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CONTRIBUTION STUDY OF THE FIXED OIL IN *LAURUS NOBILIS* L. WIDE SPREAD IN SYRIA

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ABSTRACT

The fixed oil was extracted from samples taken from two regions with different heights in Syria by using petroleum ether in ultrasonic bath. Gas Chromatography was used in order to determine the analytical different of the chemical components of these fixed oils. Also, the results were compared with the components of a commercial sample of *Laurus* fixed oil. The analytical chemical study of the components of the fixed oils showed the richness of *Laurus* nobilis with Lauric acid, linoleic acid and linoleic acid, which is consistent with the reference studies.

KEYWORDS: *Laurus* nobilis, GC, Fixed oil.

INTRODUCTION

Laurus an aromatic, evergreen (Vardapetyan et al. 2013) and perennial (Zeković et al. 2009), shrub or small tree (Nurba et al. 2005), usually growing up to height of from 3 to 15 m high (9 to 50 feet) at a slow rate (Moghtader et al. 2013). It belongs to Lauraceae family, which comprises numerous aromatic and medicinal plants (Hogg et al. 1974). The genus *Laurus* is consists of the two species *Laurus* azorica and *Laurus* nobilis (Basak et al. 2013). *Laurus* nobilis is a very important medicinal plant and there is a comprehensive monograph about this plant in many Herbal Pharmacopoeia (Ghannadi et al. 2002), and it has a long history of folk use in the treatment of many ailments (Moghtader et al. 2013). *Laurus* Leaves appear in January (Moghtader et al. 2013), alternate, they are 5–12 cm long and 1.8–5 cm wide, oblong, leathery, on both surfaces, lateral veins 10–12 pairs, margin slightly undulate, apex acute or acuminate, with a darkgreen color (Peter et al. 2010). The leaves do not fall during winter (Basak et al. 2013), they have a strong pungent odor because they contain essential oil up to 3%. The leaves can be used either dried or fresh but the flavour is stronger in dried and grinded leaves, but the leaves which are stored longer than a year they will lose their flavour (Bown, 2001). *Laurus* fruits: are small and olive-like, fleshy, dark purple when mature, ovoid, thin, brittle, wrinkled pericarp, which when broken discloses the seed kernel, the seed-coats adhering to the inner surface of the pericarp. *Laurus* fruits contain both fixed and volatile oils. The essential oil is existing up to 1% (Baytop, 2000), while *Laurus* fruits contain around 26% fixed oil (İlisulu K., 1992), this oil is a green, granular, melting at 40°C, to a darkgreen aromatic fluid, and consisting of a semi-solid fat.

Fixed Oils comprise mainly of triglycerides (Carmen et al. 2011), a small portion of non-glyceridic co-constituent like free fatty acids, partial glycerides (mono- and diglycerides) and a wide range of minor components. These minor components include sterols glucosides, tocopherols, hydrocarbons, phosphatides (phospholipids), hydrocarbons, waxes, pigments (gossypol, chlorophyll), vitamins (carotene), protein fragments as well as resinous and mucilaginous materials and heavy metals (Martincic et al. 2001). The fatty acids esterified with glycerol comprise about 95% of most oils and the fatty acids composition is the most important chemical characteristic of an oil (Ranken et al. 1997). There are many studies on the chemical composition of the fixed oil obtained from *Laurus* grown in Mediterranean and Europe (Table 1), showed that the geographical, genetic and environmental factors, are key factors on the *Laurus* fixed oil characterizations in the yield and composition (Marzouki et al. 2008; Nurbas et al. 2005; Ozcan et al. 2010: Diasa et al. 2014).

Table 1: the fatty acids composition of *Laurus* fixed oil in other studies.

Region	Marzouki 2008	Nurbas 2005		Ozcan 2010	Diasa 2014
Compound		**Symbol**		**Concentration %**	
Decanoic acid	C10:0	0.4	-----	-----	0.29
Lauric acid	C12:0	27.7	7.5	38.08	0.54
Myristic acid	C14:0	1.0	0.4	2.66	1.31
Palmitic acid	C16:0	17.1	25	14.33	13.47
Palmitoleic acid	C16:1n9	0.3	0.7	-----	0.50
Stearic acid	C18:0	-----	-----	-----	0.62
Oleic acid	C18:1n9	27.2	39.6	-----	3.78
Linoleic acid	C18:2n9,12	21.5	22.1	40.79	12.40
Linolenic acid	C18:3n9,12,15	1.2	-----	-----	51.59
Arachidic acid	C20:0	0.2	Trace	1.62	1.11
Arachidonic acid	C20:1	0.7	-----	-----	0.15

Laurus nobilis is distributed in western Syria from the northwest to the southwest. There are also indevidual numbers scattered here and there in the middle region near the coast. Kesab is a town in Syria famous of Laurel Soap manufacture, and it is characterized by its extended forests on Mount Al-Aqraa, such as Fouroloq forests. Kesab is 800 meters above sea level, and overlooking at the Mediterranean Sea. *Laurus* samples were harvested from the Al-Sajra area with coordinates (35°55′30″N 35°59′19″E). Slinfah is a Syrian city with a height of 1130 meters above sea level and it is famous with Oak forests (Quercus) and has many natural plant growing. *Laurus* samples were harvested from an area with coordinates (35°36′1″N 36°10′43″E).

The need of the medicinal plants to use in treatment and food industries is increasing, due to increasing of population and to spread new types of diseases, also due to the resistance caused by pathogens such as bacteria which become stubborn against some types of antibiotics. The increasing demand for plants caused to wanton harvesting of plants, which led to decrease the enumeration of these plants, and the spread of commercial cheat, and this led to a sharp decline in the population and natural spread, which led to the spread of fraud in the trade of these herbs, which called for the need to develop analytical chemical to ensure the purity of these samples and free of fraud. The Chromatography techniques are the most important to determine the purity of the fixed oil, which are extracted from *Laurus* fruits.

The fixed oil of *Laurus* berries are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, and for the production of perfumed soaps (Bozan et al. 2007) such as Aleppo´s Soap, and candle manufacture because of their fatty acid content (Hafizoglu and Reunanen, 1993). Also, it is used externally to treat sprains, bruises etc, and is sometimes used as ear drops to relieve pain (Grieve, 1984).

Given the scarcity of the chemical studies on *Laurus* in Syria, which has not been reported to date. The aim of the current study was to form comprehensive diagnostic methods for *Laurus* fixed oil.

MATERIALS AND METHODS

Plant samples

The samples of *Laurus* leaves and fruits were collected from two different Areas in Syria, the first from Kesab which located at 800 m above the sea level with the Coordinates (35°55′30″N 35°59′19″E), and the second from Slinfah which located at 1130 m above the sea level with the Coordinates (35°36′1″N 36°10′43″E).

The Samples were air-dried at room temperature in the shade for some weeks. They had a final moisture content of 10.0 %. Before using them, the dried samples were grinded in a blender. At the end of the milling process, the particle sizes were in the range of 0.8–0.9 mm.

Fixed oil extraction

Fruits oil is extracted by using petroleum ether in ultrasonic bath 15 min up for twice. Then it filtered and evaporated by using a rotary evaporator at 50°C. The fixed oil Dried and stored in the dark at room temperature until use.

Fixed oil analysis

The fixed oils components are converted to the simplest convenient volatile derivative, usually methyl esters. 50 microns of fixed oil sample was Taken and diluted to 2 microns by hexane, it mixed and then the fixed oils were esterified using methanol sodium hydrate solution (29g NaOH in 250 ml methanol). The solution was centrifuged for 2mins at 4000 rpm. The upper layer hexane was taken and washed with water, and then the upper layer (hexane) was taken again and dried on anhydrous sodium sulfate. 1 microns of hexane containing fatty acids was injected in GC. Gas chromatographic (GC) analysis were carried out on a Shimadzu GC 17 AFW with FID detector and a DB23 capillary column (60 m×0.25 mm; film thickness 0.25 µm). The carrier gas was helium with a flow rate of 0.81 ml/min.

The oven temperature for first 4 min. was kept at 75 °C and then increased at a rate of 3 °C/min. until 180 °C and keep on for 10 min., injector and detector temperature were set at 260 °C.

RESULTS AND DISCUSSION

Fixed oil amount

The percentage of the amount of *Laurus* fruits fixed oil were ranged between 26.5% and 30.4% as seen in the (table 2), where *Laurus* which grow in Slinfah gives the high rate of fruits fixed oil:

Table 2: The fixed Oil amount of *Laurus* fruits.

Region	Slinfah	Kesab
Coordinates	35°55′30″N 35°59′19″E	35°36′1″N 36°10′43″E
F.O mL/100g	30.4	26.5

Fixed oil analysis

As seen in the (table 3), 11 different compounds (fig. 1) were determined. There was no marked difference in the composition of laurel fixed oil samples, which was extracted in laboratory, while the percentage of the compounds of this samples were far away from the percentage of the commercial sample compounds, and this give a sign of the impurity of the commercial sample.

Table 3: the fatty acids composition of *Laurus* fixed oil.

Region	Kesab	Slinfah		Commercial sample
Coordinates	35°55′30″N 35°59′19″E	35°36′1″N 36°10′43″E		--------
Height above sea level	000 m	1130 m		--------
Compound	Symbol	Concentration %		
Decanoic acid	C10:0	0.146	0.146	0.146
Lauric acid	C12:0	14.041	11.971	3.453
Myristic acid	C14:0	0.694	0.546	0.426
Palmitic acid	C16:0	16.576	20.251	16.967
Palmitoleic acid	C16:1n9	0.253	1.014	0.403
Stearic acid	C18:0	0.905	0.905	0.905
Oleic acid	C18:1n9	48.650	37.099	32.799
Linoleic acid	C18:2n9,12	16.976	22.641	44.197
Linolenic acid	C18:3n9,12,15	1.094	1.094	1.094
Arachidic acid	C20:0	0.114	0.114	0.114
Arachidonic acid	C20:1	0.295	0.295	0.295

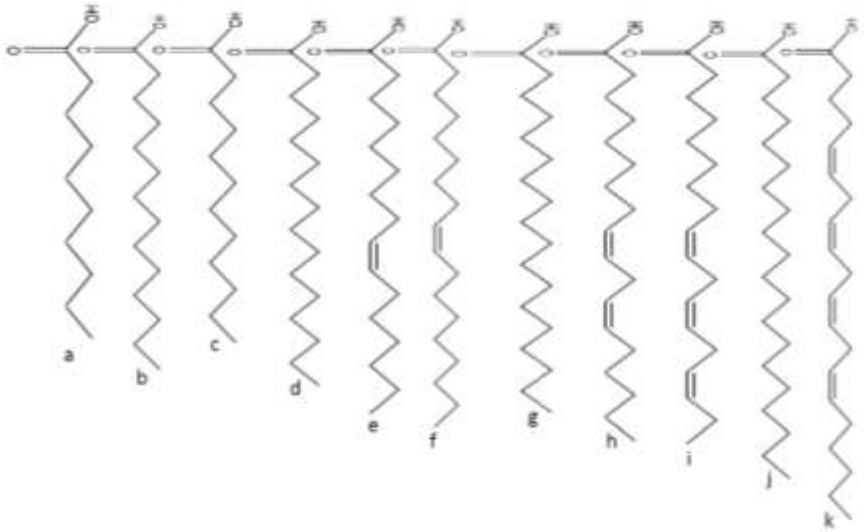

Fig. 1: The chemical structure of the fatty acids of *Laurus* fixed oil, a: Decanoic acid, b: Lauric acid, c: Myristic acid, d: Palmitic acid, e: Palmitoleic acid, f: Stearic acid, g: Oleic acid, h: Linoleic acid, i: Linolenic acid, j: Arachidic acid, k: Arachidonic acid.

CONCLUSION AND DISCUSSION

In this study, the fixed oils of the fruits of *Laurus* were obtained using Ultra sound bath and analyzed by gas chromatography with flame ionization detection (GC-FID) to determine their chemical composition and to identify of their chemo-types. The yield of the fixed oil extracted from *Laurus* fruits varied from 26.5% in Kesab to 30.4% in Slinfah (Table 2), these percentages can be

compared with Yılmaz (2017) who confirmed that the yield of the fixed oil extracted from *Laurus* fruits range between 28.37% and 42.08%. The differences could be attributed to the geographical Factors (characteristics of the ecological zone), as the fruits of *Laurus*, which grew in Slinfah gave the highest rate of fixed oil than the other area.

The comparison of Syrian samples did not reveal any big difference in their qualitative composition, but collection of samples from different areas in Syria allowed monitoring the change of the oil quantative contents. Eleven components were identified. The fatty acids in the fruits of *Laurus* mainly consist of Oleic acid (37.1 - 48,7%), Linoleic acid (17,0 - 22,6%), Palmitic acid (16,6 - 20,3%), and Lauric acid (11,9 - 14.0%).

The composition of fixed oils showed a similar pattern to those published for other geographical regions (Table 1).

The results of these study on *Laurus* fixed oil ensure the importance of the constituents of fixed oil in medicinal, nutritional and pharmaceutical branches, for example: Linoleic acid (omega-6: which exists in vegetable oils, poppy seed, safflower, sunflower, and corn oils) and Linolenic acid (omega 3: which exists in the seed oil of evening primrose, rapeseed, blackcurrant seed oil, soy beans, walnuts, flax seed, and hemp seed) are considered as an essential fatty acid, as the human body cannot synthesize them from other food components (Burr, 1930). Furthermore. a poor diet with Linoleic acid leads to mild skin scaling, hair loss (Cunnane et al. 1997) and poor wound healing (Ruthig, 1999), and many studies have confirmed that Linoleic acid has anti-inflammatory, acne reductive, and moisturizering properties when applied topically on the skin (Diezel et al. 1993; Letawe et al.1998; Darmstadt et al. 2002). On the other hand, Lauric acid (which exists also in coconut oil, palm kernel oil and in a various mammal's milk (Beare-Rogers et al. 2001; Anneken et al. 2006)). is leads to increase in high-density lipoprotein (HDL) (Mensink et al. 2003) and also it is a useful component in a treatment for acne. Otherwise, Oleic acid (omega 9: which exists in olive oil, pecan oil, canola oil, peanut oil, macadamia oil, sunflower oil, grape seed oil, poppy seed oil (Untoro et al. 2006), and sesame oil (Alfred, 2000)) is used as an excipient in pharmaceuticals, and as an emulsifying or solubilizing agent in aerosol products (Smolinske, 1992). And also, the other fatty acids which are present in a small percentage give *Laurus* fixed oil great importance, for example Myristic acid (which exists in nutmeg butter, palm kernel oil, coconut oil, and animal fats) is used in cosmetic and topical medicinal preparations due to its absorption properties. And so, with Stearic acid, which is one of the most common saturated fatty acids found in nature following palmitic acid (Gunstone et al. 2007), where is used to produce cosmetics and detergents such as soaps, shampoos and shaving cream.

The presence of all these fatty acids together in one component (*Laurus* fixed oil) give it a great importance as a source for pharmaceutical and cosmetic industries.

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