Terpenoids for medicine

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Chapter 2

Analysis of essential oil vapors produced by a vaporizing device

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Abstract

In order to improve the ability to study the pharmacology of essential oils reproducible administration forms are needed. Vaporizers are devices that heat up plant material in order to release their volatile components from plant matrix for inhalation. In this study we analyzed the vapor produced by a vaporizing device from a selection of common herbs using gas chromatography. *Lavandula angustifolia, Thymus vulgaris, Matricaria recutita, Salvia officinalis*, *Eucalyptus globulus,* and *Melissa officinalis* were selected as model plants for this study because of their well studied essential oils. *Lavandula. angustifolia, T. vulgaris, M. recutita,* and *S. officinalis* all produced detectable (>0.1 mg/g) levels of essential oil components in the vapor. Levels of compounds increased with temperature. These results suggest that vaporizing devices may be useful for the controlled administration of plant essential oils.

Introduction

Many medicinal and food plants contain volatile compounds commonly known as essential oils. Essential oils have many practical uses ranging from herbal medicines to cosmetics and foodstuffs. Aromatherapy involves inhaling volatile compounds either from whole plant preparations or pure essential oils for therapeutic effects or relaxation (Lahlou, 2004a; Lahlou, 2004b). Potential therapeutic effects of inhaled essential oils include antimicrobial, antiviral, anti-carcinogenic, sedative, analgesic, anxiolytic, and anti-inflammatory effects (Edris, 2007). Despite a long history of using essential oils the molecular mechanisms of action and therapeutic efficacy of many plant volatiles are largely unknown (Maffei et al., 2011). Although some clinical trials have been performed on essential oils administered in an aromatherapy setting, most have weak experimental designs and thus the effectiveness of such approaches is questionable (Yim et al., 2009; Cooke and Ernst, 2000).

One approach to improve research into essentials oils is to use methodology that allows for controlled dosing and manipulation of the essential oil or herb under study. Safe and effective methods for administering essential oils may be possible with the use of vaporizing devices. Vaporizers are devices that heat plant material below combustion temperatures in order to volatilize plant compounds for inhalation, ideally without generating carcinogenic polynuclear aromatic hydrocarbons (PAH) associated with smoked plant material. The Volcano® is a sophisticated vaporizing device that utilizes a temperature controlled heat flow to fill a plastic bag with plant vapors. After filling the bag it is removed from the heat source and connected to a mouthpiece for inhalation. Vaporizer technology has been employed for the inhalation of cannabinoids, the main compounds found in *Cannabis sativa* in a clinical setting (Abrams et al., 2007).

The purpose of this study is to examine whether or not the volcano can reproducibly volatilize essential oil components found in 6 common medicinal plants. Gas chromatography with flame ionization detection (GC-FID) and mass spectrometry (GC-MS) was used to analyze the major components found in the essential oil and vapor of common herbs with well-known essential oils. *Lavandula angustifolia* (lavender), *Thymus vulgaris* (thyme), *Matricaria recutita* (chamomile), *Salvia officinalis* (sage), *Eucalyptus globulus,* and *Melissa officinalis* (lemon balm) were chosen for this study.

Thymus vulgaris is a popular herb used in cooking as well as industry. The essential oil has many interesting biological properties including antimicrobial, antifungal, and antioxidant activity. Monoterpenoids make up the majority of *T. vulgaris* essential oil with thymol being the major component as well as carvacrol, *p*-cymene, and γ-terpinene (Dawidowicz et al., 2008). Both thymol and carvacrol have been shown to be antimicrobial against oral bacteria. When utilized in combination they display a synergistic enhancement of antimicrobial activity (Didry et al., 1994).

The essential oil of *L. angustifolia* is well studied due to its extensive use in

foods and cosmetics as well as for its medicinal properties. Linalool is a major component of *L. angustifolia* essential oil along with eucalyptol and camphor (Da Porto et al., 2009). There is some clinical evidence that administration of *L. angustifolia* essential oil by aromatherapy has a significant relaxation effect (Shiina et al., 2008). Linalool has been shown to exhibit anticonvulsant properties (Silva Brum et al., 2001). Anti-inflammatory and analgesic effects have also been demonstrated in mice after administration of *L. angustifolia* essential oil (Hajhashemi et al., 2003). Anxiolytic effects have also been observed after the inhalation of *L. angustifolia* essential oils in rats (Shaw et al., 2007). The essential oil of *S. officinalis* has been shown to inhibit human acetylcholinesterase (AChE) and butyrylcholine esterase (BuChE) (Savelev et al., 2004). In a double blind clinical trial daily administration of a *S. officinalis* tincture significantly reduced cognitive impairment in patients with Alzheimer's disease which may be mediated by AChE or BuChE activity (Akhondzadeh and Abbasi, 2006).

Matricaria recutita is a popular herb used for a variety of minor ailments such as indigestion, inflammation, and for its antimicrobial action. The oil is composed of mostly sesquiterpenoids such as α-bisabolol, oxides of α-bisabolol, and chamazulene (Ganzera et al., 2006). *Matricaria recutita* may be helpful to women delivering birth for pain relief, as well as for relaxing and sedative effects (McKay and Blumberg, 2006). *Eucalyptus globulus* essential oil is mainly composed of eucalyptol a monoterpenoid and has been traditionally used for treatment of respiratory illnesses. The oil has analgesic, anti-inflammatory, and antimicrobial effects (Silva et al., 2003; Cermelli et al., 2008). *Melissa officinalis* essential oil contains the monoterpenoids citral and citronellal, as well as the biologically active sesquiterpenoid β-caryophyllene. The herb is often used for the treatment of digestive ailments and for its relaxing properties (Sadraei et al., 2003).

Materials and Methods

Chemicals and reagents

All organic solvents were of analytical reagent grade and purchased from Biosolve BV (Valkenswaard, The Netherlands). Camphor, carvacrol, 1,8-cineol (eucalyptol), (+)-borneol, (-)-linalool, α-humulene (humulene), α-bisabolol, β-pinene and thymoquinone were purchased from Sigma Aldrich (Steinheim, Germany). β-Caryophyllene (caryophyllene) ≥ 80% purity was purchased from SAFC Supply Solutions (Saint Louis MO, USA). Chamazulen, thymol and geraniol were purchased from Chromadex (Boulder CO, USA). Stock solutions of each compound were made in ethanol and stored in -20 °C.

Plant materials

Thymus vulgaris, *L. angustifolia*, *M. recutita*, *E. globulus* and *M. officinalis* were purchased from A.J. Van Der Pigge Drogisterij (Haarlem, The Netherlands). *Salvia officinalis* and an additional sample of *M. officinalis* were purchased from Jacob Hooy (Limmen, The Netherlands). *Lavandula angustifolia* and *M. recutita* were

supplied as dried mostly flower top material. *Thymus vulgaris, E. globulus* and *M. officinalis* were supplied as dried leaf material. *Salvia officinalis* was supplied as a mixture of dried leaves, flower material and stems.

The volcano vaporizer and vapor collection

The volcano was purchased from Storz & Bickel GmbH & Co (Tuttlingen, Germany). The device was used in a manner similar to that described in the manual provided by the manufacturer. In order to assess the effect of temperature on vaporization 3 different temperature settings were used 100, 150 and 200 °C. One gram of plant material was heated on the volcano vaporizer per sample until the bag was full, which took approximately 45 seconds. Once the bag was full it was removed from the heat flow. Vapors were collected immediately by placing the volcano mouth piece onto the bag and attaching it to a plastic filter holder which held a glass fiber filter (44 mm in diameter). Vapor was gradually sucked onto the glass fiber filter under vacuum. Each sample was performed in triplicate at each temperature setting with a new g of plant material and fresh glass fiber filter. Between each vapor collection volcano parts exposed to vapor and plant material were ultrasonicated for 30 seconds in EtOH, rinsed with EtOH and dried under a stream of air. The bag was also filled with hot air and emptied 3 times to ensure that no residual vapors remained from previous plant samples. Vapor samples were extracted by placing glass fiber filters into 15 ml falcon tubes and extracting the filter two times with 5 ml ethanol with 15 min of gentle shaking during each extraction. Both ethanol extracts were pooled to a volume total volume of 10 ml. Samples were then centrifuged for 5 min at 2000 rpm. Supernatant was transferred to a glass vial for GC-MS and GC-FID analysis.

Determination of plant essential oil content

In order to determine the total essential oil content of each plant 20 g of plant material was steam distilled in a Clevenger apparatus for 3 h. Plant essential oils yields were determined in samples with a volume ≥ 100 µl. Essential oils were diluted in EtOH or hexane to various concentrations and analyzed by GC-FID and GC-MS. The density of each essential oil was measured by weighing 10 µl of oil on an analytical balance (0.01 mg). Dilutions in ethanol for thymol, linalool, eucalyptol, and geraniol ranging from 0.05 mg/ml to 1 mg/ml and dilutions for chamazulen, carvacrol, camphor, and α-humulene ranging from 0.1 mg/ml to 1 mg/ml were used for quantitative analysis on the GC-FID. Reproducibility of the GC-FID was determined by injecting a *L. angustifolia* vapor sample (200 °C) 3 times and calculating % relative standard deviation (%RSD) of eucalyptol, linalool, and geraniol.

Gas chromatographic analysis

An Agilent gas chromatograph 6890 series with an FID detector was used for quantitative essential oil and vapor analysis (Agilent Technologies Inc., Santa Clara, CA, USA). The instrument was equipped with a 7683 series injector and autosampler, plus a 6890 series integrator. A Varian VA-5ms (5% phenyl polysiloxane) column with a length of 30 m, an I.D. of 0.25 mm and a film thickness of 0.25 µm was used for all

samples (Varian Inc., Walnut Creek, CA, USA). Injection volume was 2 µl and split ratio was 1:20. Temperature program was the following: injector 180 °C, FID detector 190 °C, oven start 60 °C, oven rise 3 °C/min, oven final 180 °C, final temperature hold 5 min and total run time was 45 min per sample.

A Varian 3800 GC equipped with an 8200 autosampler and a Saturn 2000 GC/MS ion trap mass detector was used for identification of essential oil and vapor components. The column was a DB-5ms with a length of 30 m, an I.D. of 0.25 mm and a film thickness of 0.25 µm. The split ratio was 1:20. Temperature program was the following; injector 230 °C, oven start 60 °C, oven rise 3 °C/min, oven final 240 °C, final temperature hold 5 min and total run time was 65 min. The ion trap temperature was 220 °C, the manifold 60 °C and the transfer line was 275 °C. Varian MS workstation version 6.9.1 software was used for instrument control and data analysis.

Table 1: Essential oil yield and % composition.

Plant	yield (% v/w)	Essential oil components
Thymus vulgaris	1.5	thymol (66%), cymene (12%), γ-terpinene (8%), carvacrol (3%), β-caryophyllene (1%)
Lavandula angustifolia	3.5	linalool (43%), eucalyptol (28%), geraniol (11%), γ-terpineol (5%), camphor (4%)
Salvia officinalis	1.5	camphor (29%), thujone (29%), eucalyptol (12%), camphene (8%), humulene (2%)
Matricaria recutita	< 1	bisabolol oxide A (77%), bisabolol oxide B (11%), α-bisabolol (8%), chamazulene (< 1%)
Eucalyptus globulus	1.7	eucalyptol (74%), α-pinene (10%)
Melissa officinalis	< 1	citronellal (5%), β-caryophyllene (10%), caryophyllene oxide (32%)

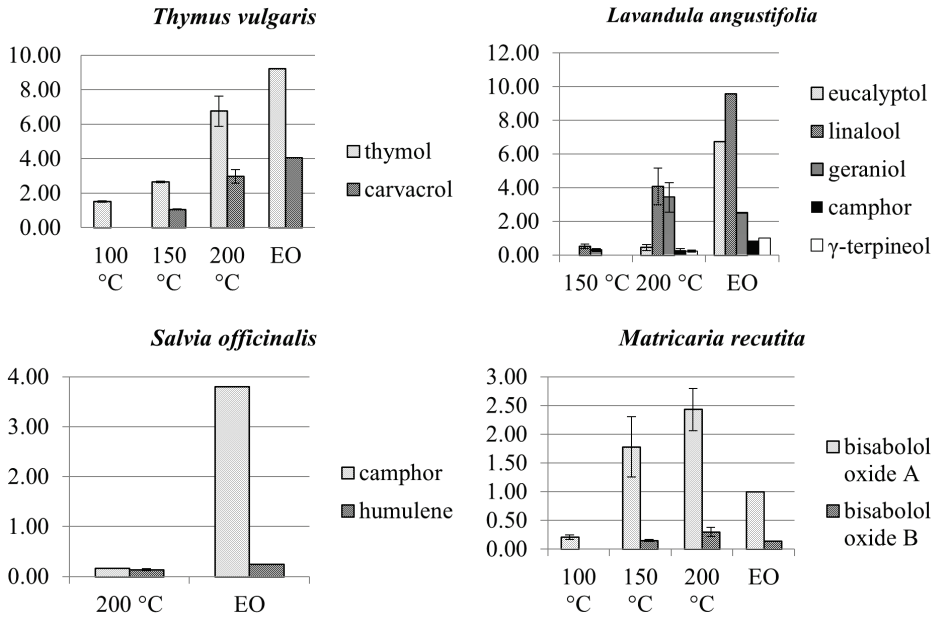
Results and Discussion

Essential oil and vapor components were identified on the basis of their mass spectrum and retention time comparison with authentic standards. Table 1 shows the yield of essential oils from each plant as well as the major components identified. The approximate percentage composition of essential oil components is based on peak area comparison in the FID detector. Standard curves were linear in the ranges analyzed (Table 2). The %RSD for eucalyptol, linalool, and geraniol from a *L. angustifolia* vapour sample (200 °C) after 3 injections was 2.1%, 1.0%, and 1.0% respectively suggesting that the GC-FID was reproducible for analysis of essential oil components. Identified essential oil components present in the vapor at levels > 0.1 mg/g were quantified in the essential oil and vapor samples (Figure 1).

Table 2. Response factor and r^2 value for standard curves.

Compound	Response factor	r^2
thymol	201550	0.996
eucalyptol	183907	0.999
linalool	200260	0.999
geraniol	191963	1.000
carvacrol	209572	0.998
camphor	198536	0.999
α-humulene	228170	1.000
chamazulene	174133	0.987

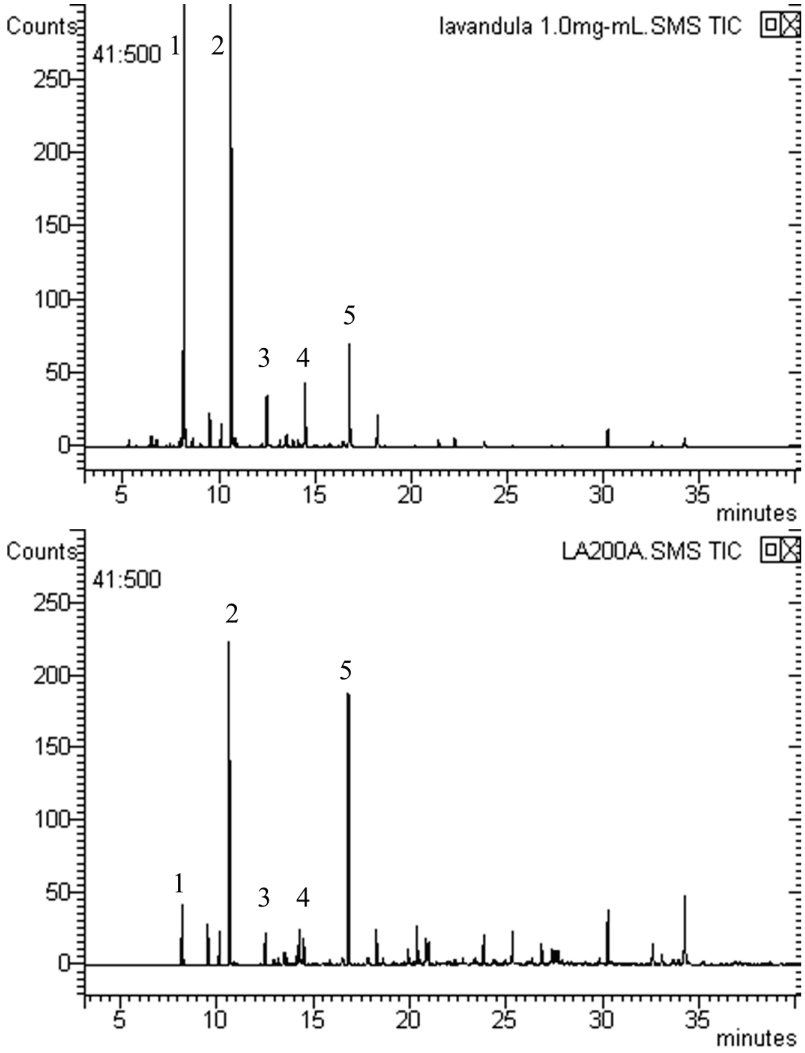
Figure 1. Quantitative analysis (mg/g of plant material) of major vapor and essential oil (EO) components.



Thymus vulgaris, *L. angustifolia*, *S. officinalis*, and *M. recutita* all had major components of their essential oils enter the vapor with levels increasing with temperature (Figure 1). A representative GC-MS chromatogram comparing *L. angustifolia* vapor with its essential oil shows the 5 major components present in the essential oil are also present in the vapor at 200 °C (Figure 2). Although qualitatively similar the vapor samples differed both in absolute and relative quantitative terms from the total essential oil content. *Lavandula angustifolia* essential oil had eucalyptol as the 2nd major component while in the vapor at 200 °C geraniol is the 2nd major component. In *M. recutita*, bisabolol oxide A and B were present in higher concentrations in the

vapor than in the essential oil. The same was true for geraniol in *L. angustifolia*. Such quantitative variation could result from differences in efficiency of steam distillation versus the vaporizing technique or from chemical conversions of the essential oil components. Some compounds volatilized very efficiently from their plant matrix in the vaporizer. At 200 °C, 73% of the total thymol found in the essential oil of *T. vulgaris* was detected in the vapor and in *L. angustifolia* 43% of the linalool. No compound varied by more than 2 mg/g in vapor samples indicating that the volcano is capable of reproducibly delivering essential oil compounds.

Figure 2. GC-MS trace *Lavandula angustifolia* essential oil (top) compared to vapor collected at 200°C (bottom). 1 = eucalyptol, 2 = linalool, 3 = camphor, 4 = terpineol, 5 = geraniol.



No essential oil components were detected at a concentration above 0.1 mg/g in vapor samples of *E. globulus* and *M. officinalis* on the GC-FID detector. *Melissa officinalis* had a low yield of essential oil (< 1%), which could explain why no components were detected in the vapor. A second batch of *M. officinalis* was purchased from a separate herb supplier and analyzed in the same manner. No essential oil components were detected in the vapor and the essential oil yield was again < 1%. This suggests that the lack of compounds detected was due to low amounts of terpenoids in the *M. officinalis* analyzed. *Eucalyptus globulus* had a higher yield of essential oil (1.7%) when compared to *M. officinalis* but no compounds were detected in vapor samples. An experiment was attempted where *E. globulus* leaves were soaked in water for 0.5 h and then vaporized in the same manner as the dry leaves but still no compounds were detected. Terpenoids in *E. globulus* are produced in secretory cavities beneath the epidermis (McCaskill and Croteau, 1998). This result suggests that the vaporizer is less efficient for volatiles from plants which are stored in secretory cavities as opposed to trichomes.

Overall these results demonstrate that volatile terpenoid components of aromatic plants can be produced by a vaporizing device. It is difficult to speculate whether or not the doses observed in the herb vapors would be enough to induce a clinical effect because so few well controlled clinical studies on monoterpenoids have been conducted. However, following oral administration of an equivalent of 1.08 mg thymol detectable levels of thymol metabolites were observed in human urine and blood (Kohlert et al., 2002). A pilot clinical trial conducted on dementia patients with orally administered *Salvia lavandulaefolia* (50 µl) 1-3 times daily was enough to produce significant improvements in cognition and inhibition of AChE *in-vivo* (Perry et al., 2003). Another recent study observed that different essential oils were capable of maintaining their antimicrobial activity in the vapor phase (Nedorostova et al., 2011). Based on such literature reports it is possible that the levels of terpenoid components produced by the vaporizer in this study are within a range that may be appropriate for further research into their effects on humans or animals.

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