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Original Article

The Development of Local Animal Fat as an Alternative Method in the Extraction of Essential Oil for Aromatherapy

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Abstract

Enfleurage is a traditional method to extract the fragrance (essential oil) from delicate flowers using odorless fat. The preparation of fats is one of the most important factors to determine the quality of extracted volatile oil. The aim of this study was to develop the optimum conditions of various kinds of local animal fats and use them to extract oil from *Michelia alba DC*. Animal fats used in this study were fats from pigs, cattle, and buffalos, all of which were extracted using dichloromethane. According to the results, buffalo fat is the best material for enfleurage because of its colorless and smooth texture which provides optimum rigidity. Buffalo fats extracted with dichloromethane in the ratio of 3:2 (w/v) for 10 days and later soaked in ethanol for 7 days showed the best yield of purified fats. In addition, the extraction of buffalo fat by the aforementioned method is easy to perform since it is highly available at very low cost and requires only a simple preparation method.

Key words : **Enfleurage, animal fat, essential oil, *Michelia alba***

Introduction

Aromatherapy is the practice of using the essential oils from any of a variety of plants in order to bring about certain changes in the body or the mood[1] of a subject or patient. Essential oils that are inhaled into the lungs are believed to offer both psychological and physical benefits; not only does the aroma of the natural essential oil stimulate the brain to trigger a reaction, but the natural constituents (naturally occurring chemicals) of the essential oil are drawn into the lungs and can also supply physical benefit.[2] An essential oil is a liquid that is generally distilled (most frequently by steam or water) from the leaves, stems, flowers, bark, roots, or other components of a plant. Essential oil extract is one of the essential products in perfume, aromatherapy and the spa business around the world, including Thailand.[3] In Thai traditional medicine, essential oils are wildly used as massage oil to initiate deeper breathing and relaxation response, improve posture and joint flexibility, increase circulation and lymph drainage and free energy flow through the body and mind.[4]

There are a number of methods used to obtain essential oils from plants, such as steam distillation, solvent extraction and enfleurage. Enfleurage is the process of extracting the aromatic soul of a plant (usu-

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ally a flower) in the gentlest way possible, using large framed glass trays of solid fats and animal fats such as lard. The blossoms are slowly persuaded to give up their scent as they die, and are replaced daily by other freshly picked blossoms until the fat is saturated with the flower's essence.[5] Despite the simplicity of the method and the quality of essential oils obtained, enfleurage currently involves high costs due to the cost of animal fats, such as spermaceti and lanolin (wool fat). For this reason, other techniques such as steam distillation and solvent extraction are much more popular than enfleurage. Enfleurage is still used for delicate flowers with a very low content of essential oils such as jasmine and tuberose.[6] However, if there were a cheaper animal fat that could used to adsorb flower fragrance instead of the present costly ones, the enfleurage method would become much more practical in Thailand and could be adapted to extract the essential oils from various aromatic plants in Thailand.

Objectives

The major objective of this study was to develop the animal fats readily available in Thailand as a replacement for spermaceti and lanolin. The study also was aimed at developing the whole process to extract and utilize animal fats using the enfleurage method. Blossom flowers of *Michelia alba* DC were the raw materials for volatile oil extraction using the animal fats developed for this study.

Materials and Methods

This experiment utilized the various kinds of readily available animal fats in Khon Kaen Province (pig fat from *Sus scrofa*, bovine fat from *Equus caballus*, and buffalo fat from *Bubalus bubalis*). Pig fat was collected exclusively from the spinal cord where the fat was more solid and less sticky. Then, the fat was cleaned and the sticky and liquid part was removed and sliced into small pieces (Figure 1A).

The other two types of fat were both collected from the testicle area of bulls where the fat was solid and white. The bovine fat was opaque and had an uneven white color (Figure 1B) whereas the buffalo fat was a homogeneous white cream color (Figure 1C). Both types of animal fat were cleaned, with the

Fig. 1 A Sliced pig fat
1 B Bovine fat
1 C Buffalo fat

fascia removed and kept for the next process.

A series of preliminary studies were conducted in order to find the animal fat most effective for extraction in terms of ratio and duration. The protocol of the study was adapted from the previous studies of essential oil extraction using purified animal fats.[7-10]

Experimental

1. Preliminary extraction study using dichloromethane

Sliced pig fat, bovine fat and buffalo fat weighing 100 g each were separated and transferred to 250 ml glass bottles; 100 ml of dichloromethane was added to each bottle, and the bottles were tightly sealed and kept 7 days for extraction. The experiment was conducted in triplicate. The dichloromethane layer of each fat sample was separately collected and transferred to aluminum trays and exposed to the sun during the day and evaporated overnight in a fume hood. After the dichloromethane was completely evaporated, the solidity, color, and texture of each extract of fat were carefully evaluated. Extracted animal fat with optimum solidity, color and texture was selected for the next study.

2. Determination of the optimum extraction ratio between fat and dichloromethane

The animal fat that showed the best characteristics in the preliminary study was selected for the study of optimum extraction ratio. The selected animal fat was extracted with dichloromethane in several ratios ranging from 1:1 (30 g fat: 30 ml solvent), 1:2 (30 g fat: 60 ml solvent), 1:3 (30 g fat: 90 ml solvent), 2:1 (60 g fat: 30 ml solvent), 2:3 (60 g fat: 90 ml solvent), 3:2 (90 g fat: 60 ml solvent) and 3:1 (90 g fat: 100 ml solvent). All of the extraction ratios were conducted in triplicate using 250 ml glass bottles. The bottles containing the animal fats and the corresponding solvent volume were left at ambient temperature for 10 hours.

Two important factors used to select the optimum extracting ratio were the percentage yield of purified fat and waste product. The stinky yellow layer containing the contaminated and dirty portion of fat occurred during the extracting process was considered as a waste product, and thus discarded.

At the end of the extraction period, the animal fat and the clean dichloromethane layer were transferred to a 500 ml beaker, with the dichloromethane layer being collected first. Then, a glass stirring rod was used to press the dichloromethane portion remaining in the animal fat and later combined with the previous layer. The total volume of dichloromethane layer was recorded and then it was transferred to the aluminum tray. The dichloromethane was removed by evaporating process in the same manner as in the previous experiment to yield the extracted fat. The extracted fat was weighed and recorded; it was divided by the original fat weight to give the percentage yield (by weight). The percentage yield of waste product was also determined by collecting the yellow layer and evaporated it in a fume hood. Only the ratio that resulted in the highest average yield of extracted fat and the lowest yield of waste product was selected for the next study.

3. Determination of the optimum duration for extracting fat

The study period to determine the optimum duration for extracting fat ranged from 1 to 30 days. The whole study was divided into three parts, each using different amounts of animal fat (30 g, 100 g and 200 g respectively). The optimum ratio selected from the previous study was a factor to determine the volume (ml) of dichloromethane used in this study.

In the first part, 30 g of selected animal fat was extracted with the corresponding volume of dichloromethane. Samples for each day were conducted in triplicate; therefore, a total of 150 samples were required during the 30-day study period. All samples (30 g for each) were transferred to 250 ml glass bottles, and the dichloromethane was added and left at ambient temperature for 1 day. The dichloromethane layer from each sample was collected the next day and later transferred to the aluminum tray and processed in the same manner as in the previous study.

The second and third parts of the study were conducted by using a larger amount of animal fat: 100 grams and 200 grams of selected animal fat,

using the exact procedure as in the study of 30 g of animal fat.

The percentage of purified fat from the three samples from each extraction period was calculated from day 1 to day 30 on the same basis as that of the previous study.

The duration that resulted in the highest average percentage of extracted fat would chosen as the optimum extracting period. All extracted fat samples were soaked with 95 percent ethanol for preservative purposes and kept for further study.

4. Essential oil extraction by enfleurage method using purified animal fat

The optimum animal fat, the extraction ratio and extraction duration determined by the previous study was used to prepare extracted fat for the enfleurage method. Purified fat (100 g) was weighed and smeared onto two glass bowls (20 centimeters in diameter); 100 g of fresh *Michelia alba* flower was put into the first bowl and then another bowl was overturned to serve as a lid covering the first bowel. The enfleurage bowl set was left overnight. On the following morning, a new set of 100 g of fresh *M. alba* flowers replaced the old one and the same process was repeated for 14 consecutive days. At the end of extraction process, the buffalo fat saturated with fragrance was then scraped out and transferred to a clean bottle; 100 ml of cooled ethanol was added to the bottle, left overnight, and then filtered to separate the ethanol extract from the fat. The aforementioned method was repeated until there was no fragrance left in the buffalo the 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 the analysis of its chemical composition.

Result and Discussion

Preliminary extraction study using dichloromethane

According to the study, the extracted pig fat appeared as a liquid in the dichloromethane layer (Figure 2A). After dichloromethane was completely evaporated, extracted pig fat appeared as a white viscous semi-solid (Figure 2B). Extracted bovine fat appeared as a white solid stain interspersed with yellow oil droplets (Figure 3A) and a yellow-white solid with intermittent yellow droplets (Figure 3B) after the solvent was removed. Extracted buffalo fat appeared as a thick, white homogenous semi-solid before the solvent was evaporated (Figure 4A) and a white homogenous solid with wax-like texture after the solvent was evaporated (Figure 4B). The details of the three extracted fats are provided in Table 1.

According to the overall physical properties of extracted fat, buffalo fat was the most satisfactory one because of its texture and solidity. Buffalo fat was therefore selected for further studies regarding the optimum extraction ratio and optimum extraction

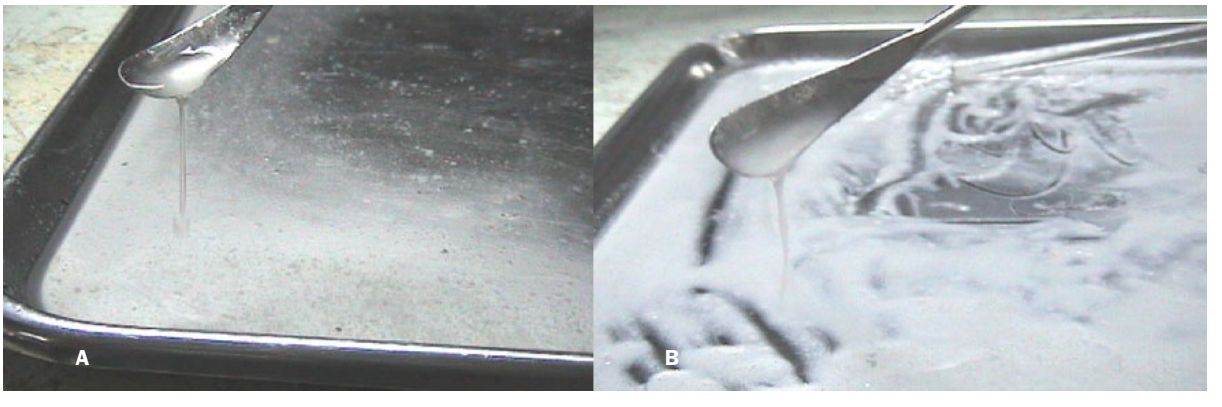

Fig. 2A Extracted pig fat in dichloromethane layer
2B Purified pig fat

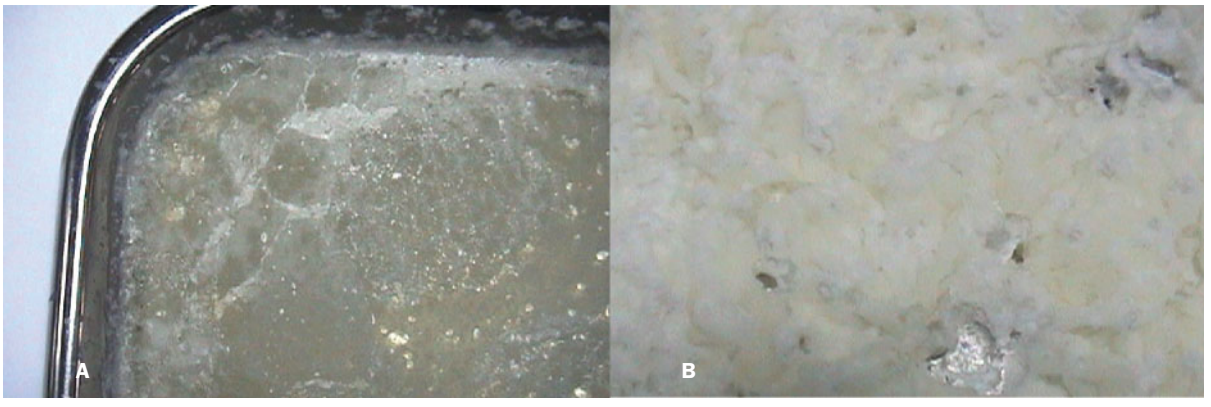

Fig. 3A Extracted bovine fat in dichloromethane layer
3B Purified bovine fat

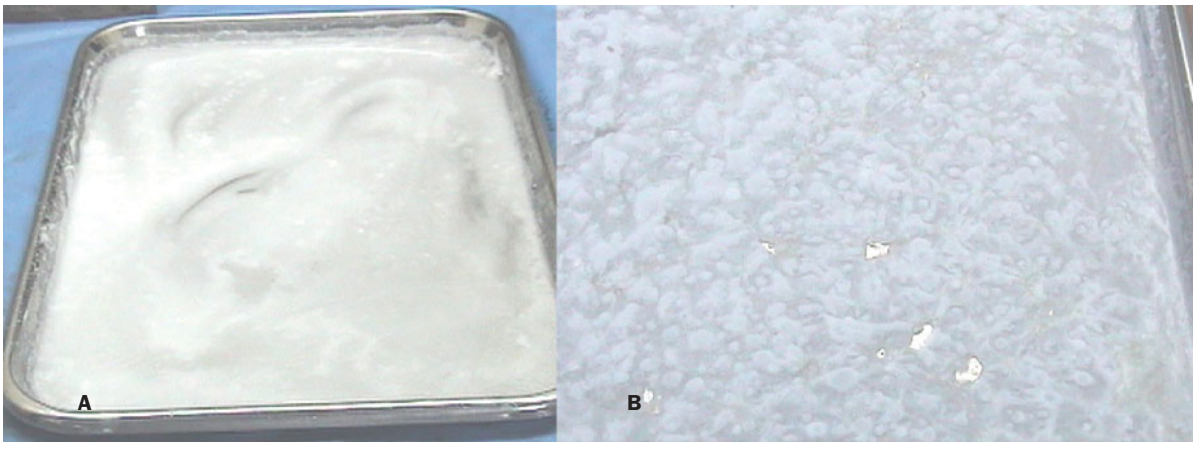

Fig. 4A Extracted buffalo fat in dichloromethane layer
4B Purified buffalo fat

Table 1 Physical properties of extracted animal fats

Type of fat	Color	Physical appearance	Odor
Pig fat	Opaque white	White semi-solid with low viscosity	Fresh meat smell
Bovine fat	White-yellow	White solid Candle-like texture mixed with yellow droplets of oil	Nearly odorless with slight meaty smell
Buffalo fat	Overall white	White solid Candle-like texture	Slightly stronger odor than bovine fat

period.

Determination of the optimum extracting ratio between fat and dichloromethane

According to this study, the ratio of fat and dichloromethane that resulted in the highest yield of purified buffalo fat was a 1:3 ratio (10.43 ± 0.40% yield) followed by a 1:2 ratio (9.30 ± 0.26% yield) and a 3:2 ratio (8.71 ± 0.02% yield), respectively (Table 2). However, another crucial factor used for choosing the optimum ratio was the yield of waste product which should be as low as possible. According to the study,

Table 2 Percentage yield of extracted fat and waste product extracted with various ratios of buffalo fat and dichloromethane

Ratio of buffalo fat (g) per volume of dichloromethane (ml)	Percentage yield of purified fat (average percentage ± standard deviation)	Percentage yield of yellow oil (waste product) (average percentage ± standard deviation)
1:1	8.23 ± 0.11	7.88 ± 0.24
1:2	9.30 ± 0.26	9.33 ± 0.15
1:3	10.43 ± 0.40	10.98 ± 0.38
2:1	7.56 ± 0.21	6.81 ± 0.10
2:3	7.93 ± 0.24	9.57 ± 0.19
3:1	6.93 ± 0.29	7.24 ± 0.22
3:2	8.71 ± 0.02	5.74 ± 0.03

fat and dichloromethane in the ratio of 1:3 and 1:2 gave a high yield of waste yellow oil 10.98 ± 0.38 percent and 9.33 ± 0.15 percent, respectively. Fat and dichloromethane in the ratio of 3:2, on the other hand, showed the lowest yield of waste product (5.74 ± 0.03%).

Overall, the higher volume of dichloromethane resulted in a higher yield of purified fat as well as waste product, which was as much as the desired product. From this result, the 1:3 and 1:2 ratios are not quite the effective ratio. Therefore, an extraction ratio of 3: 2 was selected as the optimum ratio because it produced the lowest yield of waste product and a comparably high yield of purified fats.

Determination of the optimum duration for buffalo fat extraction

Buffalo fat weighing 30 g, 100 g and 200 g. were extracted with 20 ml, 67 ml and 134 ml amounts of dichloromethane, respectively, to get the ratio of 3:2 (grams of fat/volume of dichloromethane), which was the optimal selected ratio from the previous study.

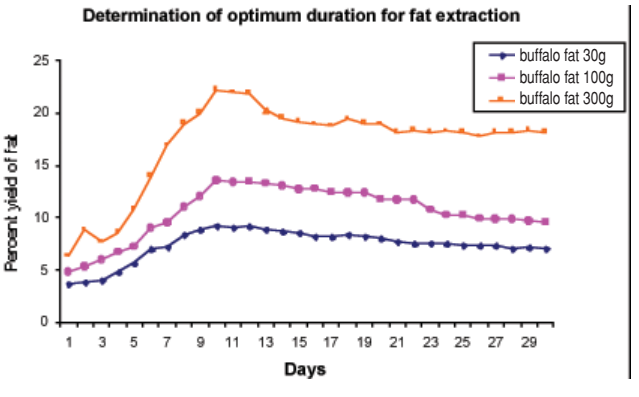

Fig. 5 Determination of optimum duration for fat extraction

According to the results, the percentage yield of fat extracted from day 1 to day 30 was of a similar pattern. Ten days was the optimum duration to give the highest yield of fat for extraction of buffalo fat in 30 g, 100 g and 200 g quantities. After day 10, the percentage yield of extracted fat declined (Figure 5). Therefore a 10-day period prased to be the optimum duration for animal fat extraction using dichloromethane as the solvent.

Essential oil extraction by enfleurage method using the purified buffalo fat

Buffalo fat, with a 3:2 extraction ratio (weight of fat/volume of solvent) and 10-day extraction period determined by the previous preliminary study, was processed to yield purified fat. The process was repeated several times in order to yield an adequate amount of product. *Michelia alba* flowers were bought from Sompong Garden House and Sila House, Tumboon Sila, Aumpher Mueng, Khon Kaen Province, Thailand in February 2007.

Ethanol was used to soak 100 g of extracted fat, as previously described, for another 7 days in order to get rid of all of the left over scent and then dried in a fume hood. Then the 100 g of purified fat was used to extract essential oil from *M. alba* flowers by the enfleurage method, as described in the Materials and

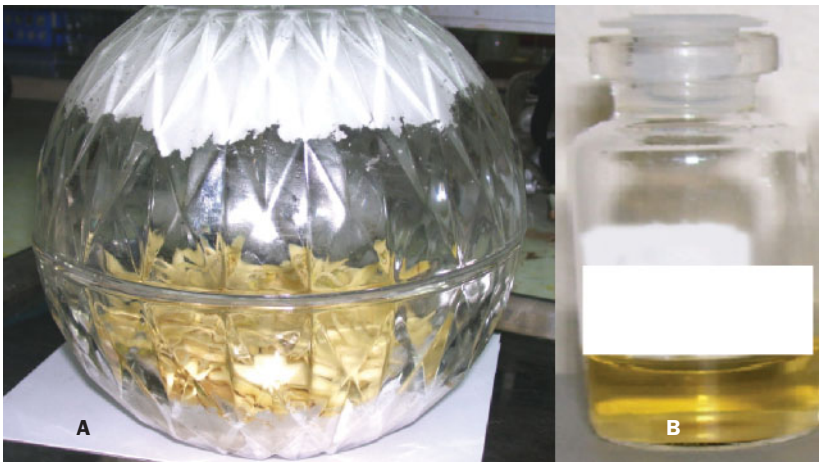

Fig. 6A Extraction of essential oil from *Michelia alba* flowers
6B Essential oils extracted from purified buffalo fat.

Methods section. The extraction of essential oils from the *M. alba* flowers, using purified buffalo fat and essential oil product, is shown in Figure 6A-B. The essential oil appeared as a light yellowish solution with an odor similar to that of fresh *M. alba* flowers.

Conclusion

The current study developed a process to prepare extracted fat for the enfleurage method. The enfleurage method in this study was adapted from the traditional method by using local animal fat to adsorb the fragrance instead of spermaceti or lanolin. According to the results, buffalo fat is an engaging material for enfleurage methods because of its colorlessness and optimum rigidity compared with pig fats and bovine fats. The extracting ratio of 3:2 (weight of buffalo fat [g] per volume of dichloromethane [ml]) was successfully applied to extract buffalo fat over the 10-day period. In addition, extracted buffalo fat was also successfully utilized to extract an essential oil from the blossoms the of *Michela alba* DC. The general appearance of the essential oil (odor and color) was highly satisfactory. To the best of our knowledge, there is no previous study regarding the use of buffalo fat as the material for the enfleurage method. Buffalo fat is considered as a waste product of slaughter houses and rarely used in Thailand. Therefore, the development of buffalo fat for the enfleurage method is a very interesting idea to increase the value of the waste product. The enfleurge method using buffalo fat as raw material may be a new alternative technique to extract high quality fragrances from flowers in Thailand.

Acknowledgement

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บทคัดย่อ

การพัฒนาไขสัตว์ในท้องถิ่นเพื่อใช้เสกัดน้ำมันหอมเพื่อใช้สำหรับสุคนธบำบัด

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**ภาควิชาเภสัชพฤกษศาสตร์และเภสัชวินิจฉัย คณะเภสัชศาสตร์ มหาวิทยาลัยขอนแก่น*

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การสกัดน้ำมันหอมวิธีอองเฟอราจเป็นภูมิปัญญาท้องถิ่นของชาวฝรั่งเศสโดยใช้ไขมันดูดซับน้ำมันหอมจากดอกไม้. กล่าวกันว่าน้ำมันหอมที่ได้จากการสกัดด้วยวิธีนี้ให้น้ำมันหอมที่มีกลิ่นคล้ายดอกไม้สดมากที่สุด ซึ่งการสกัดน้ำมันหอมด้วยวิธีอองเฟอราจนั้นขั้นตอนที่สำคัญอย่างยิ่งคือการเตรียมไข เนื่องจากคุณภาพและปริมาณของผลผลิตขึ้นกับคุณภาพของไขที่ใช้สกัด. งานวิจัยนี้มีวัตถุประสงค์เพื่อนำไขสัตว์ที่หาได้ง่ายในท้องถิ่น หรือมีในประเทศไทยมาพัฒนา เพื่อนำไปใช้สกัดน้ำมันหอมจากดอกจำปีด้วยวิธีอองเฟอราจโดยการสกัดไขด้วยไดคลอโรมีเธน ซึ่งไขมันสัตว์ที่ใช้ในการทดลองนี้คือ ไขมันหมู, ไขมันวัว และไขมันควาย, พบว่าไขมันควายเป็นไขที่เหมาะสมที่สุด เนื่องจากเป็นไขที่ไม่แข็งและไม่เหลวเกินไป สีขาวสะอาด ปราศจากกลิ่น โดยการสกัดไขมันควายใช้อัตราส่วนระหว่างน้ำหนักไขมันควายกับปริมาตรของไดคลอโรมีเธนคือ อัตราส่วน ๓ : ๒ ระยะเวลาในการสกัด ๑๐ วัน จะได้ร้อยละของผลิตภัณฑ์สูง และมีปริมาณผลิตภัณฑ์ที่ไม่ต้องการต่ำ นำไขที่ได้ไปแช่ในเอธานอล ๗ วัน จะได้ไขที่ขาวสะอาด ปราศจากกลิ่น, นำมาสกัดน้ำมันหอมจากดอกจำปีได้จริง. ดังนั้นไขมันควายซึ่งเป็นไขมันที่หาได้ง่ายในประเทศไทย สามารถนำมาเตรียมเพื่อใช้สกัดน้ำมันหอมจากดอกไม้, วิธีการเตรียมเป็นวิธีที่ทำได้ง่าย ราคาถูกเพราะจากส่วนที่นำมาใช้สกัดเป็นไขมันที่ไม่ได้นำมาใช้ประโยชน์ เนื่องจากหากมีผู้สนใจนำความรู้พื้นฐานนี้ไปประยุกต์ในระดับอุตสาหกรรมขนาดย่อมก็สามารถทำได้ง่าย ซึ่งจะเป็นผลดีในอนาคตต่อไป.

คำสำคัญ : **อองเฟอราจ, ไขมันควาย, น้ำมันหอม, จำปี**