การสกัดแยกน้ำมันหอมระเหยและสารหอมจากดอกพุด

Essential Oil and Scents Extraction from *Gardenia jasminoides* J. Ellis Flowers

อุดมลักษณ์ สุขอัตตะ[1], อุไรวรรณ ดิลกคุณานันท์[1], สรัญญา วัชโรทัย[2],
วิชัย หฤทัยธนาสันติ์[3],พจมาน พิศเพียงจันทร์[1], พุฒิตา พันจี[1], ประภัสสร รักถาวร[1]

Udomlak Sukkatta[1],Uraiwan Dilokkunanant[1], Srunya Vajrodaya[2],
Vichai Haruethaitanasan[3], Potechaman Pitpiangchan[1], Putthita Punjee[1], Prapasson Rukthaworn[1]

บทคัดย่อ

การศึกษาการสกัดน้ำมันหอมระเหยและสารหอมจากดอกพุดด้วยวิธีการกลั่นสองแบบ คือ กลั่นด้วยน้ำและไอน้ำ และกลั่นด้วยไอน้ำ การสกัดด้วยไขมันคือ ไขมันเย็นและไขมันร้อน และการสกัดด้วยตัวทำละลายอินทรีย์สองชนิด คือ เฮกเซน และปิโตรเลียมอีเทอร์ และเปรียบเทียบองค์ประกอบทางเคมีของน้ำมันหอมระเหยและสารหอมจากดอกพุดโดย GC-MS พบว่า จากการกลั่นทั้งสองวิธีได้น้ำมันหอมระเหย 0.0200 และ0.0026 เปอร์เซ็นต์ ตามลำดับ ส่วนการสกัดด้วยไขมันเย็น ไขมันร้อน เฮกเซน และปิโตรเลียมอีเทอร์ สามารถสกัดสารหอมที่เรียกว่า คอนกรีตและแอบโซลูตได้ 0.2404, 12.3724, 0.0600 และ 0.0446 เปอร์เซ็นต์ ตามลำดับ สารองค์ประกอบหลักที่พบในน้ำมันหอมระเหยจากดอกพุดคือ (Z)-3-hexenyl tiglate และ linalool แอบโซลูตที่สกัดได้จากไขมันร้อนและไขมันเย็นมีองค์ประกอบหลักคือ methyl benzoate ส่วนองค์ประกอบหลักของแอบโซลูตที่สกัดได้จากเฮกเซน และปิโตรเลียมอีเทอร์ คือ (Z)-3-hexenyl tiglate

ABSTRACT

Study on essential oil and scent extraction from gardenia flower by using distillation method that were water and steam distillation and steam distillation, enfleurage extraction that were cold and hot enfleurage and solvent extraction with hexane and petroleum ether. The chemical composition of essential oil and scent extract were determined with Gas chromatography-mass spectrometry (GC-MS). The result showed that percentage yield of oil obtained by water and steam distillation and steam distillation were 0.0026 and 0.0200% v/w (gram fresh weight), absolutes getting from cold enfleurage, hot enfleurage, hexane and petroleum ether extractions were 0.2438%, 12.5904 %, 0.0600% and 0.0446%, respectively. The main chemical compounds of gardenia oil were (Z)-3-hexenyl tiglate and linalool. Main chemical components detected in gardenia absolutes getting from enfleurage extraction was methyl benzoate, while (Z)-3-hexenyl tiglate were found to be the main chemical components in absolutes extracted by hexane and petroleum ether.

Keywords: Gardenia, essential oil, absolute enfleurage, solvent extraction, chemical composition

Email-address : aapuls@ku.ac.th

[1] หน่วยปฏิบัติการเทคโนโลยีสมุนไพรและความหลากหลายทางชีวภาพ สถาบันผลิตผลเกษตรฯ มหาวิทยาลัยเกษตรศาสตร์
Herb and Biodiverstiy Technology Unit, KAPI, Kasetsart University

[2] ภาควิชาพฤกษศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยเกษตรศาสตร์
Department of Botany, Faculty of Science, Kasetsart University

[3] ภาควิชาพัฒนาผลิตภัณฑ์ คณะอุตสาหกรรมเกษตร มหาวิทยาลัยเกษตรศาสตร์
Department of Product Development, Faculty of Agroindustry, Kasetsart University

INTRODUCTION

Gardenia jasminoides J. Ellis (commonly known as Gardenia angusta (L.) Merr.) is in family Rubiaceae and also simply known as Gardenia and Cape Jasmine, in Thailand called "Pudson". It is a woody shrub and evergreen tropical plant with fragrant flowers and handsome foliage. This plant is a native of East Asia and widely cultivate as a garden ornamental in tropical regions or in temperate greenhouses (Koo *et al*., 2006; Staples and Herbst, 2005). Flowers of Gardenia are waxy, white and fragrant with sweet smell like some jasmine flowers. Flowering occurs over a long period of time, from May to July. The cut flowers used as decorations and in corsages by florists. (Koo *et al*., 2006; Criley *et al*., 2008). Flower of Gardenia have potential as a source of natural perfume. Gardenia essential oil is one of the most precious oils in the world. The aroma and fragrance are usually used as aromatherapy product, absolute of gardenia is used extensively by the natural perfume industry (Power, 2010). There are many methods of gardenia oil and absolute extractions depend on the objective for used. The objective of this work would like to compare the yield of gardenia oil, percentage of absolute, chemical component of gardenia oil and absolutes obtained by distillations, effleurage extraction and by solvent extraction from gardenia flowers.

MATERIALS AND METHODS

Sample preparation

Gardenia flowers were bought from Pak Klong Talad market, Bangkok, Thailand. The flowers were carefully cut from the stem and separated petels form receptacle gardenia flower.

Extraction by distillation

Five hundred grams of fresh *Gardenia* flowers were extracted for four hours by using each of two methods; steam distillation and water and steam distillation. (Boutekedjiret *et al*, 2003).The final essential oils were separated by sodium sulphate anhydrous and kept at 4 ^{o}C until GC-MS analysis.

Extraction by Enfleurage (applied from Pensuk *et al*., 2007; Paibon *et al*., 2010)

Cold Enfleurage

A layer of fat palm stearin was spread on a plate of glass. Petals of gardenia were carefully placed on top of the layer of fat; they are placed as close together as possible. Then glass-fat-flower was placed on top of the flower material as the sandwich and must set at room temperature for one day. At the end of one day, the sandwich was carefully dismantled and the used flowers are removed. Fresh flower are then placed. The process was repeated until the fat was saturated with scent of the flowers. Six ratios of flowers and the palm stearins : 1,000, 1,500, 2,000, 2,500, 3,000 and 3,500 g/200 ml, were studied the optimum point of absorbing the flowering scents. The scented fat was mixed with alcohol for separate aromatic substances and removed the solvent by vacuum evaporator to leave the absolute.

Hot Enfleurage

The optimum ratio of flowers and palm oil were studied in the high yield condition were studied. Four hundred milliliters of palm oil was heated up to 60 °C for 30 min. Six treatments of 150, 200, 250, 300, 350, and 400 g flowers/ 400 ml of palm oils were investigated. The mixture was cooled down and kept in refrigerator overnight. The oil was agitated for several days. The resulting oil was filtered and was extracted by alcohol to obtain the absolute.

Extraction by solvent

Hexane and petroleum ether were used to extracting the scents from gardenia flowers. Six treatments of 50, 60, 70, 80, 90 and 100 g flowers were soaked with 1 liter of each solvent on 30 minutes. Removing the residue flowers and removing the solvent were done by rotary evaporator to obtain concrete. Gardenia absolutes were separated from concrete by alcohol. The optimum ratios between flowers and solvents were determined (Pensuk *et al*., 2007).

The comparison of yield extracts from each methods of enfleurages and solvents extraction were assessed. The saturated point of fat, oil and solvent in order to absorb the scents from gardenia were determined. In addition physical appearances such as characteristics, color and scent of all extracts were observed.

GC-MS analysis

GC-MS analyses were performed with a Shimadzu QP 5050A gas chromatograph equipped with a DB-5 capillary column (60 m x 0.25 mm; coating thickness 0.25 µm). Analytical conditions were injector and transfer line temperatures 250 and 250°C, respectively, programmed from 60°C (isothermal for 3 min), with an increase of 3°C min^{-1}, to 120°C and then increase of 4°C min^{-1}, to 200°C; carrier gas helium at 1.0 ml/min, split ratio 1:7. The components were identified by computer matching of their mass spectral fragmentation patterns with those stored in the spectrometer database using the National Institute of Standards and Technology Mass Spectral database.

RESULTS AND DISCUSSIONS

Extraction by distillation

The percentage yield of the essential oil of *G. jasminoides* flowers from steam distillation and water and steam distillation method were 0.0026, 0.0200% v/w (gram fresh weight) respectively (Figure 1). Water and steam distillation gave higher oil yield than steam distillation. Extraction of gardenia flowers by distillation method gave small amount of oil yield. Although the distillation method has traditionally been applied for the recovery of essential oils from plant materials. One of the disadvantages of the distillation method is that essential oils undergo chemical alterations and the heat sensitive compounds can easily be destroyed resulted in low quantity of oil yield (Meshkatalsadat *et al*, 2010) Oils obtained by both distillation methods were deep yellow liquid with gardenia flower odor (Table 1). Nine and six constituents of gardenia oil obtained from water and steam distillation and steam distillation have been identified by using GC-MS, in which (*Z*)-*3*-hexenyl tiglate and linalool are the main components of gardenia oil. Chemical compositions of these oil from different method were mostly similar, whereas relative concentration of the identified compounds is apparently different. The (*Z*)-*3*-hexenyl tiglate from gardenia oil which extracted by steam distillation was higher percentage (65.68%) than the yield from water and steam distillation (54.82%) (Table 1). This data correlate with those of Chaichana *et al*. (2009) they reported that main composition of gardenia oil were linalool, *alpha*-farnesene, *z*-3-hexenyl tiglate and *trans*-*beta*-ocimene.

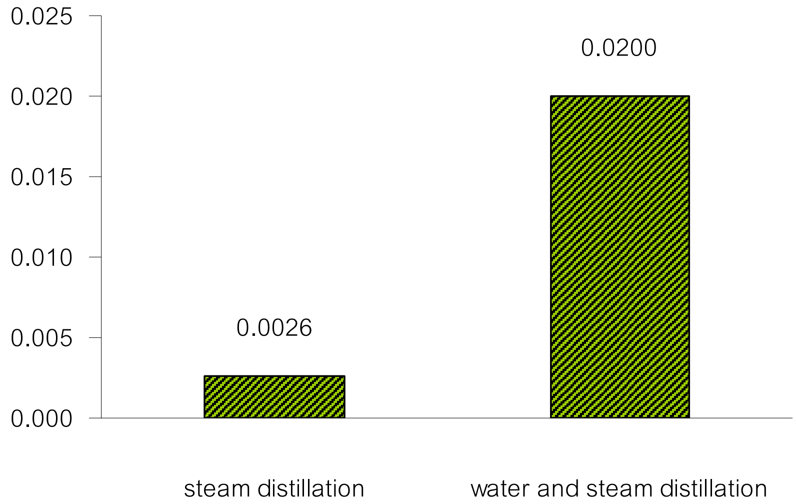
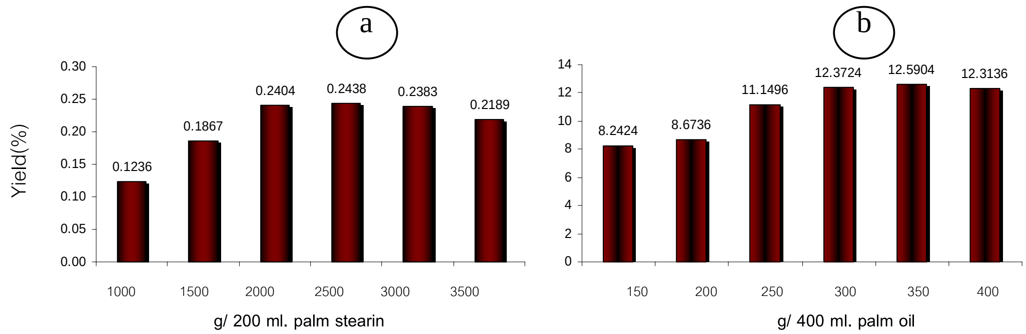


Figure 1 Gardenia oils getting from distillation method

Extraction by enfleurage

In the enfleurage extraction process the scent from the gardenia flower petals slowly migrate to the palm wax and plam oil until the palm wax and plam oil is completely saturated with the scent of the gardenia. The cold enfleurage extraction of gardenia showed that the saturated point of palm stearin wax was 2500 g flowers/200 ml and its yield was 0.2438% (Figure 2a). While the ratio 350g/400 ml was the optimum ratio of flower and plam oil in hot enfleurage and its yield was 12.5904 %. The results indicated that hot enfleurage gave higher yield of gardenia scents than cold enfleurage. This probably due to absolutes from both methods contained palm wax and palm oil which was extracted by ethanol used in the process. The wax could be removed by filtrating or centrifuge (Pensuk *et a*l., 2007) while the oil was hard to remove effected to high yields of absolute from hot enfleurage. The absolute of gardenia obtained from cold enfleurage was yellow oil with sweet odor like natural gardenia flower while absolute of hot enfleurage gave orange oily substance with a little pungent odor. The chemical constituents of gardenia absolute were detected (Table 2 and Figure 2). There were 4 and 2 compositions in absolute from cold and hot enfleurage. The major compound of both absolutes were ocimene, methyl benzoate, (Z)-3-hexenyl tiglate and alpha-farnesene. It was found that (Z)-3-hexenyl tiglate and alpha farnesene were appear only in the cold enflerage.



Fingure 2 Gardenia absolute getting from cold (a) and hot (b) enfleurage extractions

Extraction by solvent

Patals of gardenia were extracted by using maceration technique with hexane and petroleum ether. The result showed that the optimum ratio of hexane extraction was 70 g flower/l the percentage yields of hexane concrete and absolute were 0.0813 and 0.0600%, respectively (Figure 3a). The saturated point of Petroleum ether to extract the scent from gardenia was 60 g flower/l and percentage yield of petroleum ether concrete and absolute were were 0.0562 and 0.0446%, respectively (Figure 3b). The characteristic of gardenia absolute from hexane extraction was waxy substance, reddish brown color and sweet odor. Petroleum ether extraction gave pink waxy substance with sweet and strong gardenia odor. The chemical constituents of gardenia absolute from hexane and petroleum ether extraction were ten and three major compounds. (Figure 4 and Table1) The main constituents of absolute from hexane and petroleum ether were methyl benzoate and (Z)-3-hexenyl tiglate (Table 1and Figure 4).

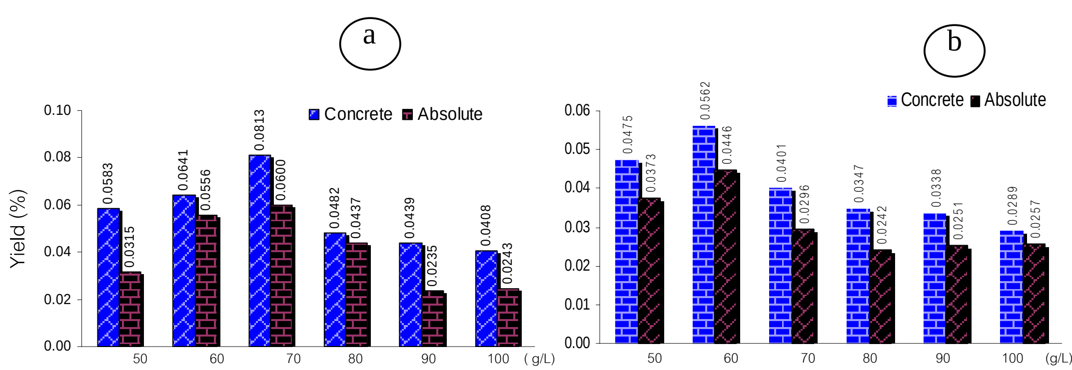


Figure 3 Concretes and absolutes getting from Hexane extraction(a) and Petroleum ether extraction (b)

Table 1 Chemical compositions of gardenia extraction from different methods.

Extraction method	Peak	Retention time	% Relative peak	Possible compounds
Water-steam	1	17.487	1.14	(*Z*) 6-octen-2-one
	2	18.211	1.33	2-isononenal
	3	18.558	3.18	methyl benzoate
	4	18.699	26.99	linalool
	5	23.039	4.39	alpha-terpineol
	6	28.543	54.82	(Z)-3-hexenyl tiglate
	7	28.704	4.79	6-tridecanol
	8	34.746	0.70	unknown[1]
	9	37.012	2.66	(*Z*)-3-hexenyl benzoate
Steam distillation	1	18.558	1.48	methyl benzoate
	2	18.677	18.91	linalool
	3	23.035	3.64	alpha-terpineol
	4	28.522	65.68	(Z)-3-hexenyl tiglate
	5	28.698	5.56	6-tridecanol
	6	37.010	4.74	(*Z*)-3-hexenyl benzoate
Hot enfleurage	1	16.246	34.43	ocimene
	2	18.567	65.57	methyl benzoate
Cold enfleurage	1	16.232	21.13	ocimene
	2	18.552	46.40	methyl benzoate
	3	28.494	29.29	(Z)-3-hexenyl tiglate
	4	34.921	3.18	alpha farnesene
Hexane	1	18.563	21.51	methyl benzoate
	2	18.681	8.82	linalool
	3	28.509	36.33	(Z)-3-hexenyl tiglate
	4	28.703	1.38	6-tridecanol
	5	30.897	1.86	tetradecanol
	6	34.616	3.78	7-deceno-5-olide
	7	35.185	7.39	2,4-di-tert-butylphenol
	8	37.018	2.51	(*Z*)-3-hexenyl benzoate
	9	37.392	9.58	nonadecanol
	10	42.954	6.83	octadecyl vinyl ether
Petroleum ether	1	18.586	12.42	methyl benzoate
	2	28.503	80.45	(Z)-3-hexenyl tiglate
	3	34.934	7.13	beta-farnesene

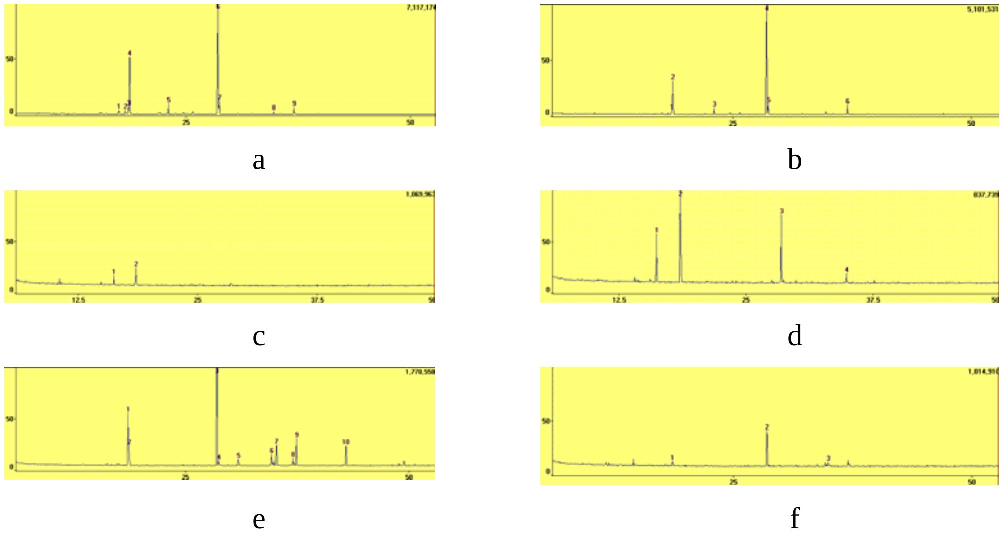


Figure 4 Chromatogram of gardenia extraction from water and steam distillation (a), steam distillation (b), cold enfluerage (c), hot enfluerage (d) hexane extraction (e) and petroleum ether extraction (f)

CONCLUSION

Percentage yield of oil from water and steam distillation and steam distillation methods were 0.0026 and 0.0200% v/w (gram fresh weight). The absolutes from cold enfleurage, hot enfleurage, hexane and petroleum ether extractions were 0.2438%, 12.5904 %, 0.0600% and 0.0446%, respectively. The main chemical compounds of gardenia oil were (*Z*)-3-hexenyl tiglate and linalool. Main chemical gardenia absolutes components from enfleurage extraction was methyl benzoate and the (*Z*)-3-hexenyl benzoate was found in main chemical components of absolutes with extraction by hexane and petroleum ether.

ACKNOWLEDGEMENT

This work was financially supported by Kasetsart University Research and Development Institute (KURDI) and Kasetsart Agricultural and Agroindustrial Product Improvement Institute

REFERENCES

Boutekedjiret, C., F. Bentahar, R. Belabbes and J. M. Bessiere. 2003. Extraction of rosemary essential oil by steam distillation and hydrodistillation. **Flavour Fragr. J.** 18: 481–484

Chaichana, J., W. Niwatananun, S. Vejabhikul., S. Somna and S. Chansakaow. 2009. Volatile constituents and biological activities of Gardenia jasminoides. **J. Health Res.** 23(3):141-145.

Criley, R.A., M.S. Roh, M. Kikuchi and R.M. Manshardt. 2008. A Comparison of *Gardenia augusta* Cultivars Using Isozymes and RAPD Markers. **Acta Hort.** 766:461-468.

Koo H.J., K.H. Lim, H.J. Jung, E.H. Park. 2006. Anti-inflammatory evaluation of gardenia extract, geniposide and genipin. **J. Ethnopharmacol.** 103:496-500.

Paibon, W., C.-A.Yimnoi, N. Tembab, W. Boonlue, K. Jampachaisri, N. Nuengchamnong N. Waranuch and K. Ingkaninan. 2010. Comparison and evaluation of volatile oils from three different extraction methods for some Thai fragrant flowers. Int. **J. Cosmet. Sci.** 1–9

Pensuk, W., T. Padumanonda and C. Pichaensoonthon. 2007. Comparison of the chemical constituents in *Michelia alba* flower oil extracted by steam distillation, hexane extraction and enfleurage method. **Journal of Thai Traditional & Alternative Medicine.** 5(1): 30-39.

Power, J. 2010. **Gardenia Essential.** http://www.aromatherapy-school.com/essential-oils/gardenia-essential-oil.html Oil, October 20, 2010

R.H.M.J. Wulijarni-Soetjipto, N. (Eds.): **Plant Resources of South-East Asia. No. 3: Dye and tannin-producing plants.** Prosea Foundation, Bogor, Indonesia. pp. 76-77.

Staples, G.W. and Herbst, D.R. 2005. **A Tropical Garden Flora.** Bishop Museum Press, 465 Honolulu, Hawaii USA.