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SUPERCRITICAL CARBON DIOXIDE EXTRACTION AND GAS CHROMATOGRAPHY ANALYSIS OF *JASMINUM SAMBAC* ESSENTIAL OIL

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Abstract

Jasmine essential oil is one of the most expensive oils that was used in cosmetics, the pharmaceutical industry, perfumery and aromatherapy. It has therapeutic properties and can be used as an analgesic, antidepressant, anti-inflammatory, antiseptic, antispasmodic and stimulant. In the present study, supercritical fluid extraction (SFE) was exploited to extract essential oil constituents from 20 kg jasmine flowers at closed bud and open flower stages. Results indicated that flowers harvested at the open stage yielded more absolute oil than those harvested at the closed bud stage. Slight variations in physical properties like refractive index, congealing point, optical rotation, and specific gravity were recorded in the essential oil extracted from flowers harvested at different stages. Gas chromatography of the jasmine oil was carried out to qualitatively and quantitatively analyze the oil constituents. Major compounds identified were citronellol, phenyl ethyl alcohol, geranial, eugenol, farnesol, geranyl acetate, citrinyl acetate, 2-phenyl ethyl acetate, citral (mixture of cis and trans), and benzyldehyde. Both harvesting stages yielded oil with differences in the percentage composition of each component, but flowers harvested at the open stage had higher yield and more components than those from the closed bud stage.

Introduction

Essential oils are plant-based volatile oils with strong aromatic components that are made up of different chemical compounds. For example, alcohols, hydrocarbons, phenols, aldehydes, esters and ketones are some of the major components of essential oil (Younis *et al.*, 2008). Oils from hundreds of plant species are available commercially (Formaceck & Kubeczka, 1982). These oils are highly volatile substances isolated by different extraction methods including supercritical fluid extraction, solvent extraction, steam distillation, cold pressing, and hot pressing (Lis-Balchin & Deans, 1997; Younis *et al.*, 2009).

Formerly, one of the most common methods for extraction of essential oils was steam distillation (Tuan & Iiangantileke, 1997; Simandi *et al*., 1999) and traditionally, distillers favoured this method and claimed that none of the newer methods extracted better quality oil, but as technological advances were made, more efficient and economical methods have been developed. Supercritical fluid extraction (SFE) is a rapid, selective and convenient method for sample preparation prior to the analysis of compounds in the volatile products of plant material. SFE is usually performed with pure or modified carbon dioxide, which facilitates offline collection of extract and online coupling with other analytical methods such as gas, liquid and supercritical fluid chromatography (Pourmortazavi & Hajimirsadeghi, 2007). The extraction of essential oil components using supercritical fluid method is considered one of most efficient methods in the food, pharmaceutical and cosmetic industries because of its superiority over solvent extraction and steam distillation (EiKani *et al.,* 1999).

The genus *Jasminum* is reported to include about 64 species (Anon., 2011), out of which 40 are indigenous to the Indian subcontinent (Irulappan, 1994). Jasmine plants are of great economic value as a field crop for the florist, landscape, medicinal and pharmaceutical industries (Green & Miller, 2009). Mostly, jasmine plants are grown in houses and gardens for ornamental purposes, and are sometimes also used for cut flowers to make garlands. However, there are a few species with fragrant flowers. Among these species, *J. grandiflorum*, *J. auriculatum* and *J. sambac* are commercially cultivated for oil extraction (Green & Miller, 2009). *Jasminum sambac* is an evergreen, shrubby vine growing up to 3 m high with shiny, pointed and very decorative leaves (Shu, 2008). Its white, intensely perfumed flowers appears in light clusters that contain a small amount of essential oil. The essential oil of jasmine is called “otto” or “attar of jasmine”; it contains over 100 constituents but the main chemical components responsible for the aroma are benzyl acetate, linalool, benzyl alcohol, eugenol, benzyl benzoate, cis-jasmone, geraniol, farnesol and trace amounts of isohytol and phytol (Lawrence, 1977).

Jasmine oil has a wide range of medicinal applications and can be used in perfumery, soaps, flavorings and the cosmetic industry (Lawless, 1995). Jasmine oil is famous for the treatment of dry, greasy, irritated and sensitive skin. Other reported medicinal uses include soothing irritating coughs, alleviating muscular pain and treating sprains (Prabuseenivesan *et al*., 2006). Therapeutically, jasmine oil is used as an antidepressant, antiseptic, antispasmodic, sedative and uterine tonic (Kang & Kim, 2002; Maxia *et al*., 2009). Jasmine essential oil contains different types of complex constituents. These constituents can be identified by gas chromatography. Gas chromatography (GC) helps to determine the relative composition of different components of essential oil and precisely measure the percentage of specific chemical ingredients. This technique proved to be an excellent tool for separation, characterization and qualitative analysis of essential oil (Françoise, 2003).

In Pakistan, the production of essential oil from jasmine is a somewhat new type of agricultural activity; therefore, there is inadequate information about production and extraction available to local producers. The present study was carried out to extract essential oil of *Jasminum sambac* using the supercritical fluid extraction (SFE) method. Objectives of the study were: to determine the physical properties of jasmine essential oil, to optimize standard conditions for maximum oil extraction from jasmine, to analyze essential oil components of jasmine by using gas chromatography analysis, and to compare quality and quantity of essential oil from flowers harvested at two different stages.

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Material and Methods

Collection and preparation of plant material: Jasmine flowers were collected at two different stages i.e., closed bud stage and fully open flower stage, for oil extraction from the agricultural fields at the University of Agriculture, Faisalabad, during the year 2009-2010. Flowers at both stages were collected in the morning before sunrise because jasmine oil is highly volatile. Petals were separated from sepals, weighed and spread in a tray under shade at room temperature in order to remove moisture, and then used for extraction.

Supercritical fluid extraction (SFE): For supercritical fluid extraction (SFE), extraction was carried out with carbon dioxide, which acts as a solvent above its critical pressure and temperature (Ambroud *et al.,* 1994). Extraction was carried out typically at 300 bar pressure (regulator was used to control the operating pressure of the supercritical CO_2) between 35-60°C, as recommended by Paroul *et al.,* (2002). The following steps were performed for the extraction process:

i. Preparation of the feed (flower) material: The flowers were carefully placed into the extractor to avoid contamination in the final product, because high-value finished products demand maximum extraction efficiency and minimal losses during handling.

ii. CO_2 conditioning: Commercially available food-grade liquid carbon dioxide (99.99% pure) was fed into the hold-up tank CO_2 cylinder. Liquid CO_2 from the hold-up tank was then fed into the high pressure metering CO_2 pump, through a pre-cooler to ensure a supply of CO_2 in sub-cooled liquid form. The high pressure liquid CO_2 from the pump discharge was then heated in an extractor to achieve supercritical temperature.

iii. Extraction: Jasmine flowers were held in the extractor with the help of a perforated basket for easy handling. The extractor was equipped with specially designed quick-acting closures for easy and fast opening and closing. The supercritical CO_2 flowed through the bed of raw material. The extract-laden supercritical CO_2 then flowed to the separator for recovery of the extract.

iv. Recovery of extract: In the separator, lower pressure and temperature conditions (i.e. 200 bar pressure and 35°C) were maintained for complete recovery of the extract (absolute oil) from the CO_2 stream. In the separator, subcritical conditions (i.e. 300 bar pressure and 45°C) were maintained. The CO_2 vapour leaving the separator was free from the extract. It was liquefied using a condenser and stored in the CO_2 hold-up tank; the absolute oil of jasmine was collected in a flask.

Data collection

Yield of absolute oil: The oil extracted includes some waxes and is called concrete oil in this condition. Natural waxes present in the concrete oil were removed by following the method of Younis *et al*., (2009) to obtain the absolute oil. Percentage yield of absolute oil was calculated based on concrete oil separately for closed bud and open flower extract.

Absolute oil percentage is given as:

$$\text{Percentage of absolute oil (on petal basis)} = \frac{\text{Weight of absolute oil}}{\text{Weight of petals}} \times 100$$

$$\text{Percentage of absolute oil (on concrete oil basis)} = \frac{\text{Weight of absolute oil}}{\text{Weight of concrete}} \times 100$$

Physical properties: The following properties of the absolute oil were recorded.

Color: Color of absolute oil was determined by the spectrophotometric method (Anon., 1987). In this method, a spectrophotometer recorded the transmittance measurement between 400 and 700 nm at regular intervals of 10 nm in the cells (optical container called a cuvette) of a given length such that the transmission factor relative to a reference solvent lies between 20 and 80 percent. Determination of the percentage transmission of the sample of jasmine essential oil in a 1 cm cell every 10 nm from 400 to 700 nm was compared with that of carbon tetrachloride as a blank, using a minimum slit width for each observation. At the points of maximum and minimum transmission, readings were taken at 1 nm distance on each side to secure precision.

Then by application of Lambert's law, the value of the transmittance for a cell of 1 cm length was calculated using the formula:

$$\text{Log} \frac{100}{T_1} \frac{1}{n} = \frac{100}{T_n} \text{Log} \underline{\qquad}$$

where T_1 is the transmission calculated for a cell of 1 cm
T_n is the transmission measured for a cell of n cm

Refractive index: The refractive index of the oil was recorded at room temperature by using Abb's refractometer (Anon., 1987).

Congealing point: The congealing point of the oil was determined by the method adopted by Younis, (2006). A few drops of oil were placed in a capillary tube, which was then suspended inside a large tube along with a standard thermometer. The inner and outer tubes were cooled together to a temperature 5°C below the expected congealing point. The liquid was gently stirred until it started to solidify. The highest temperature recorded during solidification was recorded as the congealing point.

Optical rotation: Optical rotation was measured by using the method of Younis, (2006). In this method, a 10 ml polarimeter tube containing oil was placed in a polarimeter between the polarizer and analyzer. Care was taken in filling the tube to avoid any air bubble, which could disturb the field of light. The analyzer was slowly turned until both halves of the field were viewed through the scope on which divisions are engraved.

The direction of rotation was determined as follows: if the analyzer was turned counter-clockwise from the zero position to obtain the final reading, the rotation was levo (+), if the analyzer was turned clockwise, the rotation was dextro (-).

Specific gravity: The specific gravity of the essential oil was measured by using a specific gravity bottle. The pre-weighed 10 ml specific gravity bottle was filled with jasmine absolute oil, leaving no air bubbles, and then weighed. The density of the oil was computed by using the formula:

$$\text{Density} = \frac{\text{Weight}}{\text{Volume}}$$

$$\text{Specific gravity} = \frac{\text{Density of the liquid at 20°C}}{\text{Density of } H_2O \text{ at the same temperature}}$$

Chemical properties

Acid number: Acid number was determined through the indicator method (Anon., 1987). The acid number was calculated by the following formula:

$$\text{Acid number} = \frac{\text{56.1 (Number of c.c. of 0.1 M KOH used in titration)}}{\text{Weight of the sample (g)}}$$

Ester number: The ester content was calculated by the following formula:

$$\text{Ester number} = \frac{28.05\ (a)}{S}$$

where:
a = number of cm^3 of 0.5 N HCl used in saponification.
s = weight of sample in grams.

Gas chromatography: Gas chromatography (GC) for qualitative and quantitative analysis of constituents of jasmine oil was also conducted. A Shimadzu 17-A gas chromatograph was used (Sp 2330 capillary columns, BPX70 - 70% phenyl column; column length 30 m; column thickness 25 µm). Other analytical conditions were: sample size (1 µl); carrier gas, N_2 flow velocity = 5 ml/min; initial column temperature, 50°C; final column temperature, 120°C. Initial hold up time was three min, final hold up time was nine min and ramp rate was 5°C/min (Fig. 1). Identification of constituents of essential oil for closed buds and open flowers was done by comparison with gas chromatograms of a mixture of standards (Fluka, 99% GC grade, Switzerland), while quantitative analyses were carried out by calculating the area under the peak using software CSW-32 (http://www.dataapex.com/products/csw32.php).

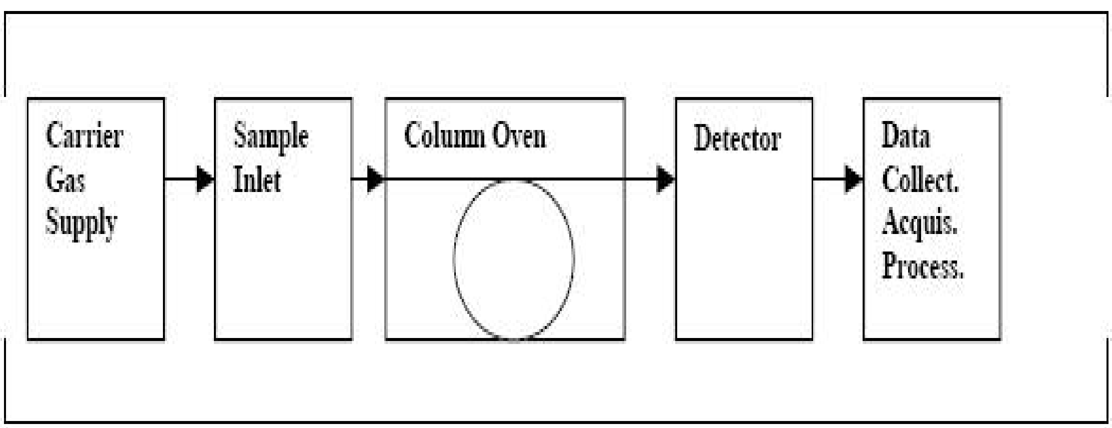

Fig. 1. Scheme of a gas chromatograph.

Results and Discussion

Physical properties of essential oil: The color of the absolute oil of jasmine from open flowers was clear yellow, in contrast to the findings of Gilbert *et al*., (1999), who found jasmine oil to be reddish brown in color. The method of extraction and environmental conditions can influence the color of oil. The color of oil obtained from closed buds of jasmine was off-whitish yellow. The refractive index at 20°C for jasmine oil extracted from open flowers was 1.47, while for essential oil extracted from closed buds, it was 1.49 (Table 1). The highest temperature at which an essential oil is solidified is called its congealing point. The congealing point for both open flower and closed bud jasmine essential oil was 17°C. Usually oil extracted through different methods has different congealing points (Joy *et al.*, 2001), but in the present study only one method was used to extract oil, so the same congealing point was observed in flowers harvested at different stages. The optical rotation of jasmine essential oil from open flowers was +3.30, while for essential oil from closed buds it was +3.50. The specific gravity at 20°C for essential oil from open flowers of jasmine was recorded as 0.9540, while for oil from closed buds it was recorded as 0.9850 (Table 1). The physical properties of essential oils are strongly influenced by variety, degree of maturity, seasonal variations, seasonal rainfall, and method of extraction and yield of oil (Richard *et al*., 1971).

Table 1. Physical and chemical properties of essential oil of *Jasminum sambac*.

Property	Closed buds	Open flowers
Color	Clear yellow	Off-whitish yellow
Refractive Index	1.47 at 20°C	1.49 at 20°C
Congealing Point	17°C	17.25°C
Optical Rotation	+3.30 at 20°C	+3.50 at 20°C
Specific Gravity	0.956 at 20°C	0.9850 at 20°C
Acid Number	6.85	6.89
Ester Number	242.58	240.02

Chemical properties of essential oil: The acid number for jasmine essential oil for both open and closed bud flowers was 6.85. It was observed that the ester number of essential oil of jasmine was 242.58 for open flowers and 240.02 for oil from closed buds (Table 1). Esters are very important in regard to the odor of the oil. In the present study, oil extracted from open flowers had the highest ester number, meaning oil from open flowers had a strong odor.

Extraction of essential oil from flowers of *Jasminum sambac* at two stages: This research was carried out with the objective of comparing the composition and quantity and quality of essential oil of jasmine obtained at two stages of growth, i.e., at closed bud stage and at the open flower stage. Using the supercritical fluid extraction method, jasmine produced 4.27 g of concrete oil [0.021% on fresh petal weight basis (PWB)] when flowers were harvested at the closed bud stage, whereas 6.79 g of concrete oil (0.033%) was recovered from fully open flowers (Fig. 2). The yield of absolute oil and absolute oil percentage showed similar patterns in flowers harvested at both stages. Jasmine flowers harvested at the fully open stage produced the highest absolute oil (4.31 g), which is 63.47% on a concrete oil basis and 0.128% on a PWB. Flowers at the closed stage produced 2.57 g of absolute oil (0.012%). The yield, the essential oil content and composition of jasmine can be influenced by flower harvesting stage and harvesting time as reported by Clark & Menary, (2006) and Younis *et al*., (2009). It is generally assumed that the material is best collected when the flowers have reached their optimal state of development (Gholamreza, *et al*., 2002). It is important to accurately determine the appropriate harvesting time and stage of flowers for better oil yield in aromatic plants.

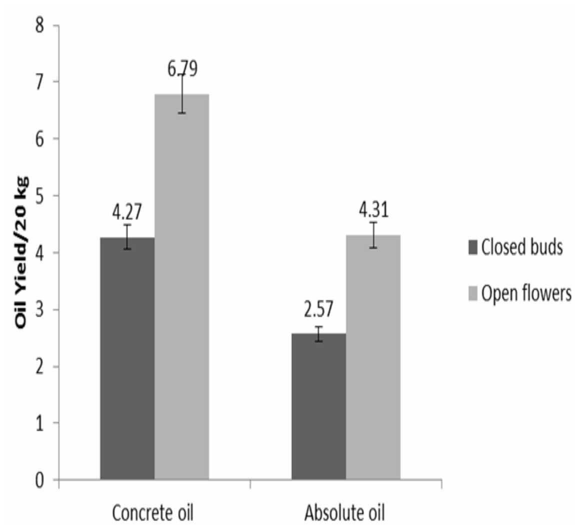

Fig. 2. Comparison of oil yield (g) of jasmine flowers harvested at two stages.

Identification of constituents of jasmine oil through gas chromatography analysis: Different constituents of essential oil of jasmine were determined using gas chromatography. The components identified through gas chromatography from jasmine closed buds and open flowers are illustrated in Table 2. In total, 14 components were identified in the jasmine essential oil extracted from open flowers, while 12 components were identified in the essential oil obtained from closed flower buds. The essential oil composition varied according to the flowering stages (Table 2), and more compounds were identified when flowers were fully opened. The major components at different flowering stages were citronellol (17.98% and 19.37), phenyl ethyl alcohol (12.98% and 14.11%), geranial (3.89% and 6.26%), eugenol (5.98% and 9.8%) and farnesol (8.91% and 8.31%). The major component of jasmine oil is citronellol, which is a famous fragrance ingredient that can be used in perfume, antiperspirant, shampoo, body lotion, toilet soap, shower gel and other toiletries, as well as in household products such as cleaners and detergents. Its use worldwide is greater than 1000 metric tons/year (Lapczynski, *et al*., 2008). In the present study, phenyl ethyl alcohol was the second major component identified (Table 2); it can be used as a biocide, antimicrobial, antiseptic, disinfectant, and as an aromatic essence and preservative in the pharmaceutical and perfume industries (Kurt, 2001). It is also used as a flavouring agent in different soft drinks, cookies and confectionary. Farnesol is also one of the major constituents of jasmine oil (Table 2); it is used in the perfume industry to accentuate the sweet odors of floral fragrances and can be used as a deodorant due to its antibacterial activity. Its method of action for enhancing perfume scent is as a co-solvent that regulates the volatility of the odorants. It also has demonstrated activity as a chemo-preventive and antitumor agent (Joo & Jetten, 2009). Eugenol, another important component of jasmine essential oil, can be used in perfumery, flavorings, and in medicines as an antiseptic and anesthetic agent.

The contents of other important active plant principles in essential oil of marigold are reported to depend considerably upon on extrinsic and intrinsic aspects, including soil and climatic situations, ontogenetic plant phases, and harvesting stage (Ester *et al*., 2008). In Finland, Holm *et al*., (1988) observed that the highest percentage of oil in dragonhead was 0.62% during the flowering stage, and the oil contained 90% oxygenated acyclic monoterpenes, i.e. geranial, geraniol, nerol, neral and geranyl acetate. In another experiment, Aziz & El-Sherbeny, (2003) documented that volatile oil of dragonhead was composed mostly (up to 93%) of acyclic oxygenated monoterpenes. Optimal harvesting time guaranteed higher contents of essential oil from mints when they were harvested just before flowering (Basker & Putievsky, 1978). The results of the current study confirm previous findings (Rohloff *et al*., 2005; Marcuni & Hanson, 2006) regarding the effect of harvesting time and stage of harvest on oil yield, oil content, and composition.

Table 2. The relative percentages of the main constituents identified through gas chromatography in the essential oil of *Jasminum sambac* flowers harvested at different stages.

Sr. #	Constituents	Closed bud stage (%)	Fully open flower stage (%)
1.	Benzyl alcohol	4.51	5.26
2.	Benzyldehyde	1.34	3.29
3.	Citral (mixture of cis and trans)	0.58	0.73
4.	Linalool	1.45	2.31
5.	2-Phenyl ethyl acetate	2.73	3.01
6.	Geraniol	3.89	6.26
7.	Eugenol	5.98	9.8
8.	Farnesol	8.91	8.31
9.	Citrinyl acetate	3.56	3.57
10.	Nerol	-	0.39
11.	Geranyl acetate	2.79	4.98
12.	Nerayl acetate	-	1.00
13.	Phenyl ethyl alcohol	12.98	14.11
14.	Citronellol	17.98	19.37

Conclusion

In this study, it was found that the SCF extraction technique proved to be an efficient method of essential oil extraction. Contents of jasmine were significantly affected by the harvesting stage of flowers. Harvesting at the closed bud stage yielded less essential oil compared to flowers harvested at the fully open stage. There were variations in constituents of essential oil at both stages. Flowers harvested at the fully open stage yielded better quality essential oil compared with flowers harvested at the closed bud stage. The quantity of oil obtained differed in both cases, as more oil was obtained at the open flower stage and less oil and wax-like substance was obtained at the closed bud stage. The quantity of absolute oil obtained at the open flower stage was almost double the quantity obtained at the closed bud stage. These results showed that the composition of essential oils in the plant at different growth stages changed and the production of a specific component of these essential oils depended on which stage of growth the plant was at when flowers were harvested.

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