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ABSTRACT

Introduction: There are two types of herbal compress balls, fresh and dried, which are normally used in combination with Thai traditional massage for relieving inflammation and pain. There is no comparing information about chemical compositions of these herbal compress balls. **Objective:** This research focused on analysis of chemical composition of two types of herbal compress balls using headspace (HS) gas chromatography–mass spectrometry (GC–MS) system at a temperature of 80°C. **Methods:** Each herbal compress sample, *Cryptolepis dubia*, *Cymbopogon citratus*, *Tamarindus indica*, *Curcuma longa*, *Zingiber montanum,* and *Citrus hystrix*, was placed in a HS vial at 80°C for 20 min, agitated at 500 rpm, and injected with splitless mode (1:10). The GC system was interfaced with Agilent 5975C inert XL EI/CI MSD at a mass range of 40.0–900.0 amu. The volatile profiles were evaluated by principle component analysis. **Results:** Freshly prepared and dried compress balls materials showed 51 volatile compounds. Some parts of herbal materials gave more amounts of compound in fresh materials, but some dried part materials gave a greater number of compounds than fresh parts. **Conclusion:** Parts of the plant matrix play an important role in the amounts and number of volatile compounds.

Keywords: Headspace gas chromatography–mass spectrometry, herbal compress ball, principal component analysis, volatile compounds

INTRODUCTION

Herbal compress balls can be used together with Thai traditional massage or by itself for target healing.[1] There are several preparations of herbal compress balls but most of them contain major medicinal plants including rhizomes of *Zingiber montanum* (Koenig) Link ex Dietr. and *Curcuma longa* L., fruit peels of *Citrus hystrix* DC., leaves of *Tamarindus indica* L., and camphor.[2] There is a comparative study among hot herbal compress, hot compress, and topical diclofenac on the treatment of myofascial pain syndrome. All treatments showed significantly decreased level of pain intensity and increased cervical range of motion and the herbal compress expressed the ability in treatment same as diclofenac. This study showed the efficiency of herbal compress same as diclofenac with no significant different healing among these treatments.[3] A clinical study was performed to compare the efficiency of Thai massage, Thai herbal compress treatment, and oral ibuprofen in a symptomatic treatment of osteoarthritis of the knee.[4] The study showed that Thai massage and Thai herbal compress treatment were as effective as oral ibuprofen and can be used as alternatives for treating osteoarthritis. A systemic review article on clinical effects of Thai herbal compress treatment was published and 13 case studies were gathered from 369 articles. This review concluded that Thai herbal compress treatment could reduce osteoarthritis and

muscle pain as effectively as nonsteroidal anti-inflammatory drugs.[5]

Herbal compress balls are available in two types, fresh and dried balls. The fresh compress ball is more popular in massage treatment but cannot be stored for more than 2 days at room temperature because of microbial and fungal growth. The dried alternative type was developed by drying herbal ingredients before making them into a compress ball. Even though dried compress balls can be stored for as long as 12 months, some useful constituents such as aromatic scents and low boiling point terpenes that are useful for healing inflammation and relieving muscle pain are decreased.[6]

HS gas chromatography–mass spectrometry (HS-GC–MS) is a powerful and efficient technique for an identification of a complex volatile oil composition because it can simultaneously perform chromatographic separation and structural identification.[7,8] Cox and coworker developed a rapid method to characterize an herbal formulation using an automated HS solid-phase microextraction and GC–MS.[9] The analytical approach allowed the detection of cannabinoids in synthetic cannabinoids (JWH-018, JWH-073) and other synthetic cannabinoid analogs from small quantities of materials (50 mg) without having to perform an extraction or know the concentration or derivatization. Further efficiency optimization using natural deep eutectic solvents as both the extraction and dilution matrix in static SHS-GC–MS was achieved for the analysis of volatile compounds in *Ipomoea cairica* sweet leaves. A total of 77 volatiles in *I. cairica* leaves were detected and identified by mass spectral matching with the US National Institute of Standards and Technology (NIST, 2014). The main compounds out of 77 volatile compounds were aliphatic and aromatics terpenoids which were β-elemene, β-caryophyllene, α-humulene, and 2, 4-di-tert-butylphenol.[10]

After searching the literatures, there is no information comparing between fresh and dried herbal compress balls chemical constituents. In this study, the composition of volatile constituents in fresh and dried Thai traditional herbal compress balls was elucidated using HS-GC–MS. The information of this experiment can support the use of Thai traditional herbal compress ball.

MATERIALS AND METHODS

Plant Materials

Thai herbal compress ball materials were prepared by the Department of Graduate Study, College of Oriental Medicine, Rangsit University, Thailand. The fresh and dried herbs were prepared from the same materials which were composed of *Z. montanum* (Koenig) Link ex Dietr. rhizomes, *C. longa* L. rhizomes, *Cymbopogon citratus* (DC.) Stapf leaves, *T. indica* L. leaves, *C. hystrix* DC. fruit peel, and *Cryptolepis dubia* (Burm.f.) M.R. Almeida vine. The samples were bought from the local market in Bangkok in August 2019 and were identified by Assoc. Prof. Dr. Wandee Gritsanapan. The voucher specimens were kept at the Department of Graduate Study, College of Oriental Medicine, Rangsit University. For the dried samples, each herb was cut into small pieces (about 0.3 cm) and was dried in a hot air oven at 50°C for 6 h or until dried.

Preparation of Sample for HS-GC–MS

Fresh herbal compress samples (0.5 g) were quickly cut in small pieces and put into 20 mL GC-HS vial for aroma volatile injection with the HS technique. GS analyses were performed with Agilent GC 7890A GS system equipped with a fused silica capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness: Mega-5MS). Injector and transfer line temperatures were 200 and 270°C, respectively. The initial temperature was 60°C for 1 min, raised to 270°C at the rate 4°C/min, and maintained at this temperature for 5 min. Helium (99.999%) was the carrier gas with a flow rate of 1 mL/min at constant flow. The number of samples in each analysis was equal to three ($n = 3$). Each fresh herbal compress sample which included *C. dubia, C. citratus*, *T. indica, C. longa*, *Z. montanum,* and *C. hystrix* was placed in an incubation at 80°C for 20 min, agitated at 500 rpm, and injected using the splitless mode at the split ratio of 1:10 and an autosampler (Agilent GC Sampler 80). For GC–MS detection, the GC system was interfaced with an Agilent 5975C inert XL EI/CI MSD; Triple-Axis detector unit operated under the EI mode (70 eV) with an ion source where the temperature was maintained at 230°C and a quadrupole analyzer (150°C) at a mass range of 40.0–900.0 amu. The dried herbal compress samples were subject to the same method as described.

Data processing and statistical analysis

All data obtained from HS-GC–MS were examined based on the ion count area of peaks exceeding 2000 count for all absolute area parameter and the statistical method used pair T-test to compare area of peaks between the fresh and the dried samples. The identification of compounds was based on the comparison of their mass spectra with those of the National Institute of Standards and Technology (NIST 2014) database library of the GC/MS system. The identification was established by interpreting the fragmentation pattern of mass spectra.

For principle component analysis (PCA), all peak area data were subjected to SPSS version 17.0 software.

RESULTS AND DISCUSSION

HS-GC–MS analysis

After an automated injection of each herbal sample into the HS-GC–MS system, the results of volatile compounds of each herb in the Thai herbal compress ball are shown in Tables 1 and 2.

Identification of Volatile Compounds in Compress Ball Herbs

The HS vial containing small pieces of each herb was kept under optimal conditions, at 80°C, which was close to the temperature of compress balls used in Thai massages. The volatile substances in the HS volume were analyzed by GC–MS as described in the method section. All data of each fresh and dried herb were identified by comparing their mass spectra with those of the National Institute of Standards and Technology (NIST 2014) database library of the GC/MS system. Five herbs shared some similar and different compounds comprising 51 volatile compounds. Each herb showed a unique characteristic

Table 1: Amounts of compounds in % total peak area of dried and fresh herbs (dried [fresh])

No.	Compounds	t_R	RI[a]	Amount (% total peak area)					
				Zingiber montanum	***Curcuma longa***	***Citrus hystrix***	***Tamarindus indica***	***Cryptolepis dubia***	***Cymbopogon citratus***
1	Hexanal	3.789	809	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (4.76±1.74)	n.d. (n.d.)	n.d. (n.d.)
2	2-Hexenal	4.763	861	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.04±0.01)	n.d. (9.26±2.62)	n.d. (n.d.)	n.d. (n.d.)
3	2-Hexenal, (*E*)-	5.027	875	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (8.61±1.63)	n.d. (n.d.)	n.d. (n.d.)
4	ð-Thujene	6.249	928	0.85±0.37 (0.48±0.012)	n.d. (n.d.)	n.d. (n.d.)	0.62±0.11 (n.d.)	n.d. (n.d.)	n.d. (n.d.)
5	α-Pinene	6.505	937	3.09±0.37 (2.12±0.0.21)	0.91±0.05 (0.69±0.06)	5.24±1.06 (2.45±0.04)	1.64±0.35 (2.33±0.23)	0.28±0.02 (1.71±0.02)	n.d. (n.d.)
6	Camphene	6.976	955	0.27±0.09 (0.35±0.080)	n.d. (n.d.)	0.29±0.03 (0.21±0.01)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
7	ð-Phellandrene	7.569	976	57.32±14.69 (52.18±4.83)	0.62±0.25 (1.12±0.27)	n.d (19.56±1.82)	n.d. (n.d.)	n.d. (21.42±0.48)	0.41±0.20 (n.d.)
8	Sabinene	7.608	978	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	15.33±1.56 (4.29±0.42)	2.89±0.88 (n.d.)	n.d. (n.d.)
9	ð-Pinene	7.783	984	3.58±0.26 (3.12±0.16)	0.52±0.11 (0.73±0.19)	n.d. (28.65±1.95)	6.67±5.86 (17.41±0.48)	3.00±0.43 (37.68±0.84)	0.67±0.31 (n.d.)
10	ð-Myrcene	7.999	992	1.91±0.14 (1.63±0.07)	0.60±0.06 (0.88±0.02)	n.d. (1.27±0.07)	10.01±1.38 (n.d.)	0.94±0.21 (n.d.)	11.93±0.84 (n.d.)
11	α-Phellandrene	8.59	1012	n.d. (n.d.)	10.46±2.58 (14.00±0.45)	0.11±0.01 (0.05±0.02)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
12	α-Terpinene	8.934	1022	n.d. (2.64±0.24)	0.90±0.15 (1.03±0.19)	n.d. (n.d.)	0.30±0.03 (1.23±0.07)	n.d. (n.d.)	n.d. (n.d.)
13	Cymene	9.214	1031	3.71±1.77 (0.77±0.25)	2.41±0.50 (5.20±0.85)	n.d. (n.d.)	0.40±0.24 (n.d.)	0.11±0.09 (n.d.)	0.02±0.01 (n.d.)
14	*D*-Limonene	9.341	1035	0.76±0.04 (0.79±0.04)	1.07±0.12 (1.59±0.16)	32.23±5.37 (25.02±2.73)	2.86±0.28 (24.27±1.94)	0.66±0.08 (31.76±1.28)	0.72±0.08 (n.d.)
15	Eucalyptol	9.421	1037	n.d. (n.d.)	5.40±1.05 (21.73±3.42)	n.d. (n.d.)	n.d. (n.d.)	0.52±0.14 (n.d.)	n.d. (0.66±0.17)
16	ð-Ocimene	9.52	1040	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	0.32±0.01 (0.53±0.11)
17	ð-Terpinene	10.26	1063	8.11±3.51 (4.99±0.56)	0.44±0.05 (0.64±0.02)	n.d. (1.62±0.30)	1.51±0.26 (2.68±014)	0.27±0.06 (0.48±0.02)	n.d. (n.d.)
18	*cis-b*-Terpineol	10.81	1080	n.d. (0.48±0.05)	n.d. (n.d.)	n.d. (0.21±0.02)	0.22±0.02 (n.d.)	n.d. (n.d.)	n.d. (0.25±0.08)
19	Terpinolene	11.13	1090	n.d. (0.81±0.09)	12.94±2.74 (14.48±2.74)	0.25±0.04 (0.31±0.04)	0.26±0.09 (n.d.)	n.d. (n.d.)	n.d. (n.d.)
20	2-Carene	11.16	1090	1.32±0.53 (0.69±0.21)	n.d. (n.d.)	n.d. (1.22±0.52)	n.d. (0.67±0.032)	n.d. (0.29±0.02)	n.d. (n.d.)
21	*p*-Cymene	11.45	1099	n.d. (n.d.)	0.28±0.05 (0.39±0.06)	n.d. (n.d.)	0.61±0.03 (n.d.)	n.d. (n.d.)	n.d. (n.d.)

(*Contd...*)

Table 1: (*Continued*)

No.	Compounds	t_R	RI[a]	Amount (% total peak area)					
				Zingiber montanum	***Curcuma longa***	***Citrus hystrix***	***Tamarindus indica***	***Cryptolepis dubia***	***Cymbopogon citratus***
22	ð -Linalool	11.75	1108	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.79±0.20)
23	Decanal	12.47	1129	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	0.26±0.015 (n.d.)	n.d. (n.d.)
24	Citronellal	13.44	1158	n.d. (n.d.)	n.d. (n.d.)	n.d. (13.77±3.03)	n.d. (n.d.)	n.d. (1.77±0.48)	n.d. (n.d.)
25	(+)-2-Bornanone	13.45	1158	n.d. (0.09±0.04)	24.79±5.097 (0.10±0.012)	n.d. (0.12±0.06)	52.17±2.60 (n.d.)	76.92±1.04 (n.d.)	n.d. (11.55±2.81)
26	*dl*-Isopulegol	13.82	1169	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	0.30±0.03 (n.d.)	n.d. (1.52±0.05)
27	*endo*-Borneol	14.45	1187	n.d. (n.d.)	n.d. (n.d.)	2.05±0.42 (0.10±0.04)	0.13±0.02 (n.d.)	0.23±0.17 (n.d.)	n.d. (1.79±0.15)
28	Terpinen-4-ol	14.6	1191	7.65±2.84 (21.44±4.11)	1.07±0.15 (0.26±0.06)	0.90±0.51 (1.42±0.78)	1.89±0.12 (2.03±0.29)	0.90±0.16 (0.33±0.03)	n.d. (0.66±0.17)
29	α-Terpineol	15.19	1209	n.d. (n.d.)	n.d. (n.d.)	0.65±0.16 (0.62±0.20)	n.d. (n.d.)	0.65±0.04 (n.d.)	n.d. (n.d.)
30	2-Decenal, (*E*)-	15.53	1436	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.22±0.14)	n.d. (n.d.)	n.d. (n.d.)
31	Citronellol	16.13	1467	n.d. (n.d.)	n.d. (n.d.)	n.d. (1.05±0.45)	n.d. (0.49±0.16)	n.d. (1.18±0.42)	n.d. (n.d.)
32	α-Citral	16.52	1488	n.d. (n.d.)	1.01±0.17 (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	28.00±0.48 (25.59±1.39)
33	Geraniol	16.93	1510	n.d. (n.d.)	0.36±0.06 (n.d.)	n.d. (0.06±0.03)	n.d. (n.d.)	n.d. (n.d.)	2.12±0.26 (2.19±1.87)
34	α-Cubebene	19.86	1348	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.04±0.01)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
35	α-Copaene	20.87	1379	n.d. (n.d.)	n.d. (n.d.)	0.69±0.22 (1.23±0.20)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
36	ð-Caryophyllene	22.33	1425	n.d. (n.d.)	2.02±0.15 (1.68±0.15)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.46±0.06)
37	*trans*-α-Bergamotene	22.63	1435	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.25±0.03)
38	ð-Copaene	22.64	1435	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.03±0.01)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
39	α-Guaiene	22.72	1437	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.02±0.01)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
40	5,9-Undecadien-2-one, 6,10-dimethyl-,(*Z*)-	22.80	1440	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.06±0.02)	n.d. (n.d.)	n.d. (n.d.)
41	(*E*)-ð-Famesene	23.2	1453	n.d. (n.d.)	0.64±0.13 (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
42	*Cis-b*-Farnesene	23.28	1456	n.d. (n.d.)	n.d. (0.57±0.16)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
43	Humulene	23.49	1462	n.d. (n.d.)	0.33±0.04 (n.d.)	n.d. (0.16±0.05)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)

(*Contd...*)

Table 1: (*Continued*)

No.	Compounds	t_R	RI[a]	Amount (% total peak area)					
				Zingiber montanum	*Curcuma longa*	*Citrus hystrix*	*Tamarindus indica*	*Cryptolepis dubia*	*Cymbopogon citratus*
44	α-Curcumene	24.21	1485	n.d. (n.d.)	2.58±0.38 (3.80±0.31)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
45	α-Zingiberene	24.60	1498	0.22±0.13 (0.33±0.04)	5.44±1.05 (6.02±1.16)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
46	Bicyclogermacrene	24.72	1502	n.d. (n.d.)	n.d. (n.d.)	n.d. (0.06±0.01)	n.d. (n.d.)	n.d. (n.d.)	n.d. (n.d.)
47	ð-Bisabolene	24.98	1511	0.11±0.04 (0.10±0.02)	1.21±0.28 (1.37±0.26)	n.d. (n.d.)	n.d. (0.90±0.08)	n.d. (n.d.)	n.d. (0.05±0.04)
48	ð-Sesquiphellandrene	25.51	1529	0.75±0.56 (1.15±0.14)	5.57±0.88 (7.51±0.88)	n.d. (0.05±0.02)	n.d. (0.66±0.05)	n.d. (n.d.)	n.d. (n.d.)
49	*Ar*-tumerone	29.76	1676	n.d. (0.12±0.08)	3.18±0.98 (3.67±0.78)	0.14±0.05 (0.02±0.01)	n.d. (0.79±0.45)	0.25±0.15 (0.25±0.08)	n.d. (0.33±0.23)
50	Tumerone	29.85	1679	n.d. (0.43±0.41)	4.56±1.14 (5.31±0.35)	0.21±0.01 (0.06±0.04)	n.d. (1.19±0.09)	1.13±0.65 (0.41±0.10)	n.d. (0.36±0.14)
51	Curlone	30.8	1713	n.d. (n.d.)	1.90±0.54 (2.26±0.29)	0.12±0.02 (n.d.)	n.d. (0.91±0.50)	0.17±0.054 (0.14±0.03)	n.d. (0.21±0.06)

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C40) on Mega-5MS column. Number of samples $n=3$

pattern of compounds as fresh and dried under HS GC–MS analysis.

Z. montanum: The main volatile compound of this herb was ð-phellandrene of which the amount in fresh and dried samples (52.18±4.83% and 57.32±14.69%, respectively) was not different. However, terpinene-4-ol in the fresh sample (21.44±4.11%) was significantly higher than that in the dried sample (7.65±2.84%). In addition, the fresh sample contained more volatile compounds than the dried one (20 vs. 14).

C. longa: For fresh turmeric rhizome, the number of volatile compounds (23 compounds) was less than that in the dried rhizome (26 compounds). However, fresh samples generally contained higher amounts of the compounds than the dried samples. For example, eucalyptol was present at a higher amount in fresh samples (21.73±3.42%) than in the dried sample (5.40±1.00%). Some compounds were found to be higher in dried materials, such as (+)-2-bornanone (24.79±5.09% in dried sample and 0.10±0.01% in fresh sample), and were likely because dried materials contained more crude herb per weight than fresh materials. Moreover, dried samples contained more volatile compounds that have a higher evaporating points, and therefore, they are present in a higher amount in the dried samples. The paired t-test was performed for fresh and dried data and there was no significant difference between these two samples ($P > 0.05$).

C. hystrix

The fresh fruit peel had more volatile compounds (up to 27 compounds) than the dried fruit peel (12 compounds). The major constituent in the fresh *C. hystrix* fruit peel was citronellal (13.77%) and it was absent in the dried one.

T. indica

Fresh leaves contained 21 volatile compounds which was more than that in dried leaves (15 compounds). Major compounds in the fresh *T. indica* leaves were ð-pinene (17.41%) and *D*-limonene (24.27%). However, dried leaves had a high amount of (+)-2-bornanone (52.17%).

C. dubia

Fresh whole plant contained less volatile constituents (12 compounds) than dried leaves (17 compounds). The major compound in dried *C. dubia* was (+)-2-bornanone (76.92%) while the fresh material had three major compounds including ð-phellandrene (21.42%), *β*-pinene (37.68%), and *D*-limonene (31.76%).

C. citratus

The fresh and dried materials of this herb showed slightly different patterns of volatile compounds in the GC chromatogram. Fresh leaves contained 17 volatile compounds, which was more than those in dried leaves (8 compounds). Both plant materials had ð-citral as a major compound (25.59% in fresh and 28.00% in dried material).

From the HS GC–MS analysis, most fresh herbal materials contained more volatile compounds than dried ones and some compounds such as terpinen-4-ol, *ar*-tumerone, and tumerone were found in all herbs. Moreover, *α*-pinene and terpinen-4-ol were found in all dried herbs. Terpinen-4-ol is considered to

Table 2: Summary of volatile compounds in dried (D) and fresh herbs (F)

No.	Compounds	t_R	RI[a]	*Zingiber montanum*	*Curcuma longa*	*Citrus hystrix*	*Tamarindus indica*	*Cryptolepis dubia*	*Cymbopogon citratus*
1	Hexanal	3.789	809	-	-	-	(F)	-	-
2	2-Hexenal	4.763	861	-	-	(F)	(F)	-	-
3	2-Hexenal, (*E*)-	5.027	875	-	-	-	(F)	-	-
4	δ-Thujene	6.249	928	D (F)	-	-	D	-	-
5	α-Pinene	6.505	937	D (F)	D (F)	D (F)	D (F)	D (F)	-
6	Camphene	6.976	955	D (F)	-	D (F)	-	-	-
7	δ-Phellandrene	7.569	976	D (F)	D (F)	(F)	-	(F)	D
8	Sabinene	7.608	978	-	-	-	D (F)	D	-
9	δ-Pinene	7.783	984	D (F)	D (F)	(F)	D (F)	D (F)	D
10	δ-Myrcene	7.999	992	D (F)	D (F)	(F)	D	D	D
11	α-Phellandrene	8.59	1012	-	D (F)	D (F)	-	-	-
12	α-Terpinene	8.934	1022	(F)	D (F)	-	D (F)	-	-
13	Cymene	9.214	1031	D (F)	D (F)	-	D	D	D
14	*D*-Limonene	9.341	1035	D (F)	D (F)	D (F)	D (F)	D (F)	D
15	Eucalyptol	9.421	1037	-	D (F)	-	-	D	(F)
16	δ-Ocimene	9.52	1040	-	-	-	-	-	D (F)
17	δ-Terpinene	10.26	1063	D (F)	D (F)	(F)	D (F)	D (F)	-
18	*cis-b*-Terpineol	10.81	1080	(F)	-	(F)	D	-	(F)
19	Terpinolene	11.13	1090	(F)	D (F)	D (F)	D	-	-
20	2-Carene	11.16	1090	D (F)	-	(F)	(F)	(F)	-
21	*p*-Cymene	11.45	1099	-	D (F)	-	D	-	-
22	δ-Linalool	11.75	1108	-	-	-	-	-	(F)
23	Decanal	12.47	1129	-	-	-	-	D	-
24	Citronellal	13.44	1158	-	-	(F)	-	(F)	-
25	(+)-2-Bornanone	13.45	1158	(F)	D (F)	(F)	D	D	(F)
26	*dl*-Isopulegol	13.82	1169	-	-	-	-	D	(F)
27	*endo*-Borneol	14.45	1187	-	-	D (F)	D	D	(F)
28	Terpinen-4-ol	14.6	1191	D (F)	D (F)	D (F)	D (F)	D (F)	(F)
29	α-Terpineol	15.19	1209	-	-	D (F)	-	D	-
30	2-Decenal, (*E*)-	15.53	1436	-	-	-	(F)	-	-
31	Citronellol	16.13	1467	-	-	(F)	(F)	(F)	-
32	δ-Citral	16.52	1488	-	D	-	-	-	D (F)
33	Geraniol	16.93	1510	-	D	(F)	-	-	D (F)
34	α-Cubebene	19.86	1348	-	-	(F)	-	-	-
35	α-Copaene	20.87	1379	-	-	D (F)	-	-	-
36	δ-Caryophyllene	22.33	1425	-	D (F)	-	-	-	(F)
37	*trans*-α-Bergamotene	22.63	1435	-	-	-	-	-	(F)
38	δ-Copaene	22.64	1435	-	-	(F)	-	-	-
39	α-Guaiene	22.72	1437	-	-	(F)	-	-	-
40	5,9-Undecadien-2-one, 6,10-dimethyl-,(*Z*)-	22.80	1440	-	-	-	(F)	-	-
41	(*E*)- δ -Famesene	23.2	1453	-	D	-	-	-	-
42	*Cis-b*-Farnesene	23.28	1456	-	(F)	-	-	-	-
43	Humulene	23.49	1462	-	D	(F)	-	-	-
44	α-Curcumene	24.21	1485	-	D (F)	-	-	-	-

(*Contd...*)

Table 2: (*Continued*)

No.	Compounds	t_R	RI[a]	*Zingiber montanum*	*Curcuma longa*	*Citrus hystrix*	*Tamarindus indica*	*Cryptolepis dubia*	*Cymbopogon citratus*
45	α-Zingiberene	24.60	1498	D (F)	D (F)	-	-	-	-
46	Bicyclogermacrene	24.72	1502	-	-	(F)	-	-	-
47	ð -Bisabolene	24.98	1511	D (F)	D (F)	-	(F)	-	(F)
48	ð -Sesquiphellandrene	25.51	1529	D (F)	D (F)	(F)	(F)	-	-
49	*Ar*-tumerone	29.76	1676	(F)	D (F)	D (F)	(F)	D (F)	(F)
50	Tumerone	29.85	1679	(F)	D (F)	D (F)	(F)	D (F)	(F)
51	Curlone	30.8	1713	-	D (F)	D	(F)	D (F)	(F)

[a]Retention indices were calculated using a homologous series of n-alkanes (C8–C40) on Mega-5MS column. Number of samples $n=3$

be one of the main active ingredients responsible for the anti-inflammatory activity of *Z. montanum* oil.[11,12] Since terpinen-4-ol is a volatile compound, some of the topically applied amount will evaporate into the air. The loss of terpinen-4-ol by evaporation reduces the topical dose available on the skin. After topical application of *Z. montanum* oil, terpinen-4-ol rapidly permeated into the dermis. The elimination half-lives were not significantly different between dosing groups. The mean dose-normalized $AUC_{0-\infty}$ between the doses of 2, 4, and 8 mg/cm^2 showed no statistically significant differences suggesting a dose proportional.[13] This information would be useful for the application of herbal compress balls in massage therapy.

Park *et al*.[14] suggested that *ar*-tumerone possessed anti-inflammatory properties as shown by the blockade of key signaling pathways in microglia. In addition, *ar*-tumerone showed an anti-dermatophytic activity.[15] Eucalyptol or 1.8-cineol was a major compound in *C. longa*. This compound was found to inhibit the cyclooxygenase pathway, which suggests the potential anti-inflammatory activity of eucalyptol.[16]

Citronellal, ð-pinene, ð-citral, and *D*-limonene were found to be antimicrobial.[17-19] (+)-2-Bornanone or camphor was found in three herbs including *C. dubia, T. indica*, and *C. longa*. This compound also has an antimicrobial activity, which was shown to work in synergy with anti-inflammatory constituents to heal the body during the compress ball application.[20]

Principal Component Analysis of Volatile Compounds

Fresh and dried herbal compress materials provided different volatile profiles, as shown in Figures 1 and 2. Significant changes in volatile profiles from the drying process allowed PCA method to differentiate between fresh and dried herbal compress materials.

In Figure 1, volatile compounds of *C. longa* (C1) and *Z. montanum* (Z1) fresh rhizomes showed high amount of some materials and different types of terpenes. Rhizome is a crucial part of a plant that can store many secondary metabolites in its matrix. This is why two fresh rhizomes stayed as isolated cluster and separated from other fresh herbs. Parts derived from herbs including C2, C3, C4, and T1 are parts that had a low capacity matrix structure (in relation to weight) for storing volatile compounds. Figure 2 shows two dried isolated

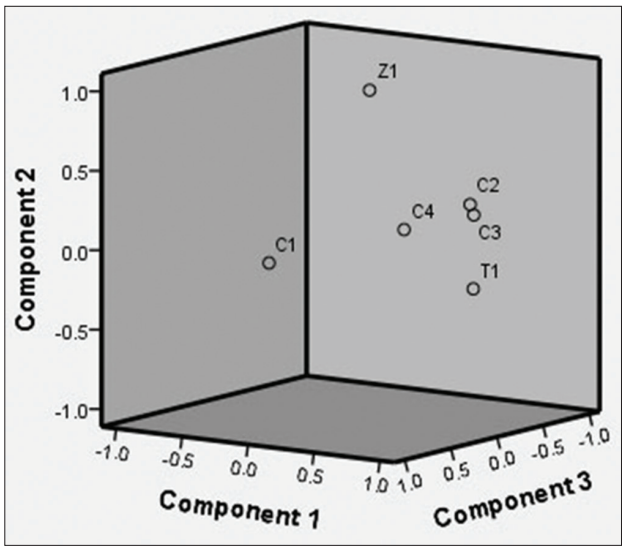

Figure 1: Principle component analysis of fresh herbal compress materials. C1 = *Curcuma longa*, C2 = *Citrus hystrix*, C3 = *Cryptolepis dubia*, C4 = *Cymbopogon citratus*, T1 = *Tamarindus indica*, Z1 = *Zingiber montanum*

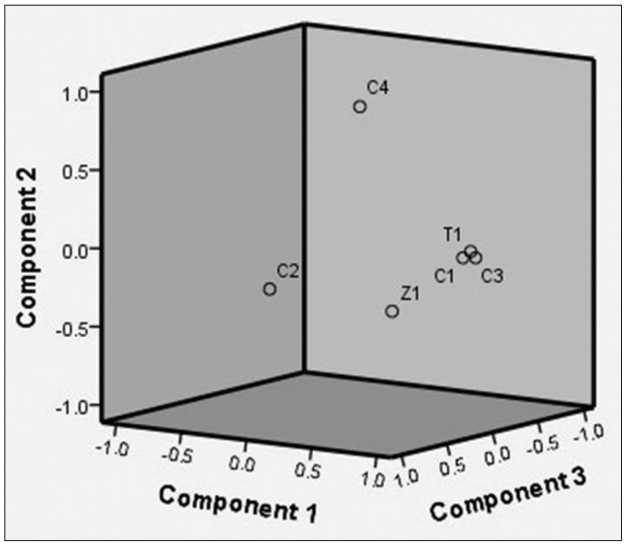

Figure 2: Principle component analysis of dried herbal compress materials. C1 = *Curcuma longa*, C2 = *Citrus hystrix*, C3 = *Cryptolepis dubia*, C4 = *Cymbopogon citratus*, T1 = *Tamarindus indica*, Z1 = *Zingiber montanum*

components, *C. hystrix* (C2) fruit peels and *C. citratus* leaves (C4), which were different from those in the fresh ones. These two plants have a matrix structure in peels and leaves that can hold great amounts of volatile compounds. Other herbs stayed as a group of clusters because their volatile compounds were lost during the drying process.

CONCLUSION

This study is the first report on comparing volatile compounds in fresh and dried herbal materials in Thai traditional compressed balls. In most herbs, fresh herbal compress balls contain higher amounts of volatile compounds than dried herbal compress balls. However, *C. dubia* and *C. longa* in the dried form had more volatile compounds than the fresh form. From the PCA analysis results, parts of the plant matrix in the fresh form can hold more volatile compounds than the dried form. Therefore, it is recommended that practitioners prepare fresh herbal compress balls before the treatment. However, in countries where fresh herbal materials are not available, ready-to-use dried herbal compress balls are suitable as an alternative compress ball in massage treatments.

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