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Vibrational spectroscopy and chemometric modeling: An economical and robust quality control method for lavender oil

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ABSTRACT

Lavandula species (Lamiaceae), commonly known as lavender, ranks among the top 10 medicinal plants used worldwide and is used and traded extensively in the flavor and fragrance, cosmetic, and aromatherapy industries. Many studies continue to confirm the phytotherapeutic potential of lavender oil in the treatment of wounds, rheumatism, muscular pains, dermatitis, acne, and eczema amongst many others. Gas chromatography coupled to mass spectrometry with a flame ionization detector (GC–MS–FID) is the conventional method for the quality assessment of lavender oil. In this study, vibrational spectroscopy methods such as mid infrared (MIR) and near infrared (NIR) in tandem with chemometric data analysis are proposed as alternative methods for the routine quality control of this commercially important essential oil. Sixty lavender oil samples were purchased from a wide range of suppliers and six major and minor compounds (1,8-cineole, (*E*)-β-ocimene, (*Z*)-β-ocimene, camphor, linalool, and linalyl acetate) were quantified using GC–MS–FID (reference data). Spectral data was acquired for both MIR (4000–550 cm^{-1}) and NIR (10 000–4000 cm^{-1}) wavelength regions and chemometric modeling applied to develop calibration models. The calibration models revealed good statistical performance where the coefficients of determination obtained for the major compounds of lavender oil were ≥0.82. Good coefficients of determination were observed for linalool $R^2 = 0.99$ (MIR) and 0.98 (NIR), as well as linalyl acetate $R^2 = 0.92$ (MIR) and 0.90 (NIR). Linalool and linalyl acetate represented about 70% of the total composition of the essential oil. Low values (≤1.6) were obtained for the root mean square error of estimation (RMSEE) and root mean square error of prediction (RMSEP). The external dataset was accurately predicted as evidenced by comparison with the reference data.

1. Introduction

Lavandula angustifolia Mill., commonly known as lavender or English lavender, originated from the Western Mediterranean region and spread to North Africa, Europe, and Western India (DAFF, 2009). The plant is cultivated in France, England, Bulgaria, Argentina, Brazil, Cyprus, Hungary, Greece, Italy, Japan, Russia, Spain, Turkey, Australia, New Zealand, and Tanzania (Macarie et al., 2007; Lawless, 2013). *Lavandula angustifolia* was used in ancient Egypt to mummify bodies and the Romans, Greeks and Persians added lavender oil to their baths. The name lavender is derived from the Latin 'lavare' which literally means 'to wash' (Van Wyk and Wink, 2004; NCCAM, 2008; Vincent, 2008). René-Maurice Gattefossé was the first scientist to recognize the value of lavender oil based on his own experience of tissue regeneration on his arm which was severely burned during a laboratory explosion (Vincent, 2008).

The oil is externally applied to treat wounds, burns, inflammation, eczema, dermatitis, migraine, nausea, sores, acne, asthma, rheumatism, and muscle pains (Lis-Balchin and Hart, 1999; Van Wyk and Wink, 2004; NCCAM, 2008). Inhalations of lavender flowers were traditionally used to cure dyspepsia and nervous disorders such as restlessness and insomnia. The reported calming actions due to its linalyl acetate (narcotic) and linalool (sedative) content may be the origin of the 'lavender herb pillow' which was traditionally used to induce sleep. Studies have shown that lavender exhibits anti-anxiety and antidepressant effects with reduced psychological distress and increased mood scores. This could be ascribed to the positive association between the distinct lavender odor and emotional state. The electro-encephalogram patterns of patients

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exposed to lavender oil odor suggested a state of 'feeling comfortable' (Cavanagh, 2005; Cavanagh and Wilkinson, 2006; Macarie et al., 2007; NCCAM, 2008). This is one of the reasons why lavender oil is included as an ingredient in countless commercial products including soap, shampoo, bath oil, lotion, bath salts, air fresheners, and cleaning products amongst others (DAFF, 2009).

Scientific studies have confirmed the antibacterial activity of lavender oil against a range of Gram-positive and Gram-negative bacteria including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Propionibacterium acnes*, *Escherichia coli*, *Shigella sonnei*, *Proteus vulgaris*, *Enterobacter aerogenes*, and *Pseudomonas aeruginosa* (Nelson, 1997; Lis-Balchin et al., 1998; Hammer et al., 1999; Cavanagh and Wilkinson, 2002). Other biological activities for lavender oil or its constituents include anticancer properties (Liston et al., 2003; Samaila et al., 2004), protection against acute ethanol-induced gastric ulcers (Barocelli et al., 2004), and spasmolytic effects (Lis-Balchin and Hart, 1999).

The essential oil of *L. angustifolia* is hydrodistilled from the fresh flowers before it blooms and is mainly composed of linalyl acetate (25.0–47.0%) and linalool (20.0–45.0%) with smaller quantities of *E*-β-ocimene (0.0–10.0%), *Z*-β-ocimene (0.0–6.0%), terpinen-4-ol (0–8.0%), lavandulyl acetate (0.0–8.0%), 3-octanone (0.0–5.0%), lavandulol (0.0–3.0%), 1,8-cineole (0.0–3.0%), α-terpineol (0.0–2.0%), camphor (0.0–1.5%), β-phellandrene (0.0–1.0%), and limonene (0.0–1.0%) (ISO, 2002). The worldwide production of lavender oil is estimated at about 200 metric tons per annum, with the major producers being Australia, Bulgaria, Canada, China, England, France, Italy, South Africa, Spain, Tanzania, and the USA (DAFF, 2009). France produces about 50–75 tons of lavender oil per annum from *L. angustifolia* while 50–60 tons are estimated to be produced in China (Beus, 2006).

Gas chromatography coupled to mass spectrometry (GC–MS) is traditionally used for the quality assessment of essential oils. However, GC–MS analysis is expensive, time-consuming, and requires considerable expertise; thus, alternative fast and efficient methods for quality control are advantageous. Vibrational spectroscopy methods including near infrared (NIR) and mid-infrared (MIR) have proved to be effective as alternative rapid quality control methods for both essential and fixed oils. Sandasi et al. (2011) used vibrational spectroscopy and chemometric data analysis to authenticate *Eriocephalus* essential oil samples, proving that *E. tenuifolius* rather than *E. punctulatus* is the source of Cape chamomile oil. The technique may also be used in the quality control of fixed oils such as extra virgin olive oil where Valli et al. (2013) used MIR and partial least squares (PLS) regression analysis to detect low quality oils based on predicting the fatty acid methyl (FAME) and ethyl (FAEE) ester content. Chemometric data analysis is applied to explore patterns of association in data (spectral and chemical) and to extract the maximal useful information from signals. First, principal component analysis (PCA) is performed on the whole dataset to identify groups or trends in the data. Then construction of calibration models through partial least square (PLS) regression analysis followed to predict the marker compounds from an external lavender oil sample. This study proposes the use of MIR and NIR spectroscopy in combination with chemometrics data analysis to rapidly quantify biomarkers used to determine the quality of lavender oil.

2. Materials and methods

2.1. Essential oils

Sixty commercial lavender oil samples were sourced from various national and international suppliers. All the samples were stored at 4 °C prior to analysis.

2.2. Gas chromatography coupled to a mass spectrometer and flame ionization detector (GC–MS–FID) analysis

To obtain reference quantification data, all the samples were analyzed using gas chromatography–mass spectrometry–flame ionization detection (GC–MS–FID) with an Agilent 6860 N GC system coupled directly to a 5973 MS (Agilent Technologies, Santa Clara, CA, USA). All the samples were diluted to a concentration of 20% using hexane. A volume of 1 μl was injected with a (200:1) split ratio at 24.79 psi and an inlet temperature of 250 °C. An HP-Innowax polyethylene glycol column (60 μm × 250 μm i.d. × 0.25 μm film thickness) incorporated in the GC system was used. The following system conditions were applied: initial oven temperature 60 °C, rising to 220 °C at a rate of 4 °C/min and held for 10 min and then rising to 240 °C at a rate of 1 °C/min; carrier gas: helium; flow rate: 1.2 ml/min; electron impact 70 eV; and a scanning range of 35 to 450 *m/z*. The percentage composition of all the compounds was obtained from the electronic integration measurement of peak areas, using flame ionization detection (FID). Compound identification was carried out using standard libraries including NIST and Mass Finder by comparing mass spectra and retention indices calculated using the *n*-alkane series (C_6–C_{20}) (Kamatou et al., 2010).

2.3. Mid infrared (MIR) spectroscopy

The attenuated total reflectance (ATR) diamond crystal surface of an Alpha-*P* Bruker spectrometer (Bruker OPTIK GmbH, Ettlingen, Germany) was covered with 10 μl of each essential oil sample. OPUS® software was used to acquire MIR spectra in absorbance mode with a wave range of 550–4000 cm^{-1}. For each sample, 32 scans were collected in duplicate using a spectral resolution of 4 cm^{-1}. The average was calculated using Microsoft® Excel and chemometric data analysis was performed using SIMCA-*P*+ 12.0 software (Umetrics AB, Malmo, Sweden) (Sandasi et al., 2010).

2.4. Near infrared (NIR) spectroscopy

The NIRFlex N500 (Büchi Labortechnik AG, Flawil, Switzerland) equipped with a liquid cell was used to acquire NIR spectra. High precision cuvettes of 0.20 mm path length (Hellma GmbH & Co, KG, Müllheim, Germany) were filled with 50 μl sample. Duplicate scans consisting of 32 scans per sample were collected in transmittance mode with the spectral resolution set at 4 cm^{-1} between the wavelengths of 10 000–4000 cm^{-1}. Data conversion to absorbance was performed using the equation: $y = \log(1/x)$; where y is the absorbance and *x* the transmittance (Sandasi et al., 2011). The average of the duplicate spectra were determined using Microsoft® Excel and chemometric analysis was performed using SIMCA-*P*+ 12.0 software (Umetrics AB, Malmo, Sweden).

2.5. Data analysis

2.5.1. Principal component analysis (PCA) model construction

Principal component analysis (PCA) is an unsupervised method or statistical procedure that orthogonally transforms observations possibly correlated from the original dataset into a new set of linearly uncorrelated variables called principal components (PC). This aims to reduce the dimensionality of the data, avoid over-fitting problems, to retain all the useful information, and diminish the signal-to-noise ratio. The analysis is sensitive to the scaling methods and univariate, center, and pareto scaling methods were used to observe cluster formation and investigate trends in the data. Score scatter plots were used to identify strong outliers while loading plots (DmodX) were used to identify moderate outliers. The correlation among the *Y*-variables was determined using PCA-*Y*.