Each location sample is divided into three parts as: dry, fresh and fruit samples. The sample coding and location is already illustrated in Table (1).

The analysis is performed as it is already described through the methodology. The process flowchart is illustrated in Figure (2). In addition, the samples of the summerseason are collected within several field trips since some samples are without fruit. The second round of analysis starts for the Wet season before June 2018.
The isolation of essential oils from bay leaves and fruits is done by steam distillation with chloroform as solvent for four hours followed by fractioning of the extractives by HPLC. Steam distillation is a special type of distillation for temperature sensitive materials like oils. The purity of the compounds is identified by analytical HPLC using a diode array detector and comparing the results with reference standards. The

extracted samples are checked by HPLC and compared with six major HPLC reference samples of primary grade. The standard reference samples are: 1,8-Cineol, β-Pinene, α-Pinene, Sabinene, α-Terpineol and Terpinine at a wavelength of 218nm.

The results of the steam distillation showed good percentages of the essential oil for the Dry season. The results of the dry leaves are within the range of 5.1-16.6% with an average of 9.3%. The results of the fresh leaves are within the range of 5.5-14.7% with an average of 8.4%. The percentage of the fruit is limited only to one sample because all the other samples are without fruit in the summer. The result of the fruit is 8.76%. Some samples are duplicated and retested in the analysis and showed similar results with little deviation. The results of the Essential Oil percentages are illustrated in Figure (3) for the dry leaves of the Dry and Wet seasons on 2017-2018.

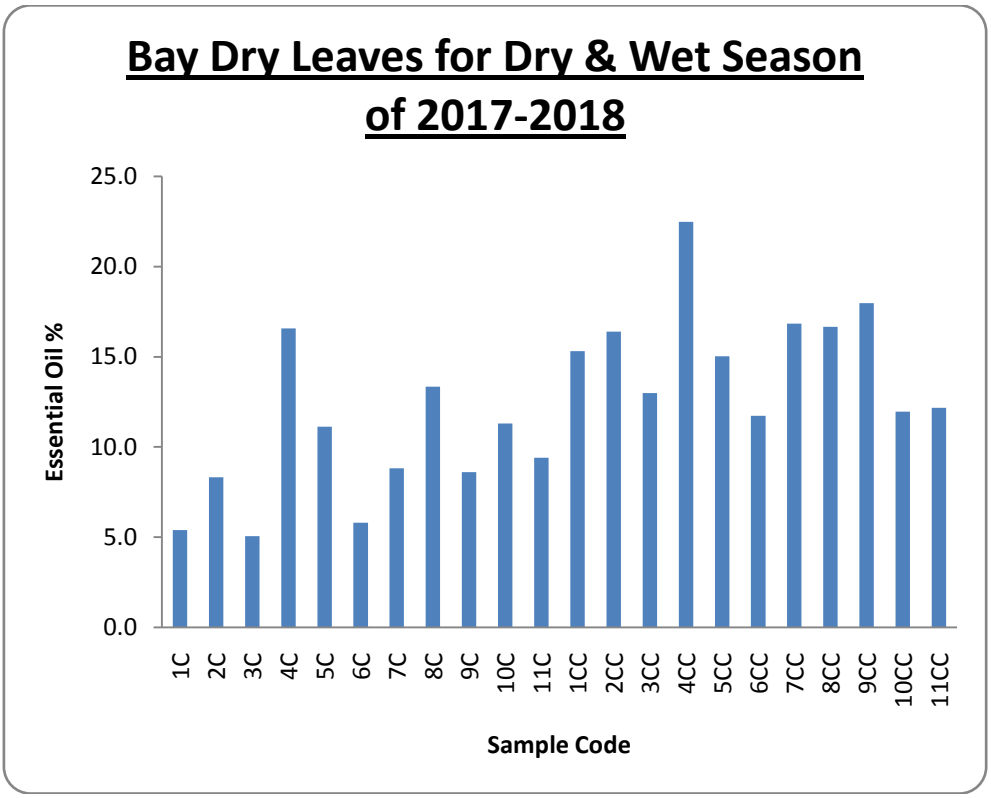

Figure (3): Essential Oil percentages for Dry leaves in Dry and Wet season of 2017-2018.

The results of the steam distillation showed good percentages of the essential oil for the Wet season. The results of the dry leaves are within the range of 11.7-22.5% with an average of 15.4%. The results of the fresh leaves are within the range of 5.6-15% with an average of 9.6%. The percentage of the fruit is limited only to one sample because all the other samples are without fruit. The result of the fruit is 17.2%. Some samples are duplicated in June 2018 for the analysis and hasparallel results. The results of the Essential Oil percentages are illustrated in Figure (4) for the fresh leaves in the Wet and Dry seasons on 2017-2018.

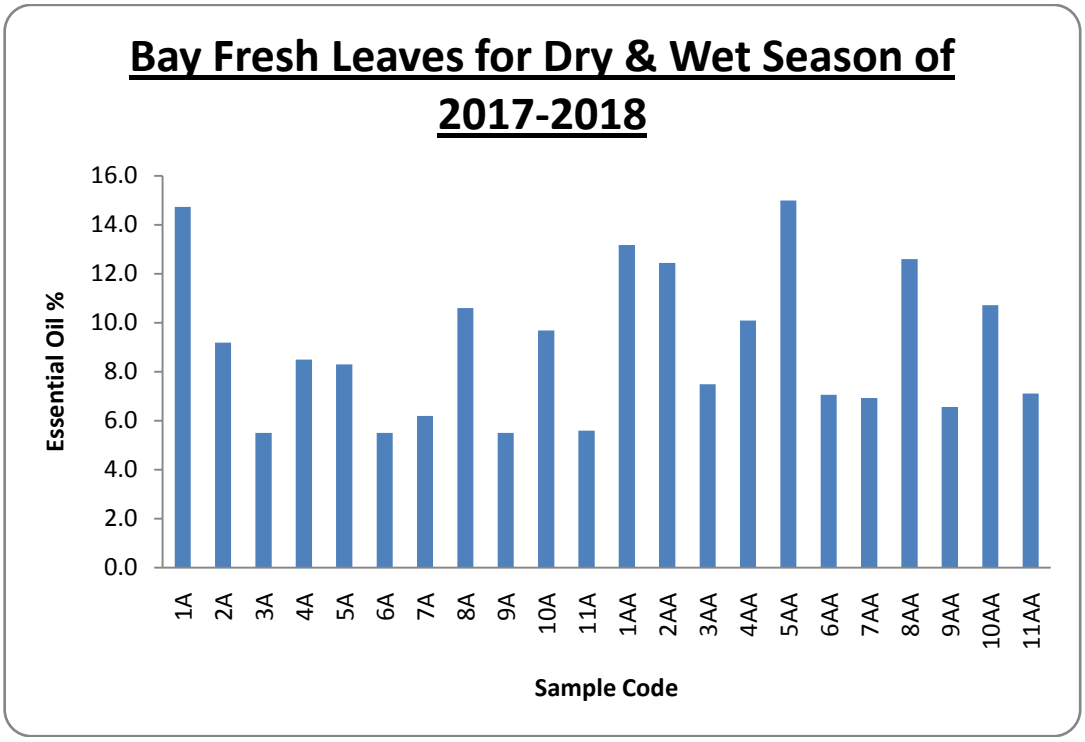

Figure (4): Essential Oil percentages for Fresh leaves in Dry and Wet season of 2017-2018.

The results of the Essential Oil percentages are illustrated in Table (2) for the Dry and Wet seasonsof 2017-2018. In general, the dry leaves of both seasons show higher Essential Oil percentages in comparison to the fresh leaves for each season. In addition, the Essential Oil percentages of the Wet season for the dry leaves and fresh leaves are higher than those of the Dryseason.

Table (2): Essential Oil percentages in the Dry and Wet Season for 2017-2018.

Dry Season					
Fresh Leaves		**Dry Leaves**		**Fruit**	
Code	%essential Oil	Code	%essential Oil	Code	%essential Oil
1A	14.7	1C	5.4		
2A	9.2	2C	8.3		
3A	5.5	3C	5.1		
4A	8.5	4C	16.6		
5A	8.3	5C	11.1	5B	8.76
6A	5.5	6C	5.8		
7A	6.2	7C	8.8		
8A	10.6	8C	13.3		
9A	5.5	9C	8.6		
10A	9.7	10C	11.3		
11A	5.6	11C	9.4		
Min	**5.5**	**Min**	**5.1**		
Max	**14.7**	**Max**	**16.6**		**8.76**
Average	**8.4**	**Average**	**9.3**		
Wet Season					
Fresh Leaves		**Dry Leaves**		**Fruit**	
Code	%essential Oil	Code	%essential Oil	Code	%essential Oil
1AA	13.2	1CC	15.3		
2AA	12.4	2CC	16.4		
3AA	7.5	3CC	13.0		
4AA	10.1	4CC	22.5		
5AA	15.0	5CC	15.0	5BB	17.2
6AA	7.1	6CC	11.7		
7AA	6.9	7CC	16.8		
8AA	12.6	8CC	16.7		
9AA	6.6	9CC	18.0		
10AA	10.7	10CC	12.0		
11AA	7.1	11CC	12.2		
Min	**5.6**	**Min**	**11.7**		
Max	**15.0**	**Max**	**22.5**		**17.2**
Average	**9.6**	**Average**	**15.4**		

The relative percentage of the oil constituents is expressed as a percentage by peak area normalization in HPLC. Identification of the chemical constituents is done by comparing the peak area with the retention time of the reference sample with that of the sample. For example, Figure (5) shows the HPLC data for the standard reference sample of α-Terpineol at the retention time of (3.06).

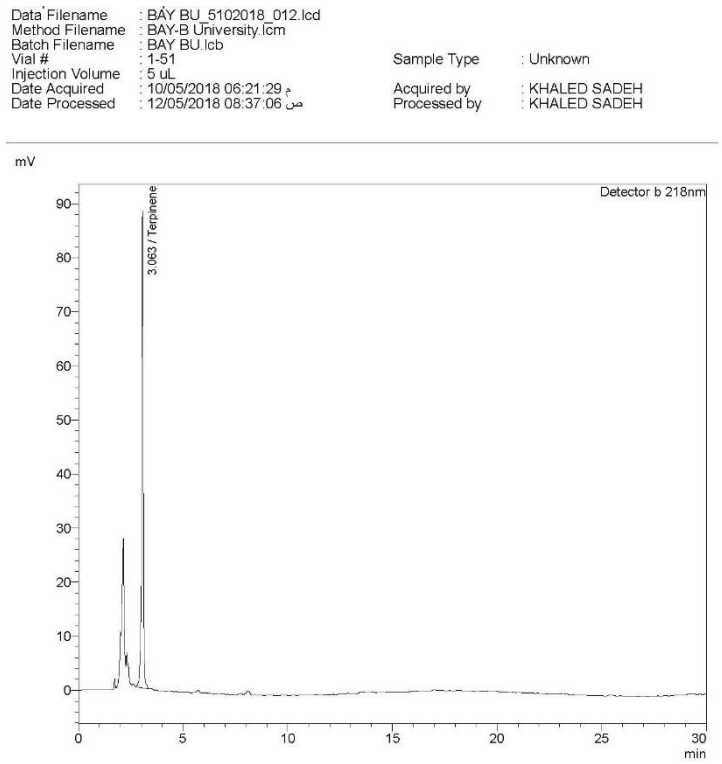

Figure (5): HPLC graph for the reference sample α-Terpineol.

The sample of (2AA) for dry leaves of the Wet season, as in Figure (6), shows similar retention time of(3.04) as α-Terpineol; its percentage is (4.46%) asα-Terpineol which is considered high percentage in comparison with other samples.

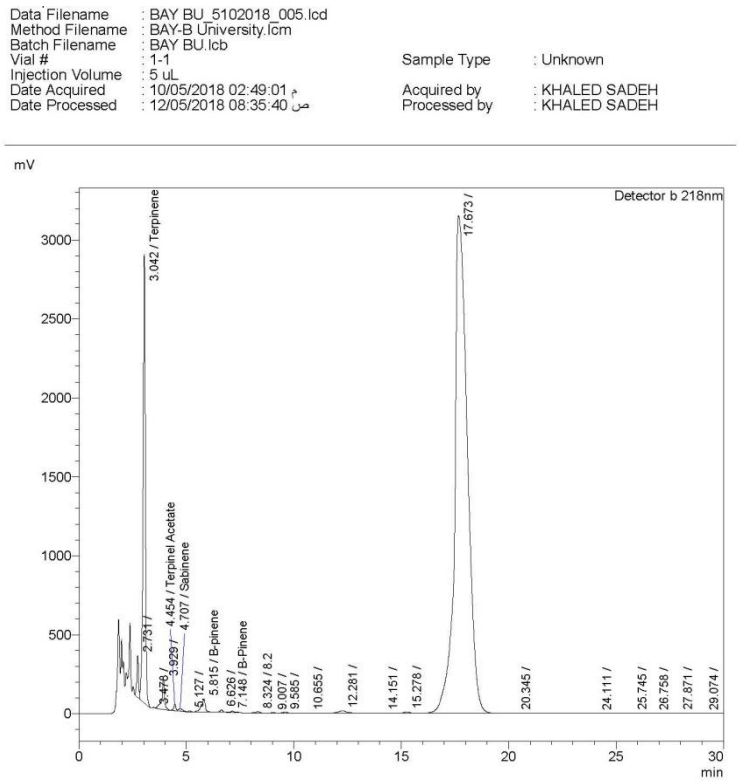

Figure (6): HPLC graph for the sample (2AA).

The overall percentages of the chemical constituents in the Wet season are less than the Dry season. This is due to the draft weather and transpiration of the plants leaves

in summer time. For instance, some samples showed high concentration of 1,8-Cineol with a maximum of 35.9% as in sample (2C). The other chemical constituents are present in very low concentration.The results are illustrated in Table (3) and Figure (7) for the Dry season.

Table (3): The percentages of the chemical constituents of dry and fresh leaves in the Dry season of 2017-2018. (N.D. = no detection)

% Chemical constituents of Dry season 2017-2018				
Sample code	**1,8-Cineol**	**α Terpineol**	**Sabinene**	**β Pinene**
2A	23.3351567	9.62447E-07	N.D	N.D
10A	32.97367521	0.000352788	0.019178376	N.D
1C	0.363589744	0.000135651	0.025405432	0.005756162
2C	35.98695157	0.000161869	N.D	0.001373996
3C	5.738632479	0.000502257	0.019022998	N.D
4C	3.296	8.24971E-05	0.007417487	0.001479732
5C	3.57585755	N.D	1.08797E-06	N.D
6C	0.021065527	1.22708E-07	0.014736967	0.002806395
7C	3.301196581	8.86714E-08	1.29118E-06	0.002214274
8C	3.776324786	1.37914E-08	N.D	N.D
11C	3.510803419	1.22138E-07	2.19981E-07	0.001613216
5B	N.D	0.003996	0.0442E-05	0.00185
Minimum	**0.021065527**	**1.37914E-08**	**2.19981E-07**	**0.001373996**
Maximum	**35.98695157**	**0.003996**	**0.025405432**	**0.005756162**
Average	**10.5344776**	**0.00047567**	**0.010720482**	**0.002441968**

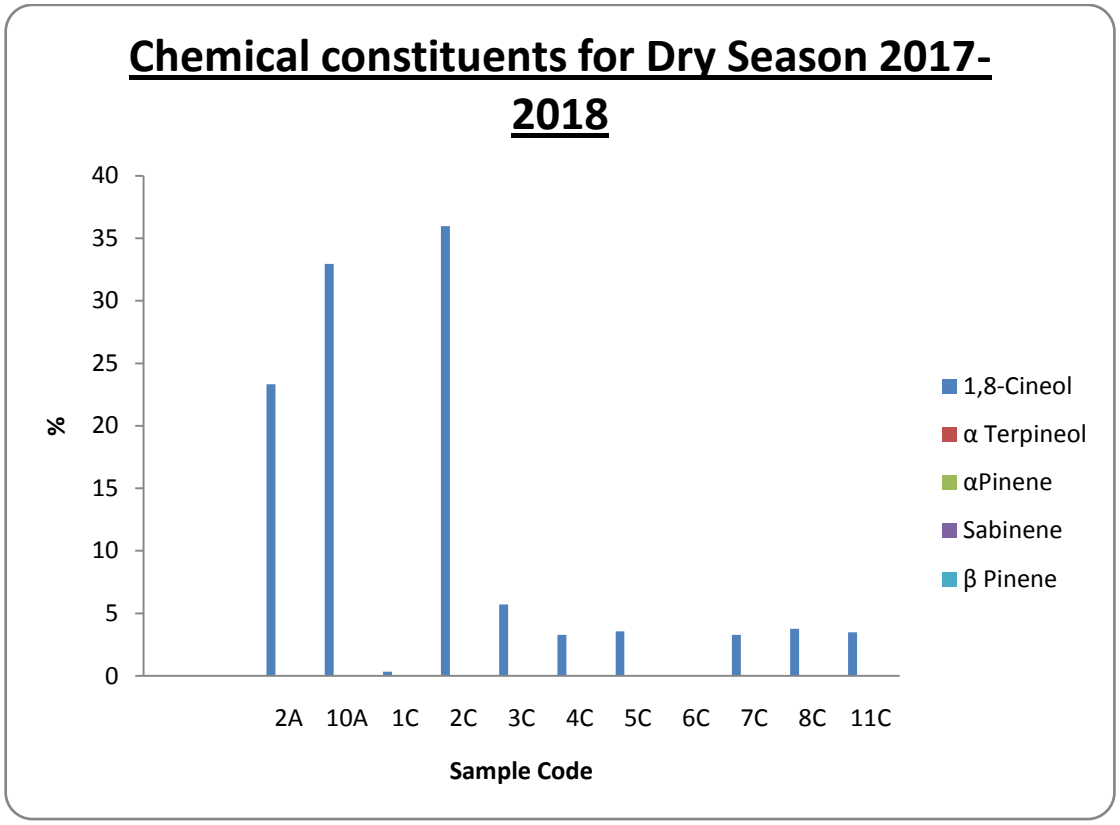

Figure (7): The percentagesof the chemical constituents for Fresh and Dry leaves in Dry season of 2017-2018.

The chemical constituents of the Wet season showed very low concentration in comparison with the draft summer. The data are illustrated in Table (4) and in Figure (8). For example, sample (2C) showed very high percentage of (35.98%) 1,8-Cineol in Dry season whereas it is in the Wet season as (2CC) negligible for (0.0039%).

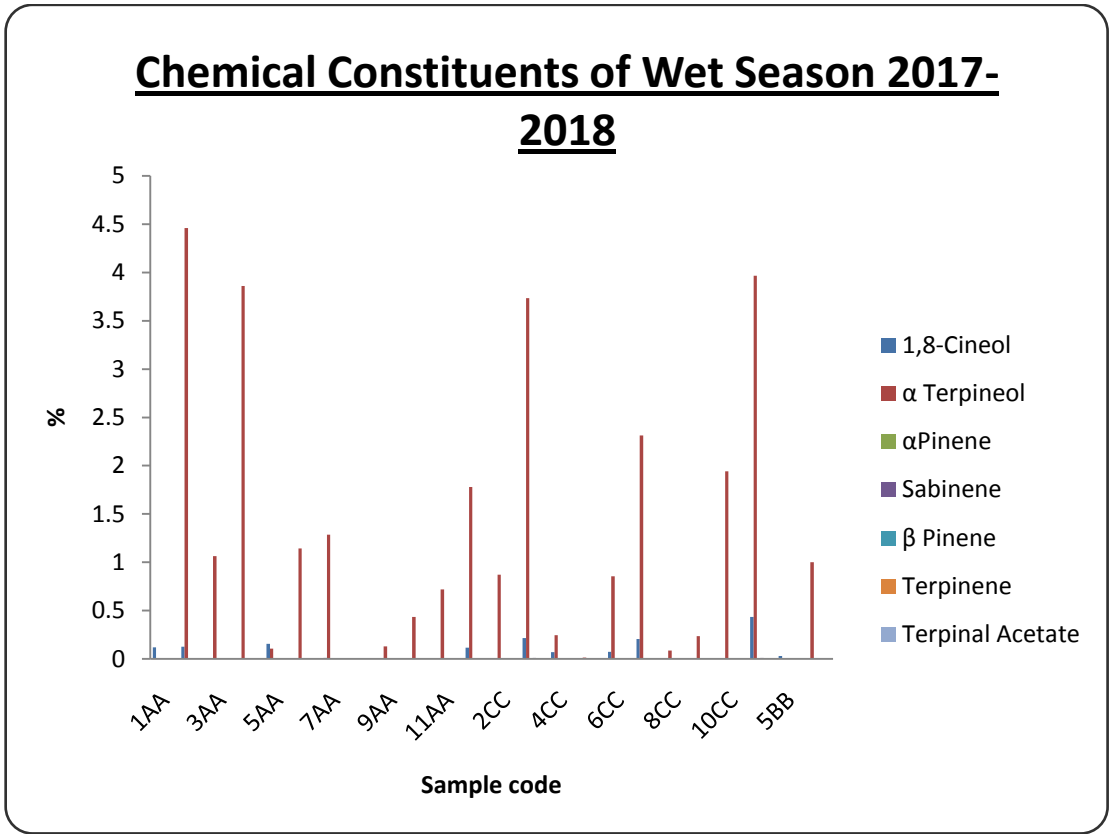

Figure (8): The percentagesof the chemical constituents for Fresh and Dry leaves in Wet seasonof 2017-2018.

The analyzed data also shows that 1,8-Cineol is the dominant chemical constituents in almost all samples of the Dry season while α–Terpineol is the dominant in the samples of the Wet season.

Table (4): The percentages of the chemical constituents of dry and fresh leaves in the Wet season of 2017-2018. (N.D. = no detection)

% Chemical constituents of Wet season 2017-2018						
Sample code	**1,8-Cineol**	**α-Terpineol**	**α-Pinene**	**Sabinene**	**β-Pinene**	**Terpinal Acetate**
1AA	0.120860483	N.D.	0.000401039	0.000830519	0.000102375	0.000480544
2AA	0.127355173	4.460138645	0.000405987	0.003874053	0.000169558	0.000317709
3AA	0.002179529	1.064212344	N.D.	0.000619285	3.54556E-05	7.28254E-05
4AA	0.003310002	3.858558507	0.000169259	0.0029691	4.05351E-05	0.000289821
5AA	0.158923885	0.105625942	0.000745621	0.003099205	0.000330685	0.000348388
6AA	0.007151568	1.143883418	N.D.	N.D.	N.D.	8.80196E-05
7AA	0.012498827	1.284802418	N.D.	0.000394623	3.22194E-05	0.000120094
9AA	N.D.	0.128595621	N.D.	0.000457904	N.D.	N.D.
10AA	0.002843261	0.43326343	N.D.	0.000951448	0.000151157	4.72769E-05

11AA	0.002467078	0.720396192	N.D.	0.001982216	4.3843E-05	5.60337E-05
1CC	0.117744465	1.778523806	0.000340056	0.00435885	0.000127991	0.000118555
2CC	0.003979988	0.872628209	N.D.	0.004084074	7.4614E-05	0.000116642
3CC	0.217001359	3.732900198	0.000649358	0.010417009	0.000383165	0.000343193
4CC	0.072876694	0.243604861	0.000295017	0.005423767	5.75123E-05	0.000118034
5CC	0.002058424	0.013686687	N.D.	0.000475621	N.D.	3.92893E-05
6CC	0.074811127	0.855350551	0.000254857	0.001908417	7.90899E-05	0.000150184
7CC	0.207768036	2.313156874	0.000874533	0.006933905	0.000249689	0.000389019
8CC	0.002660702	0.086732929	6.66213E-05	0.006977603	N.D.	7.63494E-05
9CC	0.010152253	0.234868299	0.000126618	0.00178441	3.25965E-05	0.000112778
10CC	0.006369857	1.940430141	0.000157832	0.00565022	2.11187E-05	0.000169148
11CC	0.435913098	3.966536357	0.002418508	0.008864385	0.000896257	0.000602307
5BB	0.005217255	0.999247817	0.001748532	0.004861235	0.001851314	0.000401484
min	**0.002058424**	**0.013686687**	**6.66213E-05**	**0.000394623**	**2.11187E-05**	**3.92893E-05**
max	**0.435913098**	**4.460138645**	**0.002418508**	**0.010417009**	**0.001851314**	**0.000602307**
average	**0.07392886**	**1.439863964**	**0.000618131**	**0.003662755**	**0.000259954**	**0.000212271**

The chemical constituents of the essential oil of the different plant locations contained the same compounds, but the quantitative differences between all main compounds are quite small. For instance, 1,8- Cineol, α-Terpinyl acetate, Sabinene, α-Terpineol, α-Pinene, and β-Pinenewhich are the basic components of the essential oil of the leaves arefound in small quantities in the fruits.

Conclusion:

Essential oils are concentrated volatile aromatic compounds produced by plants that give their pleasant scents. Bethlehem, as part of the Mediterranean area, is famous in several aromatic plants rich in several essential oils with medical applications such as Laurus nobilis L. known as Bay tree. The dried leaves and essential oils are used extensively in the food processing for seasoning of meat products, soups and fishes. The essential oil of leaves has antibacterial and antimicrobial properties too. The main chemical constituents of the essential oil include: 1,8-Cineole, Pinene, Sabinene, Linalool, Eugenol, Terpinyl acetate, Methyl Eugenol, as well as other esters and terponoids. Essential oils are extracted from bay leaves and fruits by steam distillation followed by HPLC for the qualitative and quantitative chemical components. The isolation of essential oils from bay leaves and fruits is done by Hydrodistillation technique with chloroform solvent. The research is the first study on the essential oil of bay leaves and fruits in Bethlehem region. The research will evaluate the major percentages of the chemical constituents of the bay essential oil.

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