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The Lebanese *Citrus aurantium*: A Promising Future in Medicinal Phytochemistry

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The West Bekaa region of Lebanon is mostly an agricultural land where traditional herbal medicine is a common practice. Many medicinal applications are especially attributed to the *Citrus aurantium* plant mainly used in the food and drink industry. In this work, the essential oil of *Citrus aurantium* is studied in an attempt to find an explanation for the different medical applications of this plant extract which is widely known among locals. Using Gas Chromatography coupled to Mass Spectrometry, twenty-six different chemicals were identified and quantified. These include the major constituent, Linalool, (29.99%), Bergamot (14.94%), Farnesol (9.86%) and D-Limonene (8.42%). The latter as well as Linalool are known to have anticancer effects, thus representing major components in anticancer treatments. Their proportions in the studied essential oil and the presence of the other components (a-thujene, a-pinene, Camphene, Sabinene, b-pinene, b-Myrcene, p-Cymene, Ocimene (E), Linalool oxide (Trans), Linalool oxide (Cis), Terpinen-4-ol, Terpineol, Nerol (Or Cis-Geraniol), Neral, Geranial, Neryl acetate, Geranyl acetate, Nerolidol, Farnesal, Eicosane, Tetracosane, Pentacosane) have amplified the importance of *Citrus aurantium* as a scientifically-proven medicinal plant thus justify its diverse medical applications.

Keyword: *Citrus aurantium*, Linalool, Limonene, Essential Oil, GC/MS.

1. Introduction

Medicinal plants are widely used in treating various diseases and illnesses. The essential oil of such plants are being extracted, and analyzed for potential anti-microbial, anti-inflammatory and even anti-oxidant effects.

Recent research reveals a growing interest in finding natural antioxidants, including volatile chemicals, in plants because they inhibit oxidative damage and may consequently prevent inflammatory conditions[1,2].

In Lebanon, locals have always used these plants and attributed many effects to each one of them, creating a wide scope of treatments based on them[3]. Tests are conducted on the essential oil of the plants. Most of the chemical constituents of these oils are characterized by a low molecular weight which allows easy transport across cell membranes to induce different biological activities including anti-fungal[4], anti-microbial, anti-inflammatory, and anti-oxidant effects[5,6].

Essential oil from *Citrus aurantium* and the monoterpene limonene found in it are widely used as flavoring agents and can be found in some common food items. This species is also used medicinally throughout the world to treat gastritis and gastric disorders[7]. In this study, the analysis of the essential oil of *Citrus aurantium*, growing in Lebanon, more specifically in the West Bekaa region, by GC/MS revealed twenty-six constituents most of them having medical importance.

The parts of this plant most often utilized by the population are the fruit peel, flowers and leaves. *Citrus aurantium* is used to treat anxiety, insomnia[8], and as an anticonvulsant[9]. It is also used to treat gastrointestinal tract disorders and for its diuretic action against tachycardia and rheumatism. Lately, more light is being shed on the anticancer effects of this plant due to the presence of Linalool and D-limonene among its constituents.

The aim of this study is to identify and quantify the constituents of the Lebanese *Citrus aurantium* essential oil using GC-MS, based on their retention time and retention index, and to investigate the medical effects of these components in order to try to find an explanation for the different remedial applications of this important medicinal plant.

2. Materials and Methods

2.1 Sample collection

Citrus aurantium was collected from Zahle in the west Bekaa region of Lebanon and authenticated by Mr. Kamal Akl (Botanist, Agricultural Engineer).

2.2 Hydrodistillation

Essential oil from the Lebanese *Citrus aurantium species* was isolated by hydrodistillation for 4 h using a Clevenger-type apparatus. The oil obtained was collected in dark glass vessels and stored at below 4 °C until chromatographic determination. For every 1 kg of *Citrus* flowers 3 ml of oil is collected.

2.3 Gas Chromatography–Mass Spectrometry

A Shimadzu QP 2010 plus gas chromatography system interfaced to a 2010 mass spectrometer was used for analysis of the samples. The separation was performed on a 30 m x 0.25 mm i.d. (internal diameter) fused silica capillary column coated with 0.25 µm film Rtx-5MS. The injector and the detector temperatures were respectively 250 and 280 °C. The oven temperature was held at 40 °C for 4 min, and programmed from 40 to 80 °C at 6 °C min^{-1} then to 150 °C at 6 °C min^{-1} and finally to 280 at 16 °C.min^{-1}. Split injection was conducted with a split ratio of 8:10. Helium was used as carrier gas, and flow-rate was 1.62 mL min^{-1}. The mass spectra were recorded over a range of 30-1000 amu (atomic mass unit) at 0.5 s $scan^{-1}$. Solvent cut time was 5 min. Ionization energy was 70 eV. The inlet and ionization source temperature were 280 °C. The chemical composition of the oil was identified by comparing their spectra with those of a NIST library and confirmed by comparing their retention indices with data published in various literatures.

3. Results and Discussion

The chromatogram of *Citrus aurantium* showed twenty-six constituents with Linalool as a major component (29.99%), Bergamot (14.94%), Farnesol (9.86%) and D-limonene (8.42%), which is responsible not only for the distinctive citrus odor and flavor, but also for major medicinal applications. Table 1 summarizes these components, their retention time, retention index as well as percent Area.

The major constituent of the Lebanese *Citrus aurantium* essential oil was found to be linalool. This fragrant liquid alcohol, $C_{10}H_{18}O$, occurs both free and in the form of esters in many essential oils and is used in perfumes, soaps, and flavoring materials. It is also a natural insecticidal similar in activity to D-limonene and exhibits antifungal properties[10]. Limonene (8.42% in our sample) is of utmost importance being one of the most common terpenes in nature and is the major constituent of an essential oil series. Due to its pleasant citric fragrance, it is commonly used as a flavoring in foods and drinks, for which it is

classified in the U.S. Code of Federal Regulation as safe. Tests on animals have proven the effectiveness of limonene against some types of cancer including gastric, mammary, pulmonary adenoma and liver.

Table 1: Chemical composition of *Citrus aurantium* growing in Lebanon

Name	Retention Index	% Area
α-Thujene	927	traces
α -Pinene	933	0.22
Camphene	953	traces
Sabinene	972	0.43
β-Pinene	978	6.09
Myrcene	991	0.59
p-Cymene	1025	0.2
Limonene	1030	8.42
Ocimene (E)	1046	0.1
Linalool oxide (Trans)	1080	0.22
Linalool oxide (Cis)	1095	0.16
Linalool	1101	29.99
Terpinen-4-ol	1180	0.11
Terpineol	1198	2.5
Nerol	1229	0.75
Neral	1238	0.04
Linalyl acetate	1250	14.94
Geranial	1268	0.21
Neryl acetate	1361	2.39
Geranyl acetate	1380	4.83
Nerolidol	1562	4.86
Farnesol	1716	9.86
Farnesal	1737	0.92
Eicosane	2000	0.03
Tetracosane	2400	0.09
Pentacosane	2500	0.02
Total	-	**87.97**

As well, Limonene has been shown to be effective in relieving gastroesophageal reflux disorder and occasional heartburn but the action mechanism has not yet been elucidated[7]. Moreover, studies have shown that Limonene has been shown to slow the growth of cancers of the pancreas, stomach, colon, skin and liver. Unfortunately these results haven't been backed up by human trials, so there isn't any proof that Limonene can fight cancer in humans[7]. A biological study of this plant's essential oil is of utmost importance, and will definitely contribute to numerous breakthroughs in Herbal medicine[7]. In a recent study, the effect of a cream containing farnesol (9.86% in our sample) and xylitol was successfully assessed on atopic dry skin[11]. Nerolidol (4.86% in our sample) and Terpineol (2.50% in our sample) are thought to possess antifungal activity against *T. mentagrophytes*, and this activity might lead to irreversible cellular

disruption[12]. The anti-anxiety effect of *Citrus aurantium* could be attributed to Neryl Acetate (2.39% in our sample), which specifically brings about the anti-spasmodic or muscle relaxant effect[13]. Bergamol (14.94% in our sample) has no relevant medical application, and, being an ester, remains only responsible for the plant's fragrance.

4. Conclusion

The identification of these components helped validating the various medicinal applications of *Citrus aurantium* among locals in the West Bekaa region of Lebanon. Thus this study proves the medical importance of this plant's essential oil and sheds more light on its anticancer effects as well as many others, amplifying the urge for further chemical and biological studies that will help in endorsing these effects and offering to the world this Lebanese species as a scientifically-proven medicinal plant.

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