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Original Article

Comparison of the Chemical Constituents in *Michelia alba* Flower Oil Extracted by Steam Distillation, Hexane Extraction and Enfleurage Method

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Abstract

White champaka (*Michelia alba* DC.) is a fragrant flower with a gentle scent that has long been utilized by Thai people; *M. alba* products remain widely popular in the Thai market. There are several methods for extracting aromatic oil from *M. alba* flowers and one of them is the enfleurage method which is believed to yield an aromatic oil with closely similar odor to fresh flowers. In this study, *M. alba* flower oil was extracted by a newly modified enfleurage method using developed buffalo fats along with other aromatic extraction methods; steam distillation and hexane extraction. The chemical composition of *M. alba* oil extracted from each technique was studied and compared using gas chromatography-mass spectrometry (GC-MS) data. According to the comparison study of *M. alba* flower oil, the enfleurage method gave a light yellow oil with similar odor to fresh *M. alba* flowers and its main composition was indole (1H) (35.5%), whereas the steam distillation method gave a colorless oil with similar odor to boiled *M. alba* flowers rather than fresh ones. Its major component was linalool (66.92%). The hexane extraction method gave a transparent oil sample with similar but more pungent odor to that of fresh *M. alba* flowers and its major compounds were 2-methyl butanoic acid and linolool (33.01% and 28.92%, respectively). Indole was also found as a minor component in *M. alba* flower oil extracted by the steam distillation technique, but was absent in oil extracted by hexane. With further comparison, linalool and 2-methyl butanoic acid were also found in oil extracted by the enfleurage method but in negligible amounts. With regard to perfumery, indole is the natural compound that increases the perceived odor strength and improves the stability of other aromatic compounds in volatile oils. The major components of indole in *M. alba* flower oil extracted by the enfleurage method could be an obvious benefit of this method. In conclusion, *M. alba* flower oil extracted by the enfleurage method, using developed buffalo fats, has a desirable quality of aromatic oil, which should meet the high demands of the aromatherapy market.

Key words: **white champaka, enfleurage, volatile oil, chemical composition, indole, buffalo fat**

Introduction

Michelia alba DC. (white champaka), Family Magnoliaceae, is a native Thai plant that is widely cultivated in home gardens as an ornamental plant. *M. alba* flowers are the source of a fragrant oil used extensively for making perfume[1,2]. The tree producing these flowers ranges from 10 to 15 meters in height and can grow up to 20 meters if cultivated in a high-moisture area. Ramification is constituted in clustering branches with brittle twigs. Cracks in the stem bark is in a reticulated pattern along the trunk. *M. alba* is an annual flowering plant; generally the flowers begin to bloom

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around 8-9 pm, with their scent becoming quickly widespread; the scent begins to fade in the afternoon. Gardeners usually collect the flowers twice a day: at 8-9 pm and at dawn[2,3]. *M. alba* acts as a cough suppressant and expectorant in induced tests in animals[3]. According to research from China, the extract from double distillation of 600 g of *M. alba* fresh flowers with 1.2 L water used in the ratio of 4:1 was successfully used to treat bronchitis patients with, a 75 per cent recovery rate[3]. Dry *M. alba* flowers are also used in Thai traditional medicine for heart and nerve maintenance and anti-motion sickness[4]. In addition to the encouraging pharmacological actions, *M. alba* is among the would's most famous fragrant flowers; its oil is one of the common ingredients in several expensive perfumes[5]. Various methods are used to extract the volatile oil from aromatic plants, the most common being steam distillation due to the convenience of the method and its low cost[6,7]. Solvent extraction is another method for extracting volatile oils applied to heat- sensitive plants in order to avoid the decomposition of fragrant compounds in the oil. However, the solvent extraction method involves a high cost owing to the necessity for removing the solvent from extracted oils, which is an expensive process[7]. Currently, solvent extraction by liquid carbon dioxide is an effective technique for yielding a high-purity volatile oil, with the solvent residue from the extracted oil being completely removed; nonetheless, this technique also involves considerable expense[7]. Another method for volatile oil extraction is a traditional one, enfleurage, originating in Grasse, France. This method utilizes animal fats to adsorb the fragrant compounds exuded by the fresh flowers[8,9]. Nowadays, with the advances in technology, the fragrance can be synthesized by chemical reaction to produce an odor similar to that of the natural fragrance. However, the natural fragrance is still in demand; several factories in France widely use the enfleurage method for fragrance extraction. The enfleurage method is reportedly the process yielding oil that has an odor most similar to that of fresh flowers[8,9]. In the enfleurage method, a large, framed plate of glass is smeared with a layer of animal fat, and petals or whole flowers are then placed on the fat. The flowers are changed daily until the fat is saturated with the fragrance and then the volatile oils are isolated from the fat[9]. Owing to the fact that the enfleurage method commonly uses spermaceti and wool fat, the cost of production is therefore very high. Furthermore, neither of these two fats are manufactured in Thailand; hence, the enfleurage method is unpopular in this country. However, if a cheaper animal fat were available that could be used to adsorb flower fragrance instead of the costly ones, the enfleurage method would likely become much more popular in Thailand.

Objectives

The main purpose of this study was to develop a practical technique at an affordable price to extract high-quality volatile oil from fresh *M. alba* flowers. Furthermore, the study was intended to compare the physical appearance and chemical constituents of the volatile oil extracted by various techniques: steam distillation, hexane (solvent) extraction and the enfleurage method. Gas chromatography-mass spectrometry data were used to determine the chemical composition of the extracted volatile oils. The study also developed an extraction procedure involving buffalo fat, which is available in Thai markets, to adsorb fat for the enfleurage method.

Materials and Methods

Source of *M. alba* flowers

M. alba flowers were bought in the Sompong Garden House and Sila House, Sila Subdistrict, Mueng District, Khon Kaen province, Thailand from October 2006 to February 2007. Samples of flowers and leaves of *M. alba* were identified by comparing them with herbarium voucher specimen number PS 4091, Faculty of Pharmaceutical Sciences, Khon Kaen University. The blossoms were collected early (6 -7 am) and used for extraction immediately after purchase. The buffalo fat used in this study was the fat near the testicle area of the Thai buffalo (*Bubalus bubalis*) also purchased in Khon Kaen province, during the period from October 2006 to February 2007.

Figure 1 Processed buffalo fat for enfleurage

Preparing buffalo fat for the enfleurage method

The buffalo fat was cleaned and unwanted parts such as blood vessel and fascia were removed. Then the fat was sliced into small pieces; 100 g of sliced fat was weighed and transferred to a glass bottle. Dichloromethane (Sigma, lab grade) (66.7 ml) was added to the bottle to attain a fat to solvent ratio of 3:2 weight/volume. Then the bottle was tightly closed and kept at ambient temperature for 10 days. The dichloromethane layer containing the extracted fat was separated from the fat by filtration. The filtrate was transferred to an aluminum tray and exposed to the sun during daytime and dried overnight in a fume hood. Extracted buffalo fat appeared to be clear white (Figure 1); it was scraped out and kept in a clean bottle. Ethanol was added to the extracted fat in a ratio of 1:1 w/v and the fat was kept for seven days prior to use. Finally, ethanol was poured out of the bottle and the extracted fat was transferred to a 250 ml beaker and placed superficially above a water bath at 50°C until the fat began to aggregate into semi-solid form. Extracted fat from the final process was kept for the enfleurage method.

Extraction of *M. alba* oil by the enfleurage method

A total of 100 g of extracted fat from the above-mentioned method was weighed and then smeared onto two glass bowls (20 centimeters in diameter); 100 g of fresh *M. alba* flowers were put into the first bowl and then another bowl was turned over to cover the first one. The enfleurage bowls were left overnight. The following morning, a new set of fresh *M. alba* flowers (100 g) replaced the old ones and the same process was repeated for 14 consecutive days. At the end of the extraction process, the buffalo fat was saturated with fragrance; it was then scraped out and transferred to a clean bottle. Thereafter, 100 ml of cooled ethanol was added to the bottle, left overnight, then filtered to separate the ethanol extract from the fat. The aforementioned method was repeated until there was no fragrance left in the buffalo fat. The combined ethanol filtrate was then evaporated using a rotary evaporator. The concentrated extract was then centrifuged and the upper layer was kept for analysis of its chemical composition.

Extraction of *M. alba* oil by steam distillation

The basic steam distillation set was connected and 300 g of fresh *M. alba* flowers was extracted with 600 ml distilled water for two hours. The final extract was cooled and centrifuged to separate the fragrant oil; then the oil was kept for the analysis of its chemical composition.

Extraction of *M. alba* oil by hexane

A total of 200 grams of fresh *M. alba* flowers was weighed and transferred to a brown bottle; 1,000 ml of hexane was added, then the bottle was left for extraction for one month; and the sample was shaken daily. After one month, the extract was filtered and the hexane (solvent) removed by rotary evaporator. The oil in the hexane extract was extracted again by partition with cooled ethanol. The ethanol layer was then collected and quickly evaporated using a rotary evaporator, followed by centrifugation, and the oil was kept for the analysis of its chemical composition.

Analysis of chemical composition of oil samples using gas chromatography-mass spectrometry (GC-MS)

Oil samples from the three extraction methods were analyzed for their chemical composition using an Agilent Technologies 6890 N GC-MS with a 5973 innert quadru-

pole mass selective detector, HP-INNOWAX column (30 m × 0.25 mm, coated film (0.25 m in thickness). The column was temperature programmed at 40-220 °C with 4°C per minute increasing rate, pulse split injector 20:1, at 230°C. The detector was El 70 eV MSD and the carrier gas was helium (10 psi) with a flow rate of 1.2 ml/min and 40-450 amu in the mass scan range. The constituents of the oil samples were identified by matching their spectra with those recorded in the MS library (Wiley 7n and Robert P. Adams).

Table 1 Physical appearance of *M. alba* oil

Extraction method	Physical appearance of the oil
Steam distillation	Clear colorless solution; immiscible with water; odor similar to boiled *M. alba* flowers
Hexane extraction	Deep orange solution; immiscible with water; odor similar to fresh *M. alba* flowers but more pungent
Enfleurage method using buffalo fat	Light yellowish solution; immiscible with water; odor similar to fresh *M. alba* flowers

Results and Discussion

Comparison of physical appearance of *M. alba* flower oil extracted by steam distillation, hexane extraction and enfleurage method

The physical appearance of the *M. alba* flower oil samples were carefully observed as shown in Table 1 and Figure 2.

Figure 2 Oil samples from three different methods
1 = oil sample from enfleurage method using buffalo fat
2 = oil sample from steam distillation
3 = oil sample from hexane extraction

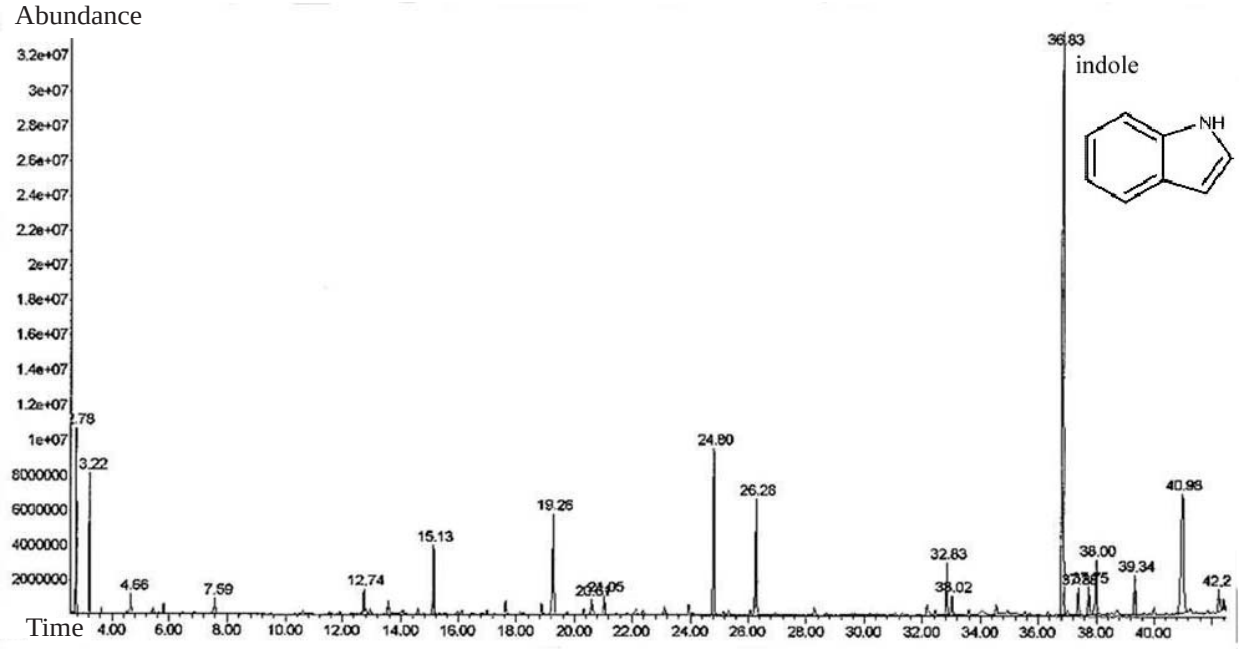

Figure 3 Total ion chromatogram of oil extracted from fresh *M. alba* flowers by the enfleurage method.

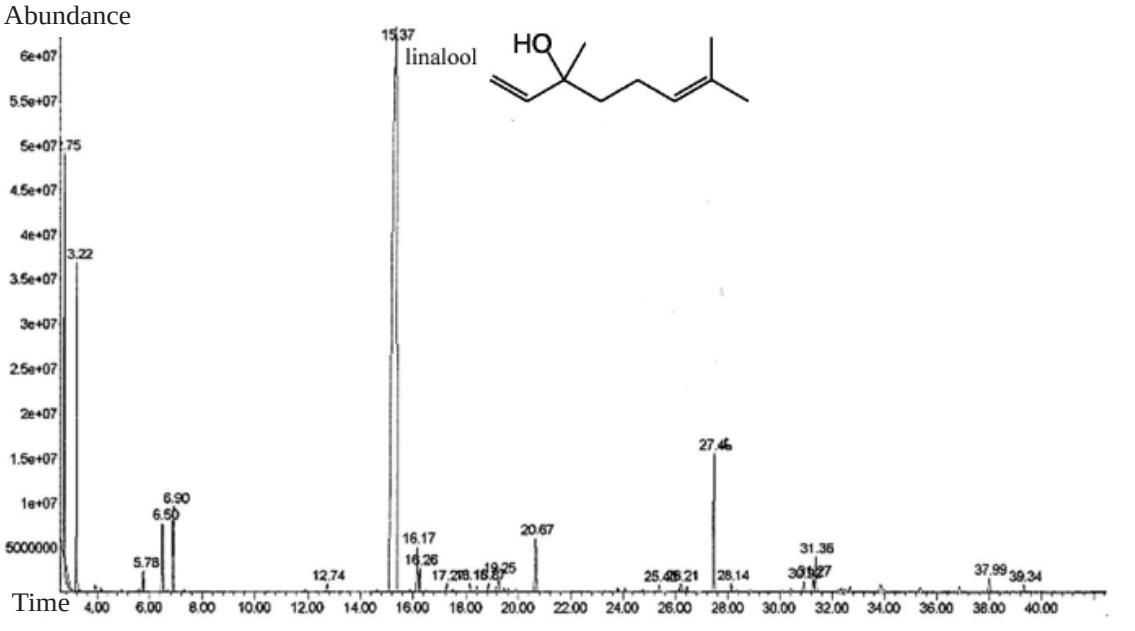

Figure 4 Total ion chromatogram of oil extracted from fresh *M. alba* flowers by steam distillation.

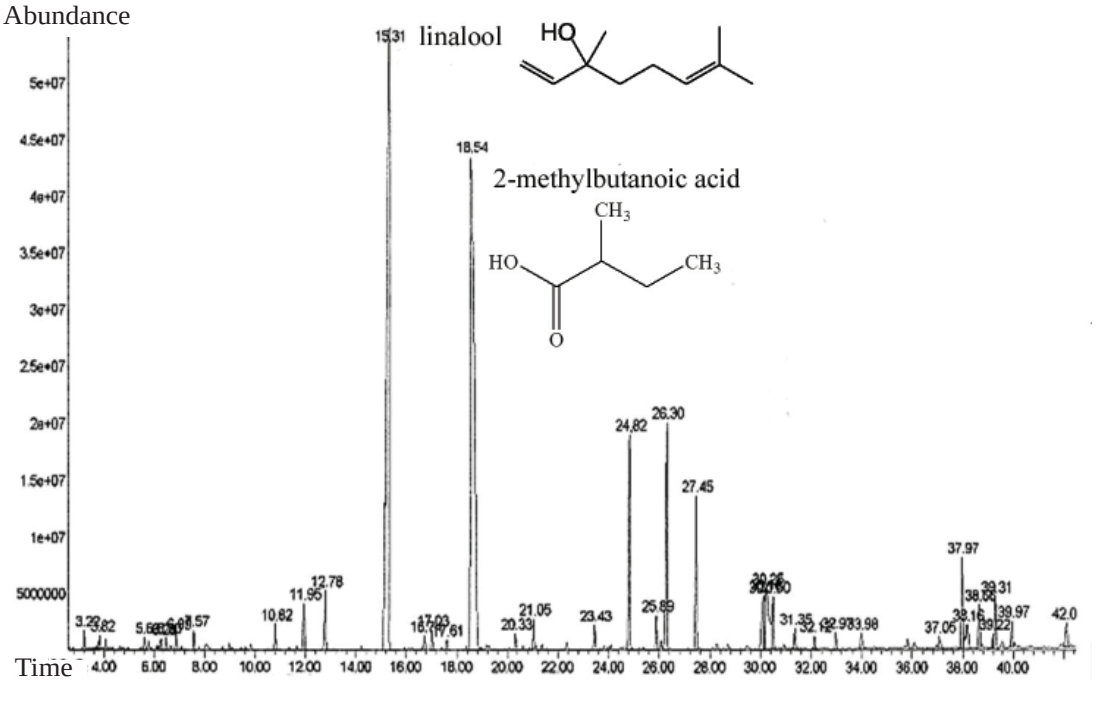

Figure 5 Total ion chromatogram of oil extracted from fresh *M. alba* flowers by hexane extraction.

Chemical composition of *M. alba* flower oil extracted by enfleurage method

According to the total ion chromatogram in Figure 3, *M. alba* flower oil consisted of the following compounds: methyl-2-methylbutyrate (5.24%), ethyl-2-methyl butyrate (3.18%), 2-methylbutyl-2-methylbutyrate (0.79 %), trans linalool oxide (< 0.5%), cis linalool oxide (0.90%), linalool (3.09%), 2-methyl butanoic acid (<0.5%), germacrene D (6.02%), phenylethyl alcohol (8.28%), phenylethyl-2-methyl butyrate (6.80%), **indole (1H-indole) (35.49%)** and hexadecanoic acid (13.18%).

Chemical composition of *M. alba* flower oil extracted by steam distillation

According to the total ion chromatogram in Figure 4, *M. alba* flower oil consisted of the following com-

pounds: methyl-2-methylbutyrate (7.77%), ethyl-2-methyl butyrate (6.76%), *cis*-ocimene (1.81), *trans*-β-ocimene (2.26%), *trans*-linalool oxide (<0.5%), *cis*-linalool oxide (0.28%), **linalool (66.92%)**, β-elemene (1.34%), trans-caryophyllene (0.72%) and eugenol (4.52%).

Chemical composition of *M. alba* flower oil extracted by hexane

According to the total ion chromatogram in Figure 5, *M. alba* flower oil consisted of the following componds: *cis* ocimene (<0.5%), *trans*-β-ocimene (<0.5%), *trans*-linalool oxide (1.14%), *cis*-linalool oxide (1.43%), **linalool (28.92%)**, methyl benzoate (0.40%), **2-methylbutanoic acid (33.01%)**, epoxylinalool (0.53%), phenyl ethylalcohol (4.52%) phenetyl-2-methyl benzoate (5.06%) and methyl eugenol (3.07%).

Comparison of chemical constituents in *M. alba* flower oil extracted by the enfleurage method, steam distillation and hexane extraction

From the comparison study using GC-MS chromatography, the constituents from the three methods were generally similar in chemical classes (esters, alcohol and terpene derivatives), but the yields varied. Linalool and derivatives were found in all oil samples with the contents range from below 0.5 to 66 per cent. The obvious difference was indole, which was found to be the major constituent in the oil sample from the enfleurage method. A blank sample of buffalo fat extract was also analyzed by GC-MS and the chromatogram showed no peak of indole (Figure 6). Therefore, the high content of indole in the oil sample from the enfleurage method was confirmed to be from the fresh *M. alba* flowers themselves.

According to previous research data of the Thailand Institute of Scientific and Technological Research[7], linalool is the major component of *M. alba* flower oil extracted by steam distillation (66.94% in content)[7] which was similar to the finding of our current study which also found linalool to be the major compound with the content being 66.92 per cent. Whereas other minor compounds were methyl-2-methylbutyrate (7.77%) and ethyl-2-methyl butyrate (6.76%), the rest of the components were found to have a content of less than 5 per cent for each component. In another study of Shang and colleagues[10], solid phase microextraction was used to analyze the volatile constituents of *M. alba* flowers and the major components were α-myrcene, (S)-limonene, (R)-fenchone, linalool, camphor, caryophyllene,

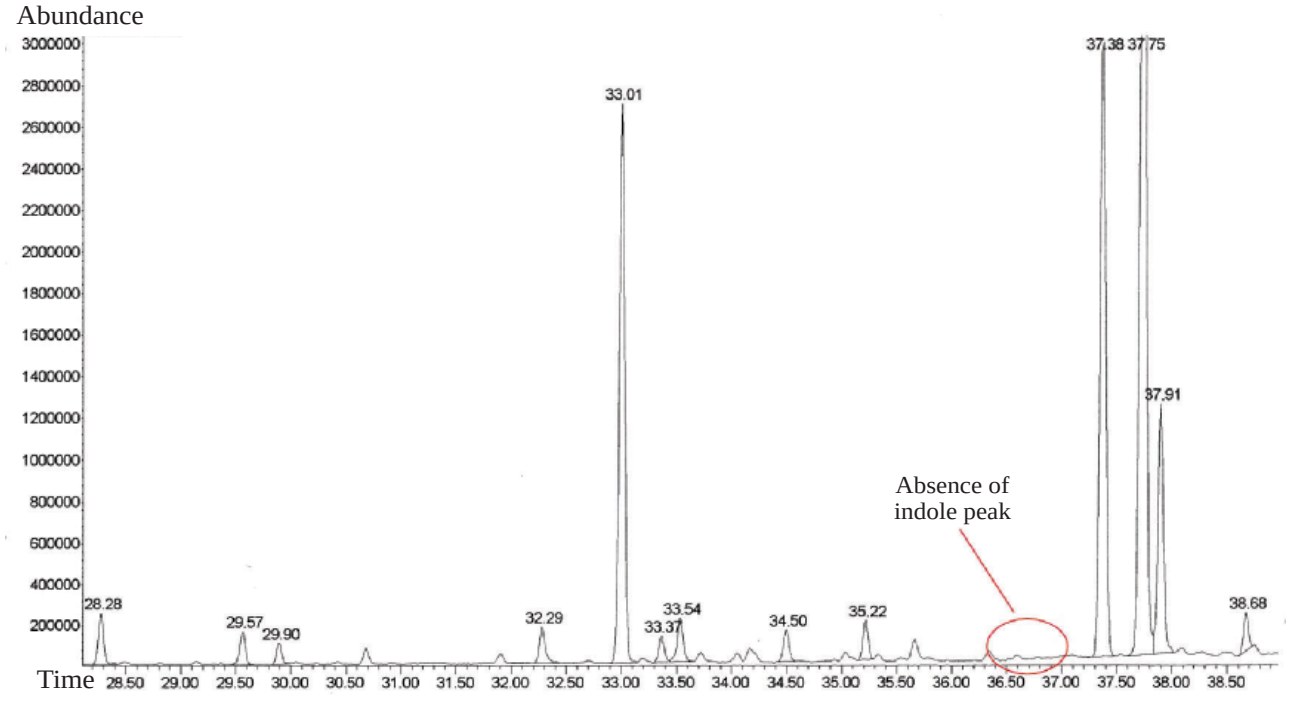

Figure 6 Total ion chromatogram of compounds in blank buffalo fat

germacrene D and others. Some compounds from that study (such as α-myrcene, (S)-limonene, (R)-fenchone) were not found in this current study due to the difference in type of GC-MS column and the variation in method conditions.

Linalool, a compound commonly found as a major component of *M. alba* flowers, is a terpene alcohol found in many flowers and spice plants with numerous commercial applications. Linalool has a pleasant floral scent with a touch of spiciness. However, in high concentration, the odor of linalool may be too strong and should be avoided by people with perfume allergy[11]. Although linalool has a pleasant scent in general, its content should be at an optimum concentration in a perfume. Interestingly, linalool and its derivatives (cis-linalool oxide, trans-linalool oxide) were found in all the oil samples from the three selected methods, which indicated that an oxidation reaction occurred during the extraction processes. Linalool derivatives were found in minor amounts (all less than 1.5%), whereas parent linalool was found in much higher amounts (28.92- 66.92%).

Indole, the exceptional component in *M. alba* flower oil from the enfleurage method, has a unique property as a fixation compound. It was one of the common ingredients in various perfume formulas used to prolong the effect of the more volatile ingredients in the formula in an attempt to equalize the rate of evaporation of the component ingredients[12]. The content of indole in the oil sample from the enfleurage method was relatively large, which may be related to its unique odor being different from that of the oil extracted by steam distillation or hexane.

According to the magnified total ion chromatograms, indole was actually found in a minuscule amount (less than 0.25%) in *M. alba* flower oil extracted by steam distillation (Figure 7) but totally absent in that produced by hexane extraction (Figure 8). The indole peak disappeared from the original GC-MS chromatogram of the oil sample from steam distillation because its abundance was below the detection limit.

When considering the extraction processes, the enfleurage method is a gentle technique. Fresh *M. alba*

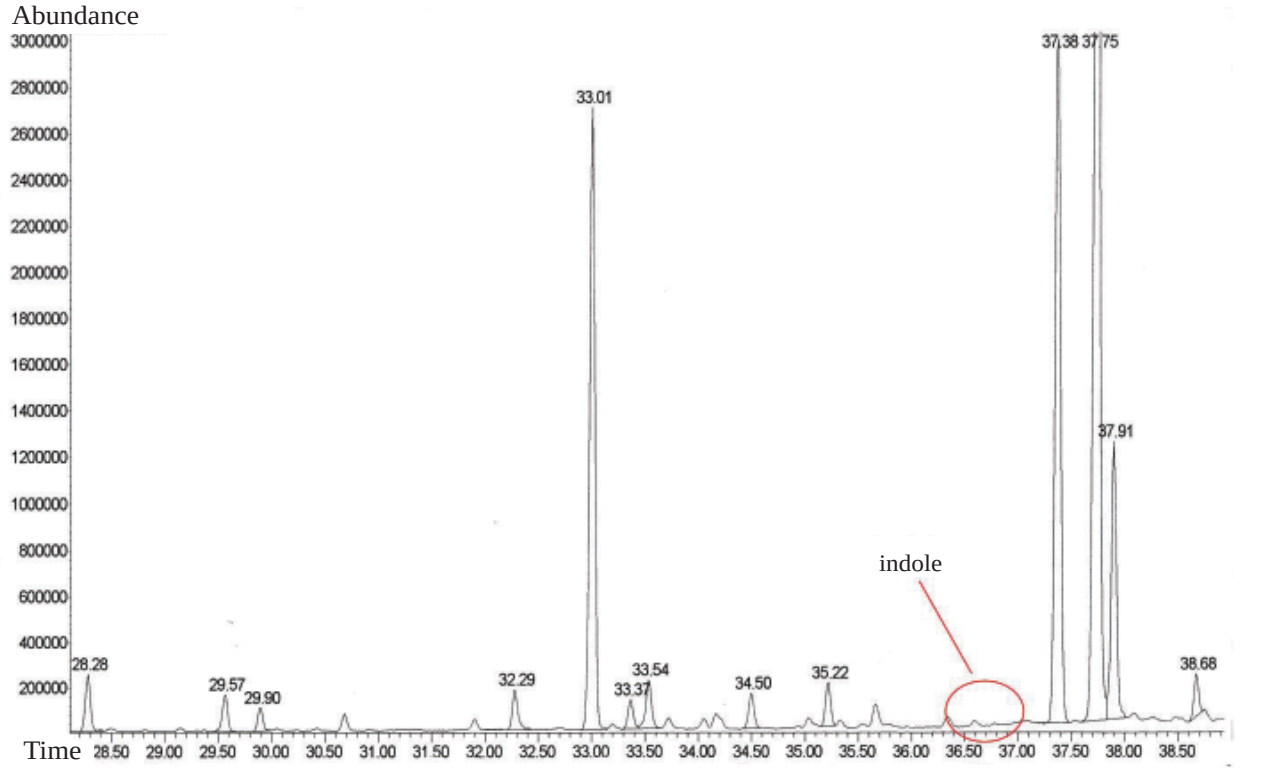

Figure 7 Magnified total ion chromatogram of oil extracted from fresh *M. alba* flowers by steam distillation (with the indole peak being in minor abundance).

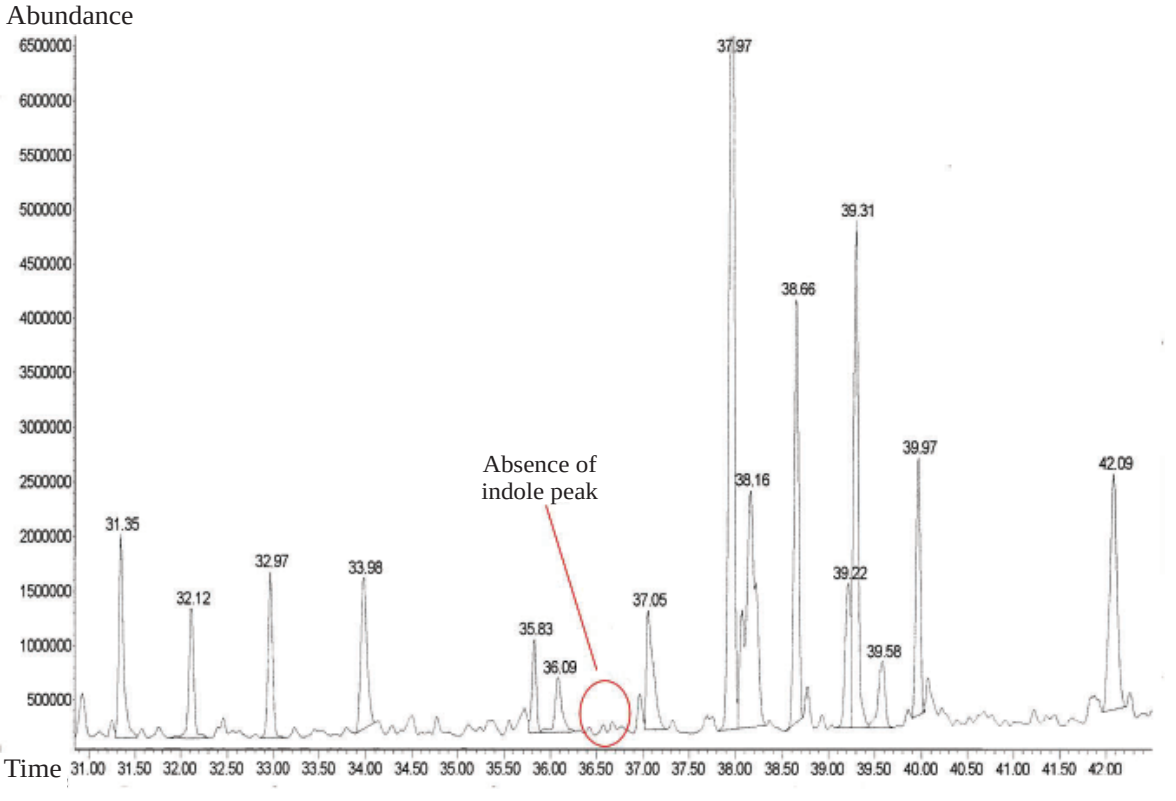

Figure 8 Magnified total ion chromatogram of oil extracted from fresh *M. alba* flowers by hexane extraction (absence of indole peak).

flowers were simply put on buffalo fat and the fragrance was gradually adsorbed without any carrier, whereas in the steam distillation process, *M. alba* flowers were boiled in water and directly exposed to high pressure steam, which may result in a loss of indole content. Indole was not found in the oil sample produced by hexane extraction, although *M. alba* flowers were not exposed to any harsh conditions. The polarity of hexane and its miscible factor may result in the absence of indole from oil extracted by this solvent. The major constituents found in *M. alba* flower oil produced by hexane extraction were 2-methylbutanoic (33.01% in content) and linalool (28.92% in content).

Owing to the fact that steam distillation is a method that was already used to extract *M. alba* flower oil in several studies, the results showed very similar chemical compositions. Hexane extraction involves a high cost to remove the solvent from the oil. One technique left to develop is the enfleurage method, which is not commonly used by Thai researchers or the industry. The good quality of oil produced by the enfleurage method in our study, in terms of odor and chemical composition, is encouraging. To the best of our knowledge, prior to our study no enfleurage method has utilized buffalo fat to adsorb the fragrance of flowers. The enfleurage method may be a new alternative technique to extract high-quality fragrance from flowers in Thailand, as buffalo fat is cheap and readily available. Further studies of the enfleurage method, with the novel extraction process of buffalo fat and other animal fats are necessary in order to develop the most suitable adsorption fat for this method.

Conclusions

The current study extracted oil from *M. alba* flowers, one of the world most famous aromatic flowers, using steam distillation, solvent extraction and the enfleurage method. The enfleurage method was adapted from the traditional method by using developed buffalo fat to adsorb the fragrance instead of spermaceti or wool fat. In comparing of GC-MS data of the oil samples obtained from these three methods, ester compounds

and terpene alcohol are found to be the most common constituents in oil samples, with some variation in their contents.

Linalool and 2-methyl butanoic acid were the two major components in oil samples obtained from steam distillation and hexane extraction (66.92 and 33.01% in content, respectively). Linalool was also found in high content in the oil sample obtained from hexane extraction (28.92%). Indole was a major compound found in the oil sample obtained from the oil produced by the enfleurage method (content of 35.49%). The major components in *M. alba* flowers, such as linalool and indole, corresponded to the indication in Thai traditional medicine for utilizing scented compounds from plants for the purpose of affecting a person's mood or health. Linolool is known as an important attractant pheromone for many insects and is used as an aromatic in many perfumes,[13] similarly indole at high dilutions has a pleasant odor and is used in perfumery as well[14].

According to the GC-MS data, the different extraction methods resulted in variations in the components and their content in the oil samples. The unexpected high yield of indole in the oil sample from the enfleurage method was an engaging result. However, an extensive organoleptic test and survey of consumer satisfaction with the oil product are required to fully assess the quality of oil obtained with this method. Future intensive studies on various animal fats and their capacity to adsorb the fragrance from aromatic flowers may also lead the way in finding more methods that can be applied to extract high quality oil from such flowers. In addition, animal fats, such as buffalo fat, cow fat and pig fat, are very cheap and available in the market, and if successfully developed to extract the aromatic compounds from flowers, they should be very useful as substitutes for traditional fats and provide an alternative method of extracting fragrant from oils flowers for the Thai perfume industry.

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บทคัดย่อ

การเปรียบเทียบสารองค์ประกอบเคมีของน้ำมันดอกจำปีที่สกัดได้จากการกลั่นด้วยไอน้ำ, สกัดด้วยตัวทำละลาย และสกัดวิธีออง เฟอราจ

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**ภาควิชาเภสัชพฤกษศาสตร์และเภสัชวินิจฉัย คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น*

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จำปี (*Michelia alba* DC.) เป็นดอกไม้หอมที่คนไทยนำมาใช้ประโยชน์จากกลิ่นหอมตั้งแต่โบราณจนถึงปัจจุบัน ดังเห็นได้จากผลิตภัณฑ์ที่มีกลิ่นหอมของดอกจำปีจะได้รับความนิยมจากผู้บริโภค. การสกัดน้ำมันหอมดอกจำปีนั้นทำได้หลายวิธี. วิธีสกัดที่เชื่อว่าจะให้น้ำมันหอมที่มีกลิ่นหอมคล้ายดอกไม้สดมากที่สุดคือการสกัดด้วยวิธีออง เฟอราจ. คณะผู้วิจัยนี้ได้ทำการศึกษาเปรียบเทียบสารเคมีของน้ำมันหอมดอกจำปีที่ได้จากการสกัด ๓ วิธี คือ การกลั่นด้วยไอน้ำ, การสกัดด้วยเฮกเซน และการสกัดวิธีออง เฟอราจ โดยใช้ไขควายที่พัฒนาให้เหมาะในการใช้สกัดน้ำมันหอม เปรียบเทียบสารองค์ประกอบทางเคมีด้วยเทคนิคแก๊สโครมาโตกราฟฟี-แมสสเปกโตรเมทรี. เมื่อศึกษาเปรียบเทียบน้ำมันหอมดอกจำปีที่ได้จากการสกัด ๓ วิธีที่ต่างกัน พบว่า น้ำมันหอมดอกจำปีจากการสกัดด้วยวิธีออง เฟอราจมีสีเหลืองอ่อน มีกลิ่นหอมเหมือนดอกจำปีสด มีสารองค์ประกอบหลัก คือ อินโดล (1H-indole) ร้อยละ ๓๕.๕; น้ำมันหอมดอกจำปีจากการกลั่นด้วยไอน้ำใสไม่มีสี มีกลิ่นเหมือนดอกจำปีต้ม ไม่เหมือนกลิ่นดอกจำปีสด มีสารองค์ประกอบหลักคือ ลินาลูล ร้อยละ ๖๖.๕; น้ำมันหอมดอกจำปีจากการสกัดด้วยเฮกเซนมีกลิ่นคล้ายดอกจำปีแต่ฉุนกว่าดอกจำปีสด สารองค์ประกอบหลัก คือ กรด ๒-เมธิล บิวทาโนอิก ร้อยละ ๓๓.๐๑ และลินาลูล ร้อยละ ๒๘.๕. น้ำมันหอมจากการกลั่นด้วยไอน้ำมีอินโดลเล็กน้อย, ซึ่งไม่พบในน้ำมันหอมจากการสกัดด้วยเฮกเซน, แต่ก็พบ ลินาลูล และกรด ๒-เมธิล บิวทาโนอิก ปริมาณเล็กน้อยในน้ำมันหอมสกัดวิธีออง เฟอราจ. อินโดล เป็นสารที่ช่วยให้สารหอมอื่นที่เป็นองค์ประกอบในน้ำมันหอมนั้นหอมมากขึ้นและช่วยตรึงกลิ่นหอมให้ติดทนยาวนานยิ่งขึ้น. ดังนั้นการพบอินโดลในน้ำมันหอมดอกจำปีจากการสกัดวิธีออง เฟอราจ แสดงให้เห็นว่าน้ำมันหอมดอกจำปีที่สกัดวิธีออง เฟอราจโดยใช้ไขควายที่พัฒนาได้สมบัติอันพึงประสงค์ของน้ำมันหอมดอกจำปีที่เป็นที่ต้องการของตลาดเครื่องหอม.

คำสำคัญ : **จำปี, ออง เฟอราจ, น้ำมันหอม, องค์ประกอบทางเคมี, อินโดล, ไขควาย**