Songklanakarin J. Sci. Technol.
39 (4), 463-470, Jul - Aug. 2017

http://www.sjst.psu.ac.th

Original Article

HPLC-QTOF-MS method for quantitative determination of active compounds in an anti-cellulite herbal compress

Ngamrayu Ngamdokmai[1], Neti Waranuch[2], Krongkarn Chootip[3], Nitra Neungchamnong[4], and Kornkanok Ingkaninan[1*]

[1] *Bioscreening Unit, Department of Pharmaceutical Chemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

[2] *Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences and Center of Excellence for Innovation in Chemistry, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

[3] *Department of Physiology, Faculty of Medical Sciences, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

[4] *Science Laboratory Centre, Faculty of Science, Naresuan University, Mueang, Phitsanulok, 65000 Thailand*

Received: 24 February 2016; Revised: 20 May 2016; Accepted: 8 July 2016

Abstract

A herbal compress used in Thai massage has been modified for use in cellulite treatment. Its main active ingredients were ginger, black pepper, java long pepper, tea and coffee. The objective of this study was to develop and validate an HPLC-QTOF-MS method for determining its active compounds, i.e., caffeine, 6-gingerol, and piperine in raw materials as well as in the formulation together with the flavouring agent, camphor. The four compounds were chromatographically separated. The analytical method was validated through selectivity, intra-, inter day precision, accuracy and matrix effect. The results showed that the herbal compress contained caffeine (2.16 mg/g), camphor (106.15 mg/g), 6-gingerol (0.76 mg/g), and piperine (4.19 mg/g). The chemical stability study revealed that herbal compresses retained >80% of their active compounds after 1 month of storage at ambient conditions. Our method can be used for quality control of the herbal compress and its raw materials.

Keywords: HPLC-QTOF-MS, herbal compress, quality control, traditional Thai medicines

1. Introduction

Traditionally in Thailand, herbal compresses or pads containing of several herbs, known as Luk-Pra-Kob in Thai, have supplemented massaging. The herbs are bundled within a cloth to form a ball with the other end rolled-up to form a short handle. The warmed herbal compresses are used to relieve pains from physical labor and exertion, stresses and strains acquired during arduous daily routines (The Institute of Thai Traditional Medicine, 1995). Nowadays, the uses of herbal compresses are expanded for spa and cosmetic businesses.

Cellulite describes the unpleasant-looking dimpling of the skin overlying subcutaneous fat around the thighs,

* Corresponding author.
Email address: k_ingkaninan@yahoo.com

buttocks and sometimes over the lower abdomen and upper arms of women. Cellulite is not a disease, but many women are concerned about its appearance (Terranova *et al.,* 2006). Because herbal compresses have found cosmetic applications, we considered that they might be useful in the amelioration of cellulite.

Accordingly, we developed a formula of herbal compress modified from the traditional formula (National Drug System Development Committee, 2013). To this were added plant products which influence cellulite i.e. increasing lipolysis, inhibiting adipogenesis, inducing vasodilation, or having anti-inflammatory effect. These plants are 1) *Zingiber officinale* Rosc. (ginger), 2) *Piper nigrum* L. (black pepper), 3) *Piper retrofractum* Vahl. (java long pepper), 4) *Camellia sinensis* L. (tea), and 5) *Coffea arabica* L. (coffee) (Table 1). The chemical structures of the active ingredients as well as camphor, which was used in the formula as a flavoring agent, are shown in Figure 1. 6-Gingerol, a main active ingredient of ginger played role for anti-inflammation (Grzanna *et al.,* 2005; Semwal *et al.,* 2015; Young *et al.,* 2005), adipogenesis inhibition (Brahma Naidu *et al.*, 2016; Impheng *et al.,* 2015; Tzeng *et al.,* 2013; Tzeng *et al.,* 2014) and vasodilation (Ghareib *et al.,* 2015), while piperine, a main active ingredient of black pepper and java long pepper, was reported to have anti-inflammation effect (Mujumdar *et al.,* 1990), increasing lipolysis (Brahma Naidu *et al.,* 2014; Malini *et al.,* 1999) and inhibition adipogenesis (Park *et al.,* 2012). Caffeine from tea and coffee could increase lipolysis (Gurley *et al.,* 2015; Herman *et al.,* 2012; Hexsel *et al.,* 2005; Mohamed *et al.,* 2014; Roure *et al.,* 2011; Velasco *et al.,* 2008) and inhibit adipogenesis (Hexsel *et al.,* 2011; Roure *et al.,* 2011). In addition, camphor can increase local blood flow in the skin (Kotaka *et al.,* 2014). These compounds served as markers for quality control and standardization of the herbal formulation to ensure their reliability and efficacy.

For this application, we developed a high performance liquid chromatography quadrupole time-of-flight mass spectrometry (HPLC-QTOF-MS) method to quantify these

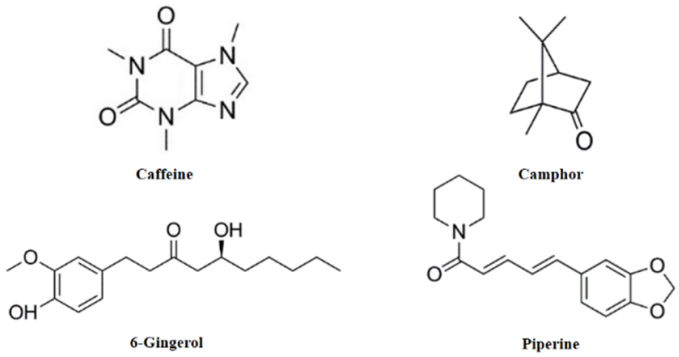

Figure 1. Chemical structures of the active ingredients as markers of the anti-cellulite herbal compress

Table 1. Principal herbs in the anti-cellulite herbal compress

Principal herbs	Part used	Active compounds	Proposed actions (with reference)
1. *Zingiber officinale* Rosc. (Ginger)	Rhizomes	6-Gingerol	Anti-inflammation (Grzanna *et al.,* 2005; Young *et al.,* 2005, Ojewole *et al.,* 2006; Lantza *et al.,* 2007) Decreasing adipogenesis (Tzeng *et al.,* 2013; Tzeng *et al.,* 2014; Impheng *et al.,* 2015; Brahma Naidu *et al.,* 2016) Vasodilation (Ghareib *et al.,* 2015)
2. *Piper nigrum* L. (Black pepper)	Fruits	Piperine	Anti-inflammation (Vasanthi *et al.,* 2010; Murlidhar *et al.,* 2013) Decreasing adipogenesis (Park *et al.,* 2012)
3. *Piper retrofractum* Vahl. (Java long pepper)	Fruits	Piperine	Anti-inflammation (Chaveerach *et al.,* 2006)
4. *Camellia sinensis* L. (Tea)	Leaves	Caffeine	Increasing lipolysis (Ko *et al.,* 2015)
5. *Coffea arabica* L. (Coffee)	Seeds	Caffeine	Increasing lipolysis (Mohamed *et al.,* 2014)

four active compounds in both the raw materials and the herbal compresses. Our method was validated and shown to be applicable to quality control of our anti-cellulite herbal formula.

2. Materials and Methods

2.1 Chemicals

Acetonitrile, water and methanol were of LC/MS grade (RCI labscan, Thailand). Formic acid was of analytical grade (Merck, Germany). Methanol was of analytical grade (RCI labscan, Thailand). Reference standards of caffeine and piperine were purchased from Sigma-Aldrich, Buchs, Switzerland. Camphor was purchased from Union Chemical, Bangkok, Thailand. 6-gingerol was purchased from Natural remedies, Bangalore, India.

2.2 Preparation of herbal materials and the herbal compress

The anti-cellulite herbal compress formula consisted of principal drugs, auxiliary drugs and flavoring agents in the ratio of 5:3:2. The names of the principal drugs are listed in Table 1. The auxiliary drugs were *Zingiber montanum* (J. Koenig) Link ex A.Dietr. rhizomes (plai), *Curcuma longa* Linn. rhizomes (turmeric), *Cymbopogon citrates* Stapf. leaves (lemon grass), *Citrus hystrix* DC. fruit peels (kaffir lime), while the flavoring agents were camphor and salts. All of these were sourced and formulated in the herbal production unit of Bangkratum Hospital (Bangkratum, Phitsanulok, Thailand). All the plant materials were dried at 45-50°C in a recirculating air oven for 3-4 days, powdered and sieved through a 60 mesh sieve and stored at -20°C until use.

2.3 Preparation of reference standard and quality control solutions

Caffeine, camphor, 6-gingerol and piperine were prepared as methanolic stock reference solutions at 1000 µg/mL, stored at 4°C until use and then appropriately diluted with methanol for calibration curves and using 3 concentrations QC1 (3 times of lower limit of quantitative (LLOQ)), QC2 (mid-range) and QC3 (80% of upper limit of quantitative (ULOQ)) for accuracy and precision study.

2.4 Preparation of sample solutions

2.4.1 Sample solutions of five main herbal ingredients

The powder of five herbal compress main ingredients i.e. ginger rhizomes, black pepper fruits, java long pepper fruits, coffee beans and tea leaves were sieved through a 60 mesh sieve. Fifty mg of each herb was accurately weighed and then extracted with methanol by shaking at 200 rpm (Scilogex, USA) for 12 hours at room temperature. The solution was filtered through filter paper (Whatman No.1). The residues were extracted with methanol on ultrasonic bath for 15 min at room temperature for two times and filtered. The filtrates were pooled, adjusted to the concentration required (2 mg/mL for tea, 5 mg/mL for black pepper and java long pepper, and 10 mg/mL for ginger and coffee). The sample solutions were filtered through a 0.2 µm nylon syringe filter (Vertical, Thailand) and then injected into the HPLC-QTOF-MS. All samples were analysed in triplicate.

2.5 Stability of anti-cellulite herbal compresses

Complete herbal compresses were stored at 28-35°C ambient humidity for 0, 14, and 30 days (*International Federation of Societies of Cosmetic Chemists* [IFSCC], 1992) and all the mixed herbs weighed and then extracted with methanol as above. The final volume was adjusted to 500 mL. The filtrate was filtered through a 0.2 µm nylon syringe filter (Vertical, Thailand). The filtrate was divided into 2 parts, part 1 was further diluted to 100-fold with MeOH for determination of 4 compounds using HPLC-QTOF-MS. And part 2 was diluted to 500-fold for determination of camphor using the same method. All determinations were conducted in triplicate.

2.6 HPLC-QTOF-MS instruments and chromatographic conditions

HPLC-QTOF-MS analysis used an Agilent series 1260 Infinity HPLC instrument (Agilent, Waldbronn, Germany) coupled to an Agilent 6540 QTOF mass spectrometer (Agilent Technologies, Singapore) equipped with an electrospray (ESI) interface. The HPLC included a binary pump, an online degasser, an auto plate-sampler and a thermostatically controlled column compartment. The chromatographic separation was achieved on a Luna C18 (2) column (4.6 mm x 150 mm, 100Å 5 µm, Phenomenex Torrance, CA, USA). Chromatographic conditions were as follows: The mobile phase was 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The gradient elution was: 0 min, 70:30, (A: B v/v); 5 min, 5:95, 15 min, 5:95. Re-equilibration between runs was 5 min. The flow rate was set at 500 µL/min and the sample volume injected was 5 µL. The MS operating parameters were as follows: gas temperature 350°C, drying gas 10 L/min, nebulizer 30 psig, capillary 3500 V, fragmentor 100 V, skimmer 65 V, OCT 1RF Vpp 750 V. All acquisition and analysis of data were controlled by MassHunter software (Agilent Technologies, USA). The sample was analyzed in positive ESI mode.

2.7 Method validation

The validation parameters were selectivity, linearity, sensitivity, precision, accuracy and matrix effect studied in accordance with guidance for dietary supplements and botanicals (Association of Official Analytical Chemists [AOAC], 2012).

2.7.1 Selectivity

The selectivity of the method was studied by adding the reference standards in the matrix sample as lemon grass and kaffir lime used as auxiliary drugs. The extract ion chromatogram at the typical *m/z* of each reference standard was used and the spectra of the reference and retention times were compared to ensure that there were no interfering peaks.

2.7.2 Linearity

The various concentrations of each reference standard were measured and the calibration linearity range determined. The calibration curves were obtained by plotting the exact ion chromatogram (EIC) peak areas versus the concentrations of the reference using linear regression $1/x^2$ weighting. The linearity of the calibration curve was evaluated by calculating the coefficient of determination (r^2).

The concentrations of caffeine and 6-gingerol used for the calibration curve were 1, 5, 25, 50, 75, and 100 µg/mL while those for camphor and piperine were 10, 20, 30, 50, 70, 100 µg/mL and 0.5, 1, 5, 15, 30, 50 µg/mL respectively. Calibration curves were made during each analysis day and the analyses were done in duplicate.

2.7.3 Lower limit of detection and lower limit of quantitation

The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) under the present chromatographic conditions were determined using a signal-to-noise ratio of 3:1 and 10:1, respectively. The LLOD is the minimum concentration at which the analyte could be detected and LLOQ is the lowest concentration at which the analyte could be reliably is quantified with acceptable accuracy.

2.7.4 Precision and accuracy

Intra-day precision and accuracy were determined by analyzing three replicates of three different QC samples (3, 60, and 80 µg/mL for caffeine and 6-gingerol; 20, 60, and 80 µg/mL for camphor; and 1.5, 10, and 20 µg/mL for piperine) on the same day. Inter-day precision and accuracy were also evaluated by analyzing three replicates of three different QC samples on three different days. Precision was expressed as the coefficient of variation (%CV) and the accuracy was expressed as percentage of the observed concentration comparing to nominal reference concentration (mean observed concentration/ nominal concentration x 100).

2.7.5 Matrix effect

The matrix effect was studied by post-extraction spiking method. The matrix effect was expressed as percent extraction recovery. The QC of each compound at three levels of concentrations was used. The "matrix" solution which were lemongrass and kaffir lime (1:1) was diluted at 1.5 mg/mL and used as the sample solution. Taking the analyte peak areas obtained by injection of reference standard solutions spiked into sample solution as A and the peak areas of reference standard solutions in neat solution as B, extraction recovery was calculated using the equation: extraction recovery (%) = (A/B) x 100. The matrix effect of less than 15% was deemed acceptable.

2.8 Statistical analysis

Data were expressed as the average ± standard deviation (SD). Statistical analysis was conducted using Student's t-test with the Statistical Package for the Social Sciences (SPSS) 16.0.

3. Results and Discussion

3.1 HPLC-QTOF-MS method validation

The herbal compress and its main ingredients were analyzed for piperine, caffeine, 6-gingerol and camphor using HPLC-QTOF-MS with an electrospray (ESI) interface. Methanol was selected as an extraction solvent as it could extract compounds in a broad range of polarity. Total ion chromatograms for both a mixture of reference standard (1 mg/mL), and extract of the compress herbal mixture (0.3 mg/mL for the anti-cellulite herbal compress and 0.06 mg/mL for camphor) showed clear peaks corresponding to caffeine (4.1 min), 6-gingerol (8.9 min), piperine (9.2 min), and camphor (9.8 min) as shown in Figure 2.

3.1.1 Selectivity

These records demonstrate no interfering peaks occur in the corresponding retention time of each analyte and the measured masses correspond to the calculated molecular weights (Table 2, Figure 2).

3.1.2 Linearity

The range of linearity of the four compounds with their LLOD and LLOQ are shown in Table 3. The coefficients of determination (r^2) for the calibration curves for all compounds were > 0.990. The signals were linear for all compounds over concentration ranges suitable of analysis of these compounds in the same sample.

3.1.3 Precision and accuracy

Intra-day and inter-day precision and accuracy at three concentrations were performed. Both precision and accuracy were within the acceptable range (%CV was not more than 15 and % accuracy was in the range of 85-110% (AOAC, 2012) (Table 4).

Table 2. Retention time and measured mass of the reference standards on the HPLC-QTOF-MS analysis

Reference standards	Retention time (min)	Measured mass
Caffeine	4.1	195.0877 $[M+H]^+$
6-gingerol	8.9	277.0300 $[M-H_2O]^+$
Piperine	9.2	286.1438 $[M+H]^+$
Camphor	9.8	153.1274 $[M+H]^+$

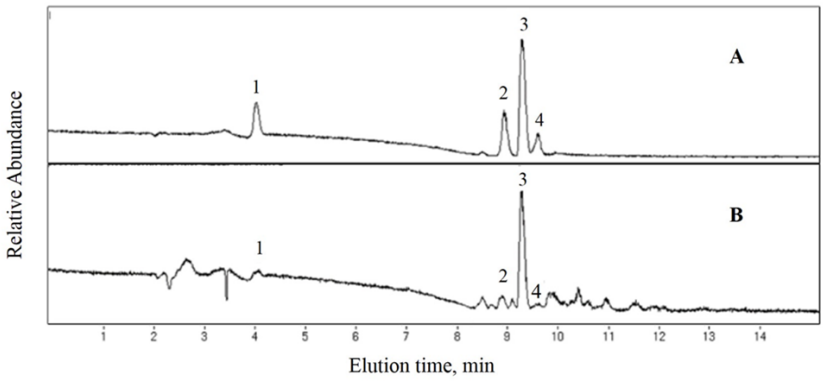

Figure 2. Total ion chromatograms from HPLC-QTOF-MS with electrospray of (A) mixture of reference standards (1 mg/mL) of caffeine (1), 6-gingerol (2), piperine (3), and camphor (4), (B) the anti-cellulite herbal compress solution (0.3 mg/mL)

Table 3. The coefficient of determination and linearity range of the calibration curves of 4 reference standards, lower limits of determination (LLOD), and their lower limits of quantitation (LLOQ) obtained by HPLC-QTOF-MS

Marker compounds	Coefficient of determination (r^2)	Range (μg/mL)	LLOD (μg/mL)	LLOQ (μg/mL)
Caffeine	0.997	1.0 – 100.0	0.50	1.00
Camphor	0.998	10.0 – 100.0	5.00	10.00
6-Gingerol	0.997	1.0 – 100.0	0.50	1.00
Piperine	0.992	0.5 – 50.0	0.10	0.50

3.1.4 Matrix effect

Matrix effects pose a significant problem in HPLC-QTOF-MS analyses of unknown samples. To test this, the matrix solution was spiked with 3 concentrations of the four reference standards and compared with the same concentrations added to methanol. On average, the recovery range was 96-110% (Table 5) suggesting that the matrix influence was negligible compared to the sample accuracies.

3.2 Quantitation of active compounds in plant extracts

The main ingredients in the anti-cellulite herbal compress, i.e., ginger, black pepper, long pepper, tea, and coffee were individually analyzed in extracts from the 5 plants (Table 6). While each extract contained substantial quantities of their respective active compound, Java long pepper also contained 6-gingerol. To our knowledge, this is the first report of this compound appearing in Java long pepper.

3.3 Active compounds in freshly made anti-cellulite compress and storage

Using our validated HPLC-QTOF-MS method, the active compounds were measured in methanolic extracts of fully formulated anti-cellulite herbal compresses and these values are shown in Table 7. Shelf-life poses a limitation on a products usefulness so we sought to assess persistence of the active ingredients within fully functional anti-cellulite herbal compresses. These were stored for 14, and 30 days at the ambient temperature (25-35°C). After 30 days, more than 95% of the compound was retained, except for 6-gingerol. It undergoes a temperature-dependent retro-aldol condensation reaction to zingerone and aliphatic aldehydes which

Table 4. The intra- and inter-day precision and accuracy data obtained by HPLC-QTOF-MS for a reference standard mixture of caffeine, camphor, 6-gingerol, or piperine at three different concentrations. Each set of concentrations was tested at three times of day.

Compounds	Concentration levels (μg/mL)	Intra-day (n=3 times)			Inter-day (n=3 days)		
		Measured concentration (μg/mL) ± SD	Precision (%CV)	Accuracy (%)	Measured concentration (μg/mL) ± SD	Precision (%CV)	Accuracy (%)
Caffeine	3	3.29 ± 0.10	0.74	109.65	3.23 ± 0.11	3.34	107.72
	60	58.79 ± 0.95	3.15	97.99	58.23 ± 1.00	1.71	97.05
	80	69.26 ± 1.82	2.63	86.58	74.19 ± 5.04	6.80	92.74
Camphor	20	19.08 ± 0.57	2.98	95.39	20.49 ± 1.31	6.39	102.44
	60	58.14 ± 0.16	0.28	96.91	58.96 ± 1.44	2.45	98.27
	80	73.46 ± 2.43	3.31	91.82	75.76 ± 3.76	4.97	94.70
6-Gingerol	3	3.27 ± 0.02	0.74	109.11	3.11 ± 0.23	7.35	103.53
	60	57.52 ± 1.81	3.15	95.87	55.22 ± 3.54	6.42	92.04
	80	73.08 ± 1.41	1.93	91.35	71.60 ± 4.28	5.98	89.50
Piperine	1.5	1.45 ± 0.04	3.05	96.78	1.45 ± 0.01	0.77	96.46
	10	10.95 ± 0.53	4.88	109.49	10.76 ± 0.18	1.69	107.60
	20	20.60 ± 0.49	2.38	102.99	20.58 ± 0.02	0.09	102.89

Table 5. The recovery of the 4 reference standards added to matrix on HPLC-QTOF-MS measurements (n=3)

Compounds	Spiked amount (μg/ml)	Recovery (%) Average ± S.D.	%CV
Caffeine	3	96.49 ± 2.36	2.45
	60	101.04 ± 1.42	1.40
	80	109.72 ± 5.22	4.76
Camphor	20	108.21 ± 0.36	0.33
	60	102.17 ± 1.41	1.38
	80	99.96 ± 2.32	2.32
6-Gingerol	3	104.28 ± 2.38	2.29
	60	109.74 ± 1.71	1.56
	80	103.66 ± 2.95	2.84
Piperine	1.5	103.15 ± 3.59	3.48
	10	99.86 ± 3.75	3.76
	20	99.76 ± 3.89	3.90

have unpleasant odours (Connell *et al.,* 1969; Seon Ok & Woo-Sik Jeong, 2012). Furthermore, the degradation of 6-gingerol is consistent with its dehydration (and 8- and 10-congeners) to 6-shogaol. The dehydration kinetics are pH and temperature dependent (Bhattarai *et al.*, 2006). Thus, HPLC-QTOF-MS used here is superior to GC/MS.

4. Conclusions

A HPLC-QTOF-MS methodology was developed and validated for quantitative determination of active compounds and quality control of the active ingredients of anti-cellulite herbal compresses containing ginger, black pepper, java long pepper, tea and coffee. The stability study using the method developed revealed that the herbal compress can be stored at room temperature for at least one month before usage.

Acknowledgements

The financial supports from Naresuan University and the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Office of the Higher Education Commission, Ministry of Education, Thailand, are acknowledged. We thank Dr. Norman Scholfield for his help in the preparation of the manuscript.

Table 6. The contents of caffeine, 6-gingerol and piperine in each dried plant material (n=3)

Plant materials	Caffeine (mg/g)	6-Gingerol (mg/g)	Piperine (mg/g)
	Average ± S.D.	Average ± S.D.	Average ± S.D.
1 Ginger	0	4.11 ± 0.22	0
2 Black pepper	0	0	10.27 ± 0.31
3 Java Long pepper	0	1.40 ± 0.08	10.03 ± 0.33
4 Tea	0	0	19.70 ± 0.22
5 Coffee	6.36 ± 0.05	0	0

Table 7. Stability of the reference standards in the anti-cellulite herbal compress kept at ambient temperature during 1 month (n = 3)

Day	Caffeine		Camphor		6-Gingerol		Piperine	
	Ave ± S.D. (mg/g)[a]	Remaining%[b]	Ave ± SD (mg/g)	Remaining%	Ave ± S.D. (mg/g)	Remaining%	Ave ± S.D. (mg/g)	Remaining%
Day 0	2.16 ± 0.05	100	106.15 ± 2.26	100	0.76 ± 0.04	100	4.19 ± 0.21	100
Day 14	2.11 ± 0.08	98	100.52 ± 3.87	95	0.75 ± 0.02*	99	4.01 ± 0.19	96
Day 30	2.08 ± 0.04	99	97.41 ± 4.94	97	0.65 ± 0.02**	87	3.92 ± 0.12	98

[a] (mg/g) refer to milligram of active compound per 1 gram powder of the anti-cellulite herbal compress.
[b] % remaining of the analytes in the herbal compress compared to that at day 0.
* $P < 0.05$ compared between Day 0 and Day 30
** $P < 0.01$ compared between Day 14 and Day 30

References

Association of Official Analytical Chemists. (2012). *Official methods of analysis*. *AOAC guidelines for single-laboratory validation of chemical methods for dietary supplements and botanicals*. Gaithersburg, MD: Author. Retrieved from http://www.aoac.org/imis15_prod/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf.

Bhattarai, S., Tran, V. H., & Duke, C. C. (2006). The stability of gingerol and shogaol in aqueous solutions. *Journal of Pharmaceutical Sciences, 90*, 1658-1664.

Brahma Naidu, P., Nemani, H., Meriga, B., Mehar, S. K., Potana, S., & Ramgopalrao, S. (2014). Mitigating efficacy of piperine in the physiological derangements of high fat diet induced obesity in Sprague Dawley rats. *Chemico-Biological Interactions, 221*, 42-51.

Brahma Naidu, P., Uddandrao, V. V. S., Ravindar Naik, R., Suresh, P., Meriga, B., Begum, M. S.,…Saravanan, G. (2016). Ameliorative potential of gingerol: Promising modulation of inflammatory factors and lipid marker enzymes expressions in HFD induced obesity in rats. *Molecular and Cellular Endocrinology, 419*, 139-147.

Chaveerach, A., Mokkamul, P., Sudmoon, R., & Tanee, T. (2006). Ethnobotany of the genus Piper (Piperaceae) in Thailand. *Ethnobotany Research and Applications, 4*, 223-231.

Connell, D. W., & Sutherland, M. D. (1969). A re-examination of gingerol, shogaol, and zingerone, the pungent principles of ginger (*Zingiber officinale* Roscoe). *Australian Journal of Chemistry, 22*, 1033-1043.

Ghareib, S. A., El-Bassossy, H. M., Elberry, A. A., Azhar, A., Watson, M. L., & Banjar, Z. M. (2015). 6-Gingerol alleviates exaggerated vasoconstriction in diabetic rat aorta through direct vasodilation and nitric oxide generation. *Drug Design, Development and Therapy, 9*, 6019–6026.

Grzanna, R., Lindmark, L., & Frondoza, C. G. (2005). Ginger-an herbal medicinal product with broad anti-inflammatory actions. *Journal of Medicinal Food, 8,* 125-132.

Gurley, B. J., Steelman, S. C., & Thomas, S. L. (2015). Multi-ingredient, Caffeine-containing Dietary Supplements: History, Safety, and Efficacy. *Clinical Therapeutics, 37,* 275-301.

Herman, A., & Herman, A. P. (2012). Caffeine's mechanisms of action and its cosmetic use. *Skin Pharmacology and Physiology, 26*, 8-14.

Hexsel, D., & Soirefmann, M. (2011). Cosmeceuticals for cellulite. *Seminars in Cutaneous Medicine and Surgery Journal Archive, 30,* 167–170.

Hexsel, D., Orlandi, C., & Zechmeister do Prado, D. (2005). Botanical extracts used in the treatment of cellulite. *Dermatologic Surgery, 31*, 866-873.

Impheng, H., Richert, L., Pekthong, D., Scholfield, C. N., Pongcharoen, S., Pungpetchara, I., &Srisawang, P. (2015). [6]-Gingerol inhibits de novo fatty acid synthesis and carnitine palmitoyltransferase-1 activity which triggers apoptosis in HepG2. *American Journal of Cancer Research, 5*, 1319-1336.

International Federation of Societies of Cosmetic Chemists. (1992). *IFSCC Monograph No. 2. The fundamentals of stability testing.* Cranford, NJ: Micelle Press. Retrieved from http://www.ifscc.org/pubs.htm.

Ko, H.-J., Lo, C.-Y., Wang, B.-J., Chiou, R. Y.-Y., & Lin, S.-M. (2015). Theaflavin-3,32-digallate, a black tea polyphenol, stimulates lipolysis associated with the induction of mitochondrial uncoupling proteins and AMPK–FoxO3A–MnSOD pathway in 3T3-L1 adipocytes. *Journal of Functional Foods, 17*, 271-282.

Kotaka, T., Kimura, S., Kashiwayanagi, M., & Iwamoto, J. (2014). Camphor Induces Cold and warm sensations with increases in skin and muscle blood flow in human. *Biological and Pharmaceutical Bulletin, 37,* 1913-1918.

Lantza, R. C., Chena, G. J., Sarihana, M., Sólyomb, A. M., Joladb, S. D., & Timmermann, B. N. (2007). The effect of extracts from ginger rhizome on inflammatory mediator production. *International Journal of Phytomedicine, 14*, 123-128.

Malini, T., Arunakaran, J., Aruldhas, M. M., & Govindarajulu, P. (1999). Effects of piperine on the lipid composition and enzymes of the pyruvate-malate cycle in the testis of the rat in vivo. *Biochemistry and Molecular Biology International. 47*, 537-545.

Mohamed, G. A., Ibrahim, S. R. M., Elkhayat, E. S., & El Dine, R. S. (2014). Natural anti-obesity agents. *Bulletin of Faculty of Pharmacy, Cairo University*, *52*, 269-284.

Mujumdar, A. M., Dhuley, J. N., Deshmukh, V. K., Raman, P. H., & Naik, S. R. (1990). Anti-inflammatory activity of pipperine. *Japanese Journal of Medical Science and Biology, 43*, 95-100.

Murlidhar, M., & Goswami, T. K. (2013). *Piper nigrum* and Piperine: An update. *Phytotherapy Research, 27*, 1121-1130.

National Drug System Development Committee. (2013). *National list of essential medicines*. Bangkok, Thailand: Author. Retrieved from http://www.thaihof.org/sites/default/files/herbal_book_56_0.pdf.

Ojewole, J. A. (2006). Analgesic, anti-inflammatory and hypoglycaemic effects of ethanol extract of *Zingiber officinale* (Roscoe) rhizomes (Zingiberaceae) in mice and rats. *Phytotherapy Research, 20*, 764–772.

Park, U.-H., Jeong, H.-S., Jo, E.-Y., Park, T., Yoon, S. K., Kim, E.-J.,…Um, S.-J. (2012). Piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPARγ activity in 3T3-L1 cells. *Journal of Agricultural and Food Chemistry, 60*, 3853-3860.

Roure, R., Oddos, T., Rossi, A., Vial, F., & Bertin, C. (2011). Evaluation of the efficacy of a topical cosmetic slimming product combining tetrahydroxypropyl ethylenediamine, caffeine, carnitine, forskolin and retinol, *In vitro, ex vivo* and *in vivo* studies. *International Journal of Cosmetic Science, 33*, 1–8.

Semwal, R. B., Semwal, D. K., Combrinck, S., & Viljoen, A. M. (2015). Gingerols and shogaols: Important nutraceutical principles from ginger. *Phytochemistry, 117,* 554-568.

Seon, O. K., & Woo-sik, J. (2012). Optimization of extraction condition for the 6-shogaol-rich extract from Ginger (*Zingiber officinale* Roscoe). *Preventive Nutrition and Food Science, 17,* 166-171.

Terranova, F., Berardesca, E., & Maibach, H. (2006). Cellulite: nature and aetiopathogenesis. *International Journal of Cosmetic Science, 28,* 157-167.

The Institute of Thai Traditional Medicine. (1995). *Thai traditional medicine training guide.* Bangkok, Thailand: The War Veterans Organization of Thailand under Royal Patronage of His Majesty the King Publishing.

Tzeng, T., & Liu, I. (2013). 6-Gingerol prevents adipogenesis and the accumulation of cytoplasmic lipid droplets in 3T3-L1 cells. *Phytomedicine,* 20481-20487.

Tzeng, T.-F., Chang, C. J., & Liu, I.-M. (2014). 6-Gingerol inhibits rosiglitazone-induced adipogenesis in 3T3-L1 adipocytes. *Phytotherapy Research, 28*, 187-192.

Vasanthi, H. R., & Parameswari, R. P. (2010). Indian spices for healthy heart–An overview. *Current Cardiology Reviews, 6*, 274-279.

Velasco, M. V. R., Tano, C. T. N., MachadoSantelli, G. M., Consiglieri, V. O., Kaneko, T. M., & Baby, A. R. (2008). Effects of caffeine and siloxanetriol alginate caffeine, as anti-cellulite agents, on fatty tissue: Histological evaluation. *Journal of Cosmetic Dermatology, 7*, 23-29.

Young, H.-Y., Luo, Y.-L., Cheng, H.-Y., Hsieh, W.-C., Liao, J.-C., & Peng, W.-H. (2005). Analgesic and anti-inflammatory activities of [6]-gingerol. *Journal of Ethnopharmacology, 96*, 207-210.