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Extraction Methods for Tuberose Oil and Their Chemical Components

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ABSTRACT

The objectives of the project were to compare essential oil extraction methods from the double-flower variety of tuberose (*Polianthes tuberosa* L.). The flowers were extracted by cold or hot enfleurage, or by solvent extraction with hexane or petroleum ether. The chemical composition of the tuberose absolutes was analyzed by gas chromatography-mass spectrometry (GC-MS). The results showed that percentage yields of tuberose oil from cold enfleurage, hot enfleurage, hexane and petroleum ether extractions were 0.3137%, 6.5808%, 0.0279% and 0.0182%, respectively. The main chemical component detected in both enfleurage absolutes was methyl benzoate, while benzyl benzoate and pentacosane were found to be the main chemical components in hexane and petroleum ether absolutes, respectively.

Key words: *Polianthes tuberosa* L., essential oil, absolute, distillation, enfleurage, solvent extraction, chemical component

INTRODUCTION

The tuberose, *Polianthes tuberosa* L., is a tuberous perennial plant with a waxy, luminous white flower in the family Agavaceae. Its flower odor is very sweet, floral and honey-like and can help give emotional strength and center the mind. It is known to improve an individual's capacity for emotional depth and can stimulate the right side of the brain and bring serenity to the mind and heart. It also contains anti-inflammatory and antispasmodic properties (Maliga, 2003).

Tuberose flowers have long been used in perfumery as a source of essential oils and aroma compounds. These aromatics are synthesized in various plant organelles and as plant protection against herbivores and infection, as well as to attract pollinators (Dudareva and Negre, 2005). At present, the demand for volatile oil is expanding. Essential oils can be extracted using a variety of methods, such as hydro distillation and solvent extraction, although some are not commonly used today, such as cold and hot enfleurage extraction (Maliga, 2003).

The objectives of this work were to use tuberose flowers to compare the scent, percentage oil yield and chemical component of tuberose absolutes obtained by cold or hot enfleurage extraction and by solvent extraction using hexane or petroleum ether.

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MATERIALS AND METHODS

Flower preparations

Tuberose panicles were cut-off and put into a water tank to keep the flowers fresh. Freshly opened blossoms were collected every day, and weighed and subjected to extraction.

Extraction methods

Enfleurage: Cold and hot enfleurage processes were used in the tuberose oil extraction. In cold enfleurage, palm wax was heated to 80°C for 2 h and poured into rectangular glass trays, with 100 ml/tray. After the palm oil had cooled down and turned to wax at room temperature, tuberose flowers were put on the wax in each tray and covered with another waxed tray. The flowers were replaced with fresh flowers every 24 h. Six pairs of the trays with 1,000, 1,500, 2,000, 2,500, 3,000 and 3,500 g of tuberose flowers were studied as shown in Table 1. The floral scents in the wax (pomade) were extracted by ethanol and the ethanol was evaporated leaving the absolute de enfleurage behind. In hot enfleurage, tuberose flowers were put into 400 ml palm oil, which was warmed at 60°C. The flowers were warmed for 30 min and cooled down at room temperature. After leaving overnight at about 8-10°C, the palm oil was warmed up to 60°C again and the previous flowers were filtered out and replaced with new flowers. The steps were repeated using 300, 400, 500, 600, 700 and 800 g of flower in each treatment. The flower scent was extracted from the pomade by the same method used for the enfleurage extraction (Gupta, 1952).

Solvent Extraction: Hexane and petroleum ether were used to extract the scents from tuberose flowers because they are strongly non-polar solvents and are frequently used in solvents to extract oils. Six different weights of 100,150, 200, 250, 300 and 350 g of flowers were soaked in 1 L of each solvent for 1 h. After removing the debris, the solvents were evaporated leaving the concrete behind. Tuberose absolute was extracted from each concrete sample using alcohol. Physical appearances, such as color, odor and other characteristics were observed for all extracts. Yields of extracts from each method were assessed, compared, and used to determine the saturation point of fat, oil and solvent to absorb the scent from the tuberose flowers**.**

Chemical composition analysis

Absolutes in petroleum ether and in hexane 100 ppm were analyzed for their main chemical components by a Shimadzu QP5050A gas chromatography mass spectrometer.

Table 1 Tuberose flowers used in enfleurage extractions.

Extraction method	Tuberose flower weight (g)					
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Cold enfleurage (200 ml wax / treatment)	1,000	1,500	2,000	2,500	3,000	3,500
Hot enfleurage (400 ml oil / treatment)	300	400	500	600	700	800

Table 2 Tuberose flowers used in solvent extractions.

Extraction method	Tuberose flower weight (g)					
	Treatment 1	Treatment 2	Treatment 3	Treatment 4	Treatment 5	Treatment 6
Hexane extraction	100	150	200	250	300	350
Petroleum ether extraction	100	150	200	250	300	350

RESULTS AND DISCUSSION

Extractions

Enfleurage: Tuberose absolutes of both cold and hot processes were similar in appearance; they were sticky, waxy orange-brown oils. The absolute from the cold extraction was lighter in color than from the hot extraction. The saturation point of palm stearin (cold enfleurage) and palm oil (hot enfleurage) for the absorption of essential oil from tuberose flowers was 2,500 g flower/200 ml palm wax and 500 g flower/400 ml palm oil, respectively and the yield varied from treatment to treatment (Figure 1) with average yields of 0.3137% and 6.5808%, respectively. However, it has been noted that the saturation point of fat depends on the essential oil content in the flowers and properties of the fat (Pensuk *et al*., 2007).

The absolute yields from cold enfleurage were less than those from hot enfleurage in all treatments. This was probably due to the heat used in the extraction process, as the wax was able to extract a greater yield from tuberose petals than in the cold process (Alchemy Works, 2007). Moreover, absolutes from both methods contained palm wax and palm oil, which was extracted by ethanol used in the process. The wax was more easily removed from the ethanol than the palm oil (Pensuk *et al.,* 2007), which may have caused the difference in yields. However, the cold enfleurage scent was more similar to that of a fresh tuberose flower than the hot enfleurage absolute, because tuberose oil is extremely delicate and thus, heating the petals would destroy the most delicate components (Handa, 2005). Cold enfleurage absolute gave the scent most strongly reminiscent of natural tuberose flowers and was safe for use; it was appropriate for use in high-grade perfume materials, However, enfleurage had the disadvantage of being very labor-intensive and expensive.

Solvent extraction: The concretes and absolutes obtained from hexane and petroleum ether extractions appeared similar. Concretes were a yellow solid with a tuberose odor and absolutes were brownish yellow with a strong tuberose scent. The scent of tuberose absolute extracted from petroleum ether was closer to that of natural flowers than from hexane. The optimum amount of 150 g flower/l for hexane and petroleum ether extraction produced yields of 0.0279% and 0.0192%, respectively (Figure 2). Solvent extraction was more cost-efficient than the enfleurage process, and so was appropriate for

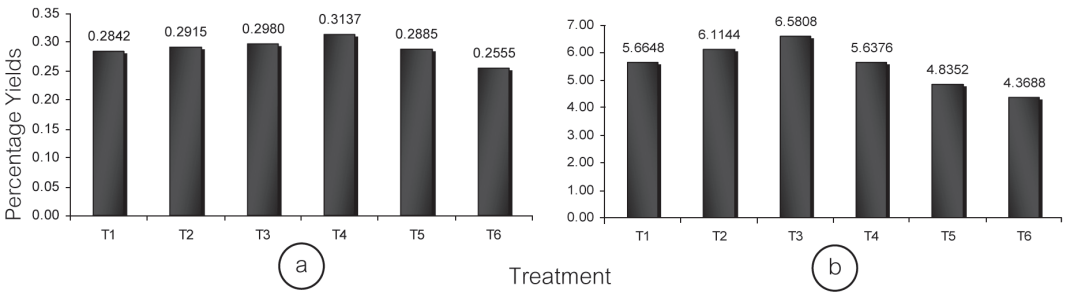

Figure 1 Yield of tuberose oil from cold (a) and hot (b) enfleurage extractions.

(a) Treatment 1 = 1000 g flowers/200 ml wax
Treatment 2 = 1500 g flowers/200 ml wax
Treatment 3 = 2000 g flowers/200 ml wax
Treatment 4 = 2500 g flowers/200 ml wax
Treatment 5 = 3000 g flowers/200 ml wax
Treatment 6 = 3500 g flowers/200 ml wax

(b) Treatment 1 = 300 g flowers/400 ml oil
Treatment 2 = 400 g flowers/400 ml oil
Treatment 3 = 500 g flowers/400 ml oil
Treatment 4 = 600 g flowers/400 ml oil
Treatment 5 = 700 g flowers/400 ml oil
Treatment 6 = 800 g flowers/400 ml oil

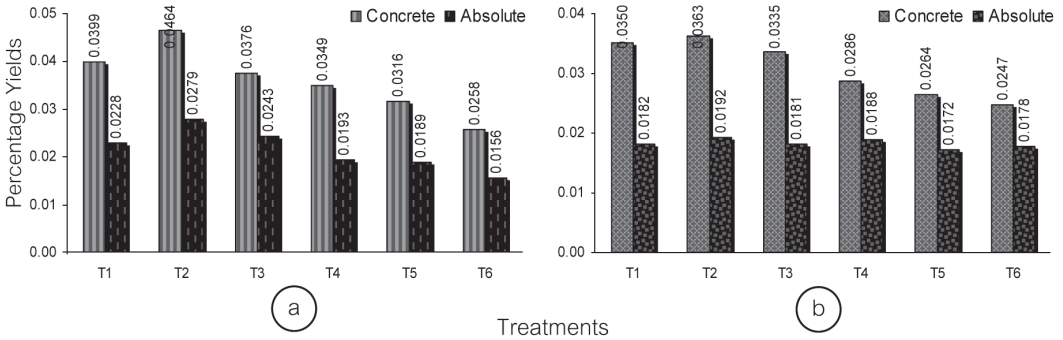

Figure 2 Tuberose concretes and absolutes obtained from hexane (a) and petroleum ether (b) extraction.
Treatment 1 = 100 g flowers/1,000 ml solvent
Treatment 2 = 150 g flowers/1,000 ml solvent
Treatment 3 = 200 g flowers/1,000 ml solvent
Treatment 4 = 250 g flowers/1,000 ml solvent
Treatment 5 = 300 g flowers/1,000 ml solvent
Treatment 6 = 350 g flowers/1,000 ml solvent

producing absolute on a pilot scale, but, this was not considered the best method for the extraction of essential oils, as the solvent was harmful and could leave a residue behind that could cause allergies and effect the immune system (Handa, 2005).

Chemical composition

Enfleurage: The chromatogram of the cold enfleurage absolute detected 10 chemicals (Figure 3 and Table 3), with the major components being methyl benzoate (30.17%), benzyl benzoate (23.64%), 7-decen-5-olide (13.33%) and methyl salicylate (12.11%).

Ten chemical constituents were also detected in the hot enfleurage absolute, with methyl benzoate being the main component again, but with a higher percentage yield (44.85%) than in the cold enfleurage absolute. Moreover, (Z)-3-hexenyl 2-oxopropanoate was a major component (27.38%) only in the hot enfleurage absolute. There were some differences in the chemical composition between the cold and hot enfleurage absolutes. However, methyl benzoate, methyl salicylate and benzyl benzoate were found in both absolutes. Among these components, methyl benzoate was considered the major characteristic of tuberose absolute, which is known as oil of niobe and tuberose. It possesses a pleasant smell, strongly reminiscent of the fruit and is mostly used in perfumery (Reverchon and Poletto, 1996). The results showed that temperature and the method of extraction affected not only the chemical composition of the absolutes, but also the percentage yield.

Solvent extraction: Fourteen chemicals were detected in the tuberose hexane absolute. Benzyl benzoate (24.25%), pentacosane (19.23%) and 7-decen-5-olide (14.96%) were the major chemical components (Figure 5 and Table 5).

There were 14 chemicals identified in the petroleum ether absolute. The main components were pentacosane (29.44%), 7-decen-5-olide (18.13%) and heptacosane (12.53%). However, benzyl benzoate represented only 10.28% (Figure 6 and Table 6). Petroleum ether extracted more wax, e.g. pentacosane and heptacosane, from plant cells than hexane. Plant wax was located in the cuticle of the epidermis. Some flowers such as jasmine and tuberose contained scent in the flower wax, which could be dissolved with some solvents (Alchemy-works, 2007). If the wax content were high, it would be difficult to obtain the essential oil by distillation. In this case, extraction by solvents extracted more oil resulting in the tuberose scent of the petroleum ether absolutes

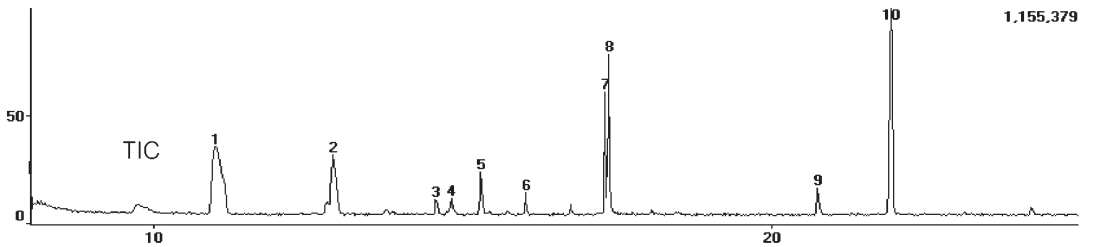

Figure 3 Chromatogram of cold enfleurage absolute.

Table 3 Chemical compositions of cold enfleurage absolute.

Peak No.	Retention time (min)	% Relative peak area	Possible compounds
1	10.996	30.17	methyl benzoate
2	12.896	12.11	methyl salicylate
3	14.567	1.78	indole
4	14.818	1.47	(E)-citral
5	15.297	4.45	methyl anthranilate
6	16.021	1.80	methyl eugenol
7	17.306	8.83	(E)-methyl isoeugenol
8	17.373	13.33	7-decen-5-olide
9	20.755	2.43	(z)-beta-farnesene
10	21.943	23.64	benzyl benzoate

Table 4 Chemical compositions of hot enfleurage absolute.

Peak No.	Retention time (min)	% Relative peak area	Possible compounds
1	10.987	44.85	methyl benzoate
2	12.904	7.18	methyl salicylate
3	14.740	2.91	2,4-decadien-1-al
4	14.815	27.38	(Z)-3-hexenyl 2-oxopropanoate
5	15.736	4.15	1-tetradecene
6	17.300	1.78	(E)-methyl isoeugenol
7	17.374	3.35	(Z)-nerolidol
8	18.460	3.39	1-hexadecene
9	21.922	3.75	benzyl benzoate
10	23.119	1.26	2-heptadecanone

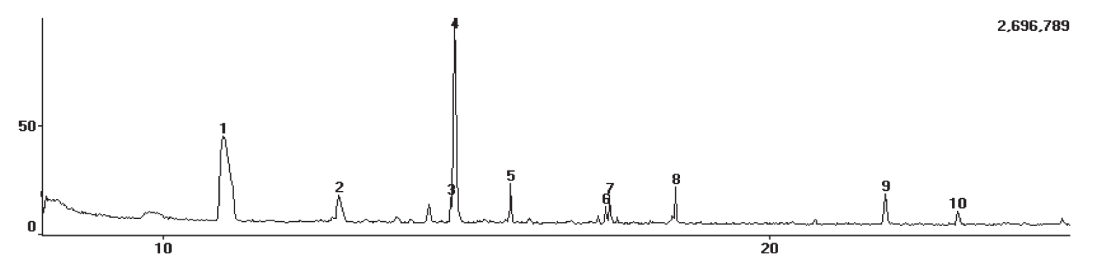

Figure 4 Chromatogram of hot enfleurage absolute.

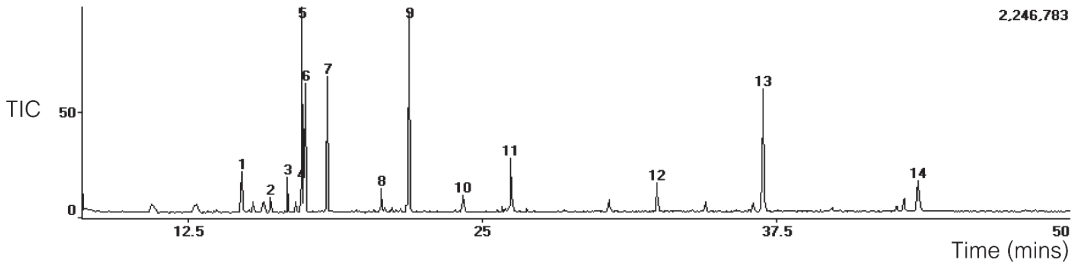

Figure 5 Chromatogram of tuberose hexane absolute.

Table 5 Chemical compositions of tuberose absolutes obtained by hexane extraction.

Peak No.	Retention time (min)	%Relative peak area	Possible compound
1	14.804	3.70	(Z)-3-hexenyl 2-oxopropanoate
2	16.009	1.07	methyl eugenol
3	16.744	2.32	methyl isoeugenol
4	17.294	1.89	(E)-methyl isoeugenol
5	17.364	14.96	7-decen-5-olide
6	17.494	8.36	2,4,-di-tert-butylphenol
7	18.449	8.85	1-hexadecene
8	20.736	1.94	alpha-farnesol
9	21.901	24.25	benzyl benzoate
10	24.198	1.39	benzyl salicylate
11	26.245	4.96	ecosanol
12	32.440	2.47	tricosane
13	36.972	19.23	pentacosane
14	43.542	4.60	heptacosane

being closer to natural flowers than from hexane absolute. In the current study, most of the chemicals identified in from both solvent extractions were similar. However, some chemicals in the tuberose absolutes from both solvents were different; methyl anthranilate, 1-tetradecene and (Z)-methyl isoeugenol were detected only in the petroleum ether absolute, while (Z)-3-hexenyl 2-oxopropanoate, 2,4,-di-tert-butylphenol and ecosanol were detected only in the hexane absolute. Several studies have shown that the tuberose absolute contained many chemical constituents, such as: benzyl benzoate, (Z)–5–decen–4-oilde, (Z,Z)–6,9–dodecadien–4–oilde, (Z)–6–dodecadien–4–oilde, eugenol, farnesol, geraniol, hecogenin, methyl benzoate, methylvanillin, nerol, (Z)–6–nonen–4–olide, (Z)–5–octen–4–oilde, piperonal, tuberoholoside and tuberolide (Nuntavan, 1996). Reverchon and Poletto (1996) reported the main chemical components of tuberose absolute from super critical fluid extraction were: 1,8-cineole, methyl benzoate, methyl salicilate, trans-methyl eugenol and benzyl benzoate. Moreover, Jumras and Possom (2003) reported tuberose oil chemical as follows: methyl benzoate, methyl anthranilate, benzyl alcohol, butyric acid, eugenol, nerol, farnesol and geraniol.

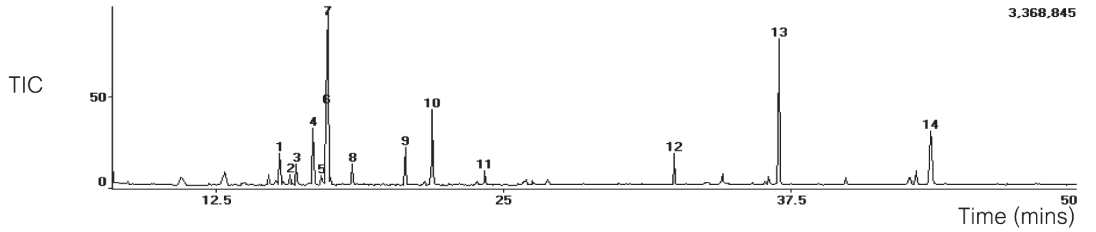

Figure 6 Chromatogram of tuberose absolute obtained from petroleum ether extraction.

Table 6 Chemical compositions of tuberose absolute obtained from petroleum ether extraction.

Peak No.	Retention time (min)	%Relative peak area	Possible compounds
1	15.297	4.15	methyl anthranilate
2	15.741	0.98	1-tetradecene
3	16.022	1.89	methyl eugenol
4	16.757	4.85	methyl isoeugenol
5	17.117	0.96	(Z)-methyl isoeugenol
6	17.311	6.05	(E)-methyl isoeugenol
7	17.385	18.13	7-decen-5-olide
8	18.463	1.69	1-hexadecene
9	20.754	4.06	alpha-farnesol
10	21.935	10.28	benzyl benzoate
11	24.217	1.34	benzyl salicylate
12	32.462	3.65	tricosane
13	37.013	29.44	pentacosane
14	43.597	12.53	heptacosane

CONCLUSIONS

The percentage yield of absolutes obtained from cold enfleurage, hot enfleurage, hexane and petroleum ether extractions were 0.3137%, 6.5808%, 0.0279%, and 0.0182%, respectively. The main chemical component detected in absolutes extracted by enfleurage was methyl benzoate, while benzyl benzoate and pentacosane were found to be the main chemical components in absolutes extracted by hexane and petroleum ether, respectively. The main chemical components of absolute extracted by petroleum ether were pentacosane, 7-decen-5-olide and heptacosane, respectively. The main components in cold enfleurage absolute were: methyl benzoate, benzyl benzoate, 7-decen-5-olide and methyl salicylate, respectively.

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