Chemical Characterization and Detection of Adulteration in Essential Oil of *Lavandula Angustifolia* Linn. by ATR-FTIR

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Abstract: *Lavandula angustifolia* Linn. (Lavender) is an important source of high-quality fragrance and possesses several pharmacological activities such as antianxiety, antidepressant and hypnotic. Because of its high market price and medicinal values, adulteration is very common with *L. angustifolia* essential oil. The aim of present study is to characterize essential oil and detect adulteration in it by ATR-FTIR method. *L. angustifolia* essential oil was isolated by the hydrodistillation method and characterized by GC/MS and FTIR. The authentic isolated essential oil of *L. angustifolia* was further adulterated by sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% *v/v*) and analyzed by FTIR. The GC/MS analysis of essential oil of *L. angustifolia* yielded 74 compounds and linalyl acetate (39.28%), linalool (26.76%), and *trans*-β-caryophyllene (4.77%) were found as major chemical compounds. The ATR-FTIR results of isolated pure essential oil of *L. angustifolia* showed characteristic peaks of linalool and linalyl acetate, the major chemical components present in it. For detection of adulteration in *L. angustifolia* essential oil, different binary-mixtures of *L. angustifolia* essential oil with sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% *v/v*) were analyzed, and the results of FTIR analysis shows very strong peaks in the range of 2800 to 3000 cm^{-1} and at 1739.31 cm^{-1} in case of adulterated mixtures compared to the pure essential oil of *L. angustifolia*. The proposed method was found a simple, economic, quick, reliable, and reproducible for the detection of adulteration in *L. angustifolia* essential oil.

Keywords: *Lavandula Angustifolia*, Lavender, Adulteration, Essential Oil, ATR-FTIR, GC/MS, Linalool.

1. Introduction

Lavandula angustifolia Linn. (Lamiaceae) commonly known as Lavender, and abundantly found in Kurdistan region of Iraq (Hamad et al., 2013). *L. angustifolia* is an important source of high-quality fragrance. Traditionally *L. angustifolia* is used for the treatment of anxiety, depression and induce sleep (Kim et al., 2021). *L. angustifolia* essential oil is also used as a flavor in food and cosmetic products (Guo and Wang, 2020).

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Linalool is the main chemical component of *L. angustifolia* essential oil and it is used industrially for synthesis of vitamin A and E (Kamatou et al., 2008). *L. angustifolia* also abundantly found throughout the world such as in Italy, France, Spain Iran, India, and Turkey. *L. angustifolia* essential oil is extensively used in fragrance industry, and in also aromatherapy (Babatabar et al., 2020). Besides *L. angustifolia* essential oil use in fragrance and aromatherapy, it possesses several pharmacological actions such as anticancer (Najibullah et al., 2021), antidiabetic (Nasiri Lari et al., 2020; Kulabas et al., 2018), antianxiety (Sayed et al., 2020), antibacterial and antioxidant (Hamad et al., 2013; de Rapper et al., 2016; Giovannini et al., 2016; Insawang et al., 2019), antifungal (Behnam et al., 2006), and anti-inflammatory (Cardia et al., 2018).

Techniques for qualitative and quantitative analysis of essential oils are limited. GC/FID and GC/MS techniques are the only choice for characterization of aroma components in essential oils of medicinal plants (Ahamad et al., 2020, Najibullah et al., 2021). GC based techniques are highly sensitive, selective and accurate for identification and quantification of essential oil constituents. However, it has several drawbacks such as it is expensive, time consuming and required skilled person to handle the machine (Ahamad, 2021; Ahamad et al., 2021). Hence, there is a need to search alternative analytical methods that could be easy, fast, reliable, sensitive, and accurate. The FTIR coupled with ATR (attenuated total reflectance) spectroscopy provides rapid, sensitive, and accurate alternative analytical method for analysis of essential oils (Rodríguez et al., 2018). ATR-FTIR spectroscopy also requires no sample preparation steps and it provides fingerprint of the sample and can be considered as an alternative way for detection of adulteration in essential oil. Essential oils exhibit a complex IR spectrum due to presence of a large number of constituents in it; hence identification of particular essential oil becomes difficult. However, every essential oil shows some unique IR peaks (Dhoot et al., 2009; Rodríguez et al., 2018). The aim of the present study is to characterize the chemical components of essential oil of *L. angustifolia* by FTIR, and also develop a method for the detection of adulteration.

2. Material and Methods

2.1 Plant Materials and Chemicals

Lavandula angustifolia Linn. aerial parts (about 1 kg) were collected in March 2021 from Erbil, Kurdistan Region, Iraq. The authenticity of all the accession was ascertained by Dr. Raad A Kaskoos, Faculty of Pharmacy, Hawler Medical University, Erbil, Iraq. For future reference, the plant sample was archived in the Faculty of Pharmacy, Tishk International University, Erbil, Iraq (voucher number: PRL/2022/08). The solvents and reagents used in the present study were of analytical grade.

2.2 Isolation of *L. angustifolia* Essential Oil

The fresh aerial parts of *L. angustifolia* washed with distilled water and 750 g of it was then used for isolation of essential oil by hydrodistillation method using Clevenger apparatus. The hydrodistillation was performed for 6 hrs. After that, the isolated essential oil was collected in a graduated tube and filtered over anhydrous sodium sulphate. Then essential oil was stored at 4 ^{o}C in the Refrigerator for further use.

2.3 FTIR Analysis of Market and Isolated Essential Oils of *L. angustifolia*

The purity of market and isolated essential oils of *L. angustifolia* were analyzed by ATR-FTIR (attenuated total reflectance-fourier transform infrared). ATR-FTIR (IRAffinity-1S, Shimadzu, Japan)

was utilized to record IR spectra of *L. angustifolia* essential oil. The spectra acquisition was performed in the spectral range of 400 to 4000 cm-1. The isolated *L. angustifolia* essential oil was placed on ATR surface and FTIR spectra was recorded using 45 scans and 4 cm-1 resolutions. The ATR surface was cleaned using hexane and background scan was recorded before each sample scan. The FTIR data obtained of isolated *L. angustifolia* essential oil was also compared with market essential oil of *L. angustifolia*. The FTIR spectrum of market *L. angustifolia* essential oil was also searched in library (IRAffinity-1S, Shimadzu, Japan).

2.4 Detection of Adulteration in *L. angustifolia* Essential Oil by FTIR

The detection of adulteration in *L. angustifolia* essential oil was performed by ATR-FTIR method. The sesame oil was selected as the solvent of adulteration based upon search results of market *L. angustifolia* essential oil that showed it is adulterated with sesame oil. For detection of adulteration in *L. angustifolia* essential oil, different binary-mixtures of various concentrations of *L. angustifolia* essential oil with sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% v/v) were prepared and FTIR spectra of each concentration were recorded.

3. Results and Discussion

3.1 Isolation and Characterization of Essential Oil of *L. angustifolia*

The essential oil of *L. angustifolia* from aerial parts was isolated by hydrodistillation method using Clevenger apparatus. The aerial parts of *L. angustifolia* gave colourless essential oil with a characteristic odour (yield 1.56±0.27% v/w). The essential oil was then characterized by GC/MS method and published elsewhere (Najibullah et al., 2021). The GC/MS analysis the of essential oil of *L. angustifolia* yielded seventy-four chemical compounds which constitute about 98.25% of total essential oil. The major chemical compounds identified in essential oil of *L. angustifolia* were linalyl acetate (39.28%), linalool (26.76%), trans-β-caryophyllene (4.77%), lavandulyl acetate (3.04%) and 1,8-cineole (2.15%).

The essential oil was also characterized by the FTIR method and results were presented in Figure 1 and Table 1, and FTIR results were in agreement with previous studies (Coates, 2006; Truzzi et al., 2021). In the present study, the IR bands were assigned on the basis of the results of a previous study conducted by Coates, 2006; Truzzi et al., 2021. Although some shift in band position was observed in current study in comparison with reported. The ATR corrections and smoothing of results were not applied. In Figure 1 and Table 1, the main functional groups with respective IR peak values were presented. The characteristic peaks for linalool and linalyl acetate, the main components of *L. angustifolia* essential oil were also listed in Figure 2, and the IR values match with the essential oil of *L. angustifolia* which is given in Table 1

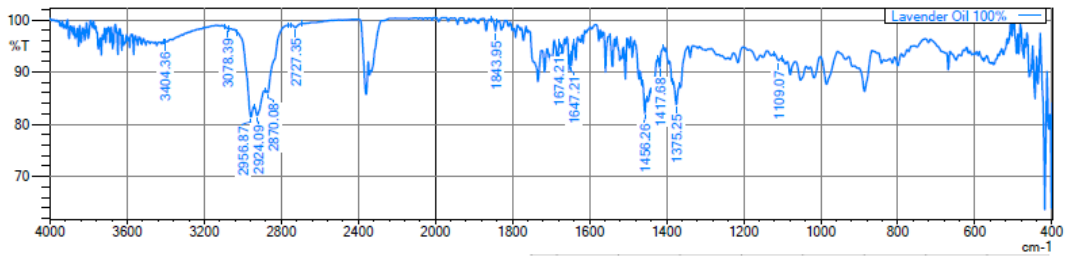
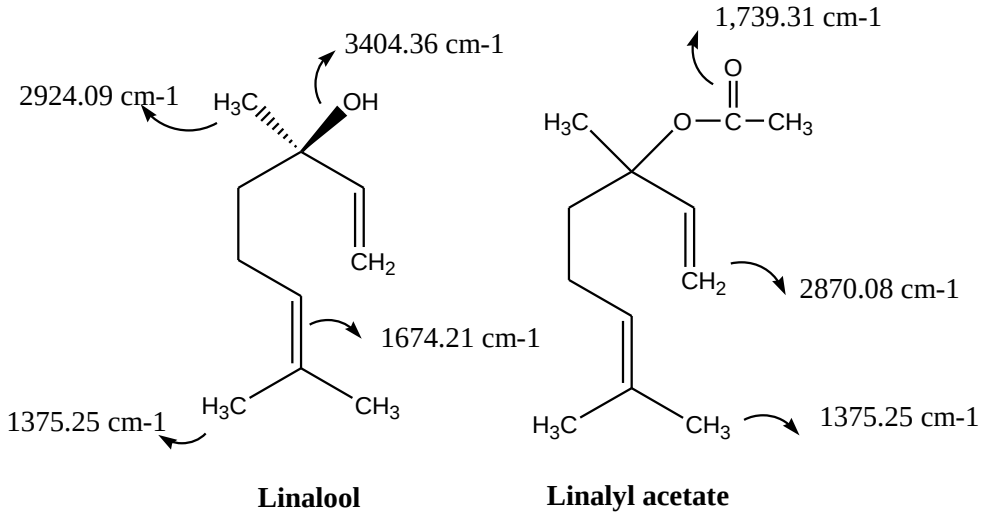


Figure 1: Characteristic FTIR spectrum of essential oil of *L. angustifolia*



Linalool **Linalyl acetate**

Figure 2: Characteristic IR peaks of linalool and linalyl acetate present in *L. angustifolia* essential oil

Table 1: Characteristic FTIR peaks, functional groups, and possible phytochemicals present in essential oil of *L. angustifolia*

S. No.	Peaks (cm^{-1})	Functional groups	Type of phytochemicals
1.	1375.25	C-H bending (characteristic band)	Hydrocarbons
2.	1417.68	C-H bending (vinyl group)	Esters (linalyl acetate)
3.	1456.26	C-H bending ($-CH_3$)	Hydrocarbons
4.	1647.21	C=C stretching (alkene)	Unsaturated hydrocarbons
5.	1674.21	C=C stretching (alkene)	Unsaturated hydrocarbons
6.	1739.31	C=O stretching (C=O)	Esters
7.	2870.08	C-H stretching ($-CH_2$)	Hydrocarbons in terpenes
8.	2924.09	C-H stretching ($-CH_3$)	Hydrocarbons in terpenes
9.	2956.87	C-H stretching ($-CH_3$)	Hydrocarbons in terpenes
10.	3078.39	C-H stretching (vinyl group)	Esters (linalyl acetate)
11.	3404.36	O-H stretching (OH)	Linalool (oxygenated terpenes)

3.2 Screening of Pure and Market Essential Oil of *L. angustifolia*

The *L. angustifolia* essential oils isolated in the laboratory and collected from the market were analyzed by ATR-FTIR, and results were presented in Figure 3. From figure 3, it is a little difficult to differentiate between pure, market essential oil of *L. angustifolia* and sesame oil. However, it is quite evident that IR peaks in the range of 2800 to 3000 cm-1 (C-H stretching) and at 1739.31 cm-1 (C=O

stretching) are very strong peaks in case of market *L. angustifolia* essential oil and sesame oil compared to pure essential oil from isolated *L. angustifolia* (Figure 3). The FTIR spectrum of market *L. angustifolia* essential oil was also searched in the FTIR library, and it matches with sesame oil (searched score 916).

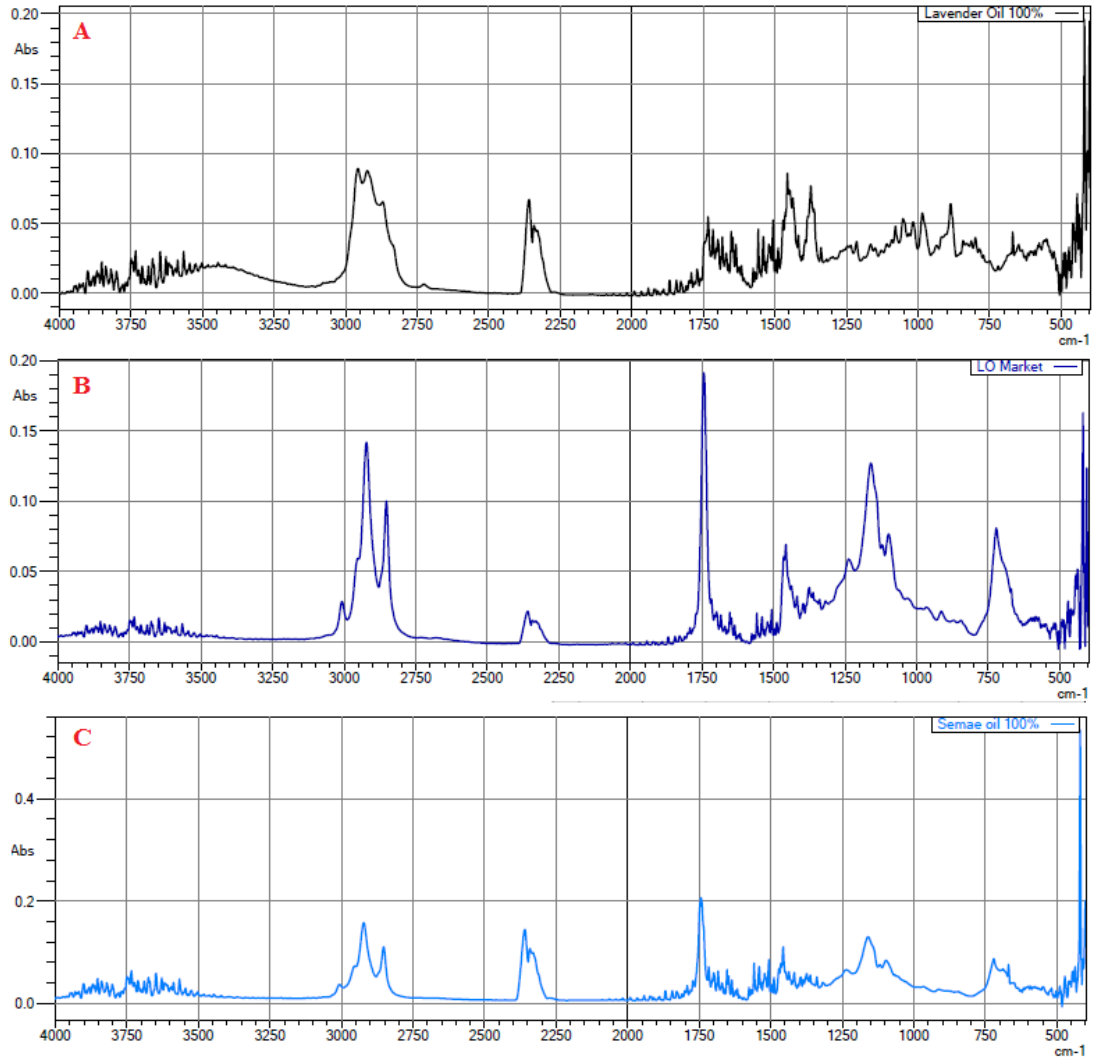


Figure 3: FTIR absorption spectra of (A) *L. angustifolia* essential oil (100%); (B) Market *L. angustifolia* essential oil; and (C) Sesame oil (100%)

3.3 Detection of Adulteration in *L. angustifolia* Essential Oil by FTIR

On the basis of the above study, sesame oil was chosen as solvent for adulteration. The *L. angustifolia* essential oil was adulterated with sesame oil (0, 5, 10, 20, 30, 40, 50 and 100% v/v), and analyzed by ATR-FTIR. The results were presented in Figure 4A-F. As evident from FTIR spectra (Figure 3 & 4), it is quite difficult to differentiate peaks of pure *L. angustifolia* essential oil with adulterated oils with sesame oil by just visual examination of their whole spectrum. However, a careful examination of the FTIR spectra, peaks at 2870.08 cm-1 (for C-H stretching in -CH2) and 2924.09 cm-1 (for C-H stretching in -CH3, usually present in hydrocarbons in terpenes), and at 1739.31 cm-1 (C=O stretching in esters) provide a clear difference between pure *L. angustifolia* essential oils with adulterated oils. It is quite evident in Figure 4A-F, the IR peaks in the range of 2800 to 3000 cm-1 and at 1739.31 cm-1

are very strong peaks in case of adulterated oil with sesame oil compared to pure essential oil from isolated *L. angustifolia.* The proposed method was found a simple, economic, quick, reliable, and reproducible for the detection of adulteration in *L. angustifolia* essential oil

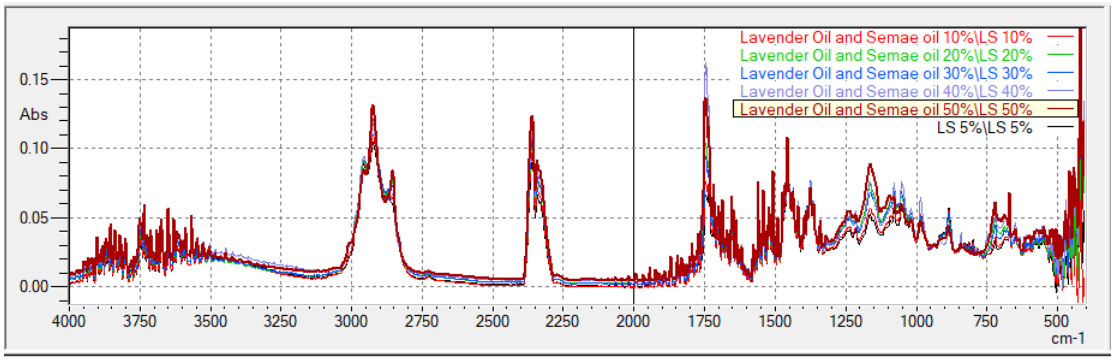


Figure 4: FTIR absorption spectra of (A) *L. angustifolia* oil adulteration with sesame oil (5% *v/v*); (B) 10% *v/v*; (C) 20% *v/v*; (D) 30% *v/v*; (E) 40% *v/v*; and (F) 50% *v/v*, respectively

4. Conclusion

Lavender is a source of high-quality fragrance and is used for the treatment of several human ailments such as anxiety, depression and also induces sleep. The GC/MS analysis of *L. angustifolia* essential oil shows the presence of linalyl acetate (39.28%), and linalool (26.76%) as major chemical compounds and FTIR analysis also shows characteristic peaks for linalool and linalyl acetate. The proposed method for detection of adulteration in *L. angustifolia* essential oil was found simple, economic, quick, reliable, and reproducible; based on a simple visual comparison of IR peaks. The most distinct peaks appear in the range of 2800 to 3000 cm-1 and at 1739.31 cm-1, in case of adulterated oil very strong peaks were observed compared to pure essential oil from isolated *L. angustifolia.*

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