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GC-MS fingerprints combined with chemometric analysis for the authentication of *Morus alba* leaves from Thailand

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ABSTRACT

Introduction: *Morus alba* L. is one of the economic plants in Thailand. Several varieties are grown in various parts of the country. Different varieties from different regions may produce dissimilar chemical fingerprints. **Objectives:** The gas chromatography coupled to mass spectrometry (GC-MS) fingerprints of 2 varieties of *M. alba* leaves from three locations in Thailand, along with tea products will be established. **Materials and Methods:** The GC-MS chromatograms combined with chemometric analysis were used to analyze the extracts of *M. alba* leaves and their tea products. **Results:** A total of 83 compounds comprising terpenes, saturated fatty acids, unsaturated fatty acids and benzofurans were found in *M. alba* leaves from both varieties. Fifteen compounds such as phytol, oleic acid, and palmitic acid, with % peak area (detected at >0.04%) from high to low, were found in var. Buriram 60. Eighteen compounds such as phytol, palmitic acid, and oleic acid, respectively, were found in var. Khun Pai. Six compounds, that is, methyl palmitate, palmitic acid, methyl linolenate, phytol, oleic acid, and stearic acid were found in the tea products. **Conclusion:** The GC-MS fingerprints were combined with chemometric analysis for the authentication and characterization of two varieties of *M. alba* leaves from Thailand.

Keywords: Gas chromatography-mass spectrometry, chemometric analysis, chemical fingerprints, mulberry, *Morus alba*

INTRODUCTION

Morus alba L. (mulberry, white mulberry) (Family Moraceae) is a dioecious shrub (sometimes monoecious) 2–5 m in height. Leaves and fruits are edible.[1,2] It is a plant of economic importance in the sericulture and silk industry. In Thailand, the Queen Sirikit Department of Sericulture (QSDS) was established in 2009 under the Ministry of Agriculture and Cooperatives to enhance the potential of the production of mulberry, silk, and their products.[3] The Office of Sericulture Research and Development, QSDS has recommended nine varieties of *M. alba* for the plantation to yield leaves mainly as food for silkworms (*Bombyx mori* L.).[4] Chemical constituents isolated from *M. alba* leaves were reported from many studies,[5-10] including biologically active compounds such as 1-deoxynojirimycin,[9] quercetin,[10] and ramulus mori polysaccharides.[11,12] 1-Deoxynojirimycin,[13] ramulus mori polysaccharides,[11,12] chlorogenic acid, rutin,[14] and loliolide[15] have shown to the lower blood sugar in animals in various experiments. Hypoglycemic effect of

M. alba leaf extracts and extracts of leaf powder enriched with 1-deoxynojirimycin were also studied in healthy volunteers and type II diabetic patients.[16-19] Reports on hypoglycemic effects led to the consumption of *M. alba* leaves in Thailand as herbal tea products.

Leaves of *M. alba* grown in different regions may contain different chemical constituents. The establishment of the chemical constituents as chemical fingerprints is beneficial for the authentication and characterization of *M. alba* leaves and their tea products. The chemical fingerprint together with principal component analysis (PCA) has been used in the quality assessment of tea and herbal products.[20-24] The phenolic fingerprints from white and black mulberry leaves were also combined with PCA for the selection of an appropriate variety of *M. alba* for agro-food industries and for the authentication of plant materials.[25] Two varieties of *M. alba* grown in three regions of Thailand, as recommended by the Office of Sericulture Research and Development, QSDS, were selected for this study. Leaves of var. Buriram 60 (BR60), a certified variety by The Department of Agriculture, Ministry of Agriculture and Cooperatives, and var. Khun Pai (KP), a native variety, were collected from QSDS plantations in Kanchanaburi (KB), Nakhon-Ratchasima (Korat or KR), and BR provinces. The objectives of this study were to (i) establish the chemical fingerprints of *M. alba* leaves var. BR60 and var. KP, (ii) employ the established chemical fingerprints for the authentication of *M. alba* commercial tea products, and (iii) combine the chemical fingerprints with the application of chemometric analysis for the characterization of the chemical constituents in *M. alba* leaves.

MATERIALS AND METHODS

Plant Materials and Tea Products

Leaves (500 g) of *M. alba* var. BR60 and var. KP were certified and obtained from the plantation of QSDS centers in KB (KB, Location: 13.987479837758197, 99.42628641246432), Nakhon-Ratchasima (KR, Location: 14.952460491437439, 102.03128216106592), and BR (BR, Location: 15.538129784070055, 103.0017258493679) provinces, Thailand during January to March 2017. The fourth leaf from the top of the plant was collected, cut in half and petiole removed, according to the procedure recommended for tea processing. Leaves were dried at 60°C in an oven for 24 h and ground into powder.

Five *M. alba* tea products were purchased from health stores, tea stores and herbal markets.

All voucher specimens were kept at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Thailand.

Solvents and Chemicals

Ethanol was of analytical grade (Merck, Darmstadt, Germany). Methanol was of HPLC grade (Merck, Darmstadt, Germany). Distilled water was used for the preparation of tea samples and solutions.

Extraction Procedure

Two grams of leaf sample were macerated in 20 ml of ethanol, shaking frequently every 10 min, for 1 h and filtered. Maceration was repeated 2 more times. The filtrates were combined and dried under vacuum with a rotary evaporator and stored in a refrigerator at 4°C until analysis.

One gram of tea sample was macerated in 10 ml of ethanol and preceded under the same procedure as the leaf sample and stored in a refrigerator at 4°C until analysis.

All samples were weighed and extracted in triplicate.

Gas Chromatography-Mass Spectrometry (GC-MS)

Sample preparation

Ethanolic extracts of leaf and tea sample were dissolved in methanol to obtain a 1 mg/ml solution. The solution was filtered through a 0.45 μm syringe filter and put into a vial for GC-MS analysis.

GC-MS condition

The chemical constituents were analyzed using an Agilent GC-MS, model 6890 N equipped with 5973 mass selective detector (EIMS with 70 eV of electron energy as ion source and a quadrupole mass analyzer). Chromatographic condition was modified from Shidong *et al*.[22] Two microliters of a sample were injected into an HP-5MS column (0.25 mm i.d. × 30 m × film thickness 0.25 μm). The initial temperature was set at 60°C, whereas the injector temperature was set at 250 °C, and throughout the process, the temperature was set to increase at a speed of 3°C/min. Helium was used as a carrier gas at a constant flow rate of 1 ml/min. The total run time was 63 min and mass scan range was 50–500 amu.

Identification of chemical constituents

Identification of compounds was done based on the comparison of mass spectra with spectra in Wiley 8th with NIST08 Library (Product number G1025B, Agilent Technologies). Compound with percentage peak area (% peak area) higher than 0.04% was determined as a constituent in the leaf extract.

Similarity Analysis

The common method based on the Pearson's correlation coefficient was used to evaluate the similarity between the chemical fingerprints of *M. alba* leaves and their commercial tea products. The Pearson's correlation coefficient of each similarity measure was calculated by Microsoft Office Excel version 2013.

Hierarchical Cluster Analysis (HCA) and PCA

HCA and PCA are widely used as unsupervised learning methods to segment the associated objects in groups based on the selected properties.

HCA was implemented with sklearn.cluster.AgglomerativeClustering in the Scikit-learn library using the Euclidean distance and Ward's method as similarity measure and agglomerative algorithm, respectively. The dendrogram was obtained by using scipy.cluster.hierarchy.dendrogram in the SciPy library.

PCA was performed with Scilab version 6.0.0 open-source software, Windows version 7.0 (available from: http://www.scilab.org). Data for HCA and PCA analyses were collected

from GC-MS chromatograms of three replicates of each sample from two varieties and were taken from the retention time interval between 5 to 70 min. Twenty-seven peaks obtained from leaves *of M. alba* and giving a percentage peak area of more than 0.04 were selected and subjected to HCA and PCA as variables.

RESULTS AND DISCUSSION

GC-MS Fingerprints of *M. alba* Leaf Extracts

The GC-MS chromatograms of the ethanolic leaf extracts from two varieties of *M. alba*, BR60 and KP, cultivated in three different locations in Thailand, that is, KB, KR, and BR provinces were analyzed to obtain 18 fingerprints.

A total of 83 compounds comprising terpenes, saturated fatty acids, unsaturated fatty acids, and benzofurans were found in both varieties of *M. alba* from all three provinces. In the leaf extracts of BR60 grown in KB, KR, and BR provinces, 19, 16, and 26 compounds, respectively, were found [Table 1]. Fifteen compounds such as phytol (or 3,7,11,15-tetramethylhexadec-2-en-1-ol) (% peak area of 10.3–41.1%), oleic acid (or (*Z*)-octadec-9-enoic acid) (3.4–32.3%), and palmitic acid (or hexadecanoic acid) (2.2–17.5%) were found in common in all leaf extracts from three provinces. In the leaf extracts of KP grown in KB, KR, and BR provinces, 23, 18, and 21 compounds, respectively, were found [Table 2]. Eighteen compounds such as phytol (6.5–34.4%), palmitic acid (4.5–17.6%), and stearic acid (or octadecanoic acid) (4.46–9.10%), with respect to % peak area, were found in common in all leaf extracts from three provinces. Twenty-seven compounds were found altogether in all leaf extracts of both varieties obtaining from all three provinces. Fourteen compounds, including phytol, palmitic acid, stearic acid, methyl linolenate (or methyl

Table 1: Retention time and % peak area of compounds found in ethanolic leaf extracts of *M. alba* var. Buriram 60 from KB, KR, and BR provinces in Thailand

Peak	Retention time (min)	% Peak area			Compound Name (or common name)
		KB	KR	BR	
1	5.79	0.09–0.23	0.06–0.21	0.04–0.05	N, N-dimethyl ethanethioamide
2	29.66	0.18–0.50	0.16–0.31	0.22–0.30	4,4,7a-trimethyl-6,7-dihydro-5*H*-1-benzofuran-2-one
3	38.26	-	-	0.11–0.36	myristic acid (or tetradecanoic acid)
4	38.61	0.21–0.46	0.24–1.07	0.45–0.54	(-)-loliolide (or (6*S*,7a*R*)- 6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5*H*-1-benzofuran-2-one)
5	40.18	0.17–0.59	-	0.21–0.41	myristaldehyde (or tetradecanal)
6	40.38	-	-	0.12–0.64	propan-2-yl-tetradecanoate
7	40.75	0.34–0.66	0.27–0.50	0.64–0.98	neophytadiene (or 7,11,15-trimethyl-3-methylidenehexadec-1-ene)
8	40.96	0.66–1.25	0.61–0.84	0.39–1.56	phytone (or 6,10,14-trimethylpentadecan-2-one)
9	42.18	-	-	0.27–0.44	unknown
10	43.04	-	-	0.07–0.34	7,9-di-tert-butyl-1-oxaspiro[4.5]deca-6,9-diene-2,8-dione
11	43.76	0.56–6.51	0.61–0.84	1.70–3.50	methyl palmitate (or methyl hexadecanoate)
12	44.99	2.21–5.02	13.91–17.50	5.08–11.28	palmitic acid (or hexadecanoic acid)
13	45.48	-	-	0.52–0.58	unknown
14	45.97	-	0.44–0.55	0.36–0.53	ethyl palmitate (or ethyl hexadecanoate)
15	48.99	0.29–3.34	0.39–0.55	0.82–2.28	methyl linoleate (or methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate)
16	49.19	2.48–18.33	2.01–3.39	4.57–12.72	methyl linolenate (or methyl (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate)
17	49.52	33.14–41.11	10.25–16.29	26.84–32.30	phytol (or 3,7,11,15-tetramethylhexadec-2-en-1-ol)
18	50.05	0.28–1.75	0.06–0.20	0.78–0.94	methyl stearate (or methyl octadecanoate)
19	50.18	0.40–1.16	2.31–2.91	0.44–0.61	linoleic acid (or (9*Z*,12*Z*)-octadeca-9,12-dienoic acid)
20	50.39	3.36–8.67	22.35–32.25	5.93–8.96	oleic acid (or (*Z*)-octadec-9-enoic acid)
21	51.20	1.59–2.22	5.91–6.57	4.70–6.61	stearic acid (or octadecanoic acid)
22	52.57	0.33–0.59	-	0.88–1.04	unknown
23	55.59	-	-	0.26–0.36	*trans*-ferulic acid (or (*E*)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid)
24	56.97	0.28–0.45	-	0.11–0.17	farnesyl acetone (or (5*E*,9*E*)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one)
25	60.74	2.22–4.54	0.74–1.23	1.58–2.45	monopalmitin (or 2,3-dihydroxypropyl hexadecanoate)
26	60.94	0.15–2.22	-	1.08–1.35	(6*E*,10*E*,14*E*,18*E*)-2,6,10,19,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene

Table 2: Retention time and % peak area of compounds found in ethanolic leaf extracts of *M. alba* var. Khun Pai from KB, KR, and BR provinces in Thailand

Peak	Retention time (min)	% Peak area			Compound Name (or common name)
		KB	KR	BR	
1	5.79	0.15–0.19	0.09–0.18	0.07–0.18	N, N-dimethyl ethanethioamide
2	29.66	0.20–0.54	0.13–0.29	0.27–0.45	4,4,7a-trimethyl-6,7-dihydro-5*H*-1-benzofuran-2-one
3	38.61	0.37–0.95	0.27–0.56	0.60–1.01	(-)-loliolide (or (6*S*,7a*R*)-6-hydroxy-4,4,7a-trimethyl-6,7-dihydro-5*H*-1-benzofuran-2-one)
4	40.18	0.53–0.82	-	-	myristaldehyde (or tetradecanal)
5	40.75	0.45–0.93	0.50–1.04	0.75–1.31	neophytadiene (or 7,11,15-trimethyl-3-methylidenehexadec-1-ene)
6	40.96	0.92–1.78	0.32–0.63	0.62–1.08	phytone (or 6,10,14-trimethylpentadecan-2-one)
7	42.18	0.14–0.42	0.20–0.30	0.22–0.52	unknown
8	43.76	2.29–3.88	0.65–1.62	0.40–2.76	methyl palmitate (or methyl hexadecanoate)
9	44.99	4.46–5.96	11.07–17.57	5.02–9.28	palmitic acid (or hexadecanoic acid)
10	45.48	0.16–0.41	-	-	unknown
11	45.81	0.14–0.21	-	-	farnesol (or 3,7,11-trimethyldodeca-2,6,10-trien-1-ol)
12	45.97	0.27–0.38	0.34–1.08	0.43–0.52	ethyl palmitate (or ethyl hexadecanoate)
13	48.99	1.54–3.33	0.32–0.59	0.28–2.30	methyl linoleate (or methyl (9*Z*,12*Z*)-octadeca-9,12-dienoate)
14	49.19	5.69–11.47	1.24–4.16	1.29–9.51	methyl linolenate (or methyl (9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate)
15	49.52	23.28–34.40	6.54–10.54	26.16–31.62	phytol (or 3,7,11,15-tetramethylhexadec-2-en-1-ol)
16	50.05	-	-	0.14–0.83	methyl stearate (or methyl octadecanoate)
17	50.18	0.69–1.16	1.59–3.68	0.45–3.63	linoleic acid (or (9*Z*,12*Z*)-octadeca-9,12-dienoic acid)
18	50.39	0.74–1.22	15.39–25.65	8.05–20.69	oleic acid (or (*Z*)-octadec-9-enoic acid)
19	51.20	7.59–9.10	6.13–7.70	4.46–5.38	stearic acid (or octadecanoic acid)
20	52.57	0.74–1.01	-	0.73–0.97	unknown
21	55.59	0.13–0.26	-	0.04–0.15	*trans*-ferulic acid (or (*E*)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid)
22	56.97	0.20–0.37	0.15–0.23	0.13–0.29	farnesyl acetone (or (5*E*,9*E*)-6,10,14-trimethylpentadeca-5,9,13-trien-2-one)
23	60.74	2.82–2.98	0.36–2.68	2.34–2.82	monopalmitin (or 2,3-dihydroxypropyl hexadecanoate)
24	60.94	0.64–2.31	1.26–4.72	0.60–1.15	(6*E*,10*E*,14*E*,18*E*)-2,6,10,19,23-pentamethyltetracosa-2,6,10,14,18,22-hexaene

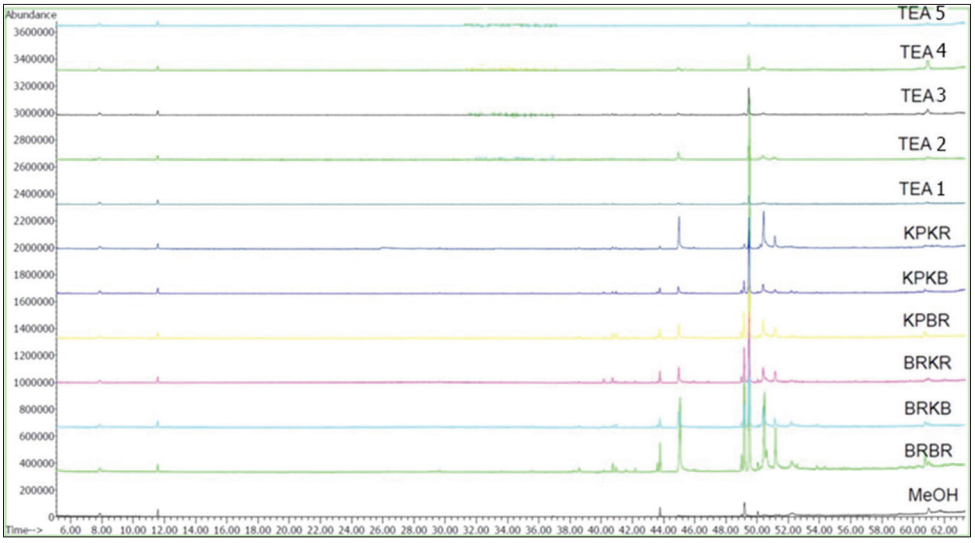

Figure 1: Chemical fingerprints of ethanolic extracts of *M. alba* leaves and tea products. (BRBR = var. BR60 from Buriram, BRKB = var. BR60 from Kanchanaburi, BRKR = var. BR60 from Nakhon-Ratchasima, KPBR = var. KP from Buriram, KPKB = var. KP from Kanchanaburi, KPKR = var. KP from Nakhon-Ratchasima provinces)

Table 3: Retention time and % peak area of main compounds found in ethanolic extracts of *M. alba* tea products

Peak	Retention time (min)	% Peak area					Compound Name (or common name)
		1	2	3	4	5	
1	43.76	0.367–1.004	0.350–0.826	0.967–1.623	0.299–0.435	0.146–0.314	methyl palmitate (or methyl hexadecanoate)
2	44.99	5.357–6.806	14.852–20.383	3.463–5.250	4.529–11.258	15.209–18.594	palmitic acid (or hexadecanoic acid)
3	49.19	0.447–1.932	0.094–1.152	1.721–2.281	0.889–1.044	0.394–0.598	methyl linolenate (or methyl (9*Z*,12*Z*,15*Z*) -octadeca-9,12,15-trienoate)
4	49.52	11.784–19.952	13.891–18.146	29.656–33.718	15.472–20.472	17.354–23.402	phytol (or 3,7,11,15 -tetramethylhexadec-2-en-1-ol)
5	50.39	6.826–10.543	14.809–22.556	3.400–5.513	2.425–24.235	16.786–28.735	oleic acid (or (*Z*)-octadec-9-enoic acid)
6	51.20	0.491–2.354	4.872–6.434	0.667–2.229	2.025–2.198	3.495–4.263	stearic acid (or octadecanoic acid)

(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoate) and oleic acid, were found in common in both varieties. However, it was remarkable that for both varieties, % peak area of phytol was much less and % peak area of palmitic acid, oleic acid, and linoleic acid (or (9*Z*,12*Z*)-octadeca-9,12-dienoic acid) were much higher in leaves from KR than in leaves from KB and BR.

The GC-MS chemical fingerprints of the ethanolic leaf extracts from two varieties of *M. alba* are shown in Figure 1.

GC-MS Fingerprints of *M. alba* Tea Extracts

The GC-MS chromatograms of the ethanolic extracts of 5 *M. alba* commercial tea products were analyzed to obtain 15 fingerprints. On the package, all tea products were labeled *M. alba* leaves without other herbal plants. Five tea products shared six compounds, including phytol (11.8–33.7%), palmitic acid (3.5–20.4%), oleic acid (2.4–28.7%), stearic acid (0.5–6.4%), methyl palmitate (or methyl hexadecanoate) (0.1–1.6%), and methyl linolenate (0.09–2.3%), which were also found in both varieties of *M. alba* leaves [Table 3]. The GC-MS chemical fingerprints of the ethanolic extracts of tea products were compared with those from *M. alba* leaves [Figure 1].

Similarity Analysis of *M. alba* Leaf Samples and Tea Extracts

The established chemical fingerprints from the extracts of both varieties of *M. alba* leaves were used for the authentication of *M. alba* commercial tea products by the similarity analysis. Pearson's correlation coefficient among *M. alba* leaves and tea products is shown in Table 4 demonstrated that the chemical constituents found in all tea products were associated with the chemical constituents presented in both varieties of *M. alba* leaves. These correlations confirmed that all tea products contained *M. alba* leaves.

Chemometric Analysis

The percentage peak area of 27 variables corresponding to all chemical constituents from GC-MS chromatograms was analyzed by HCA and PCA. HCA was carried out to obtain hierarchical associations among *M. alba* leaves as a dendogram

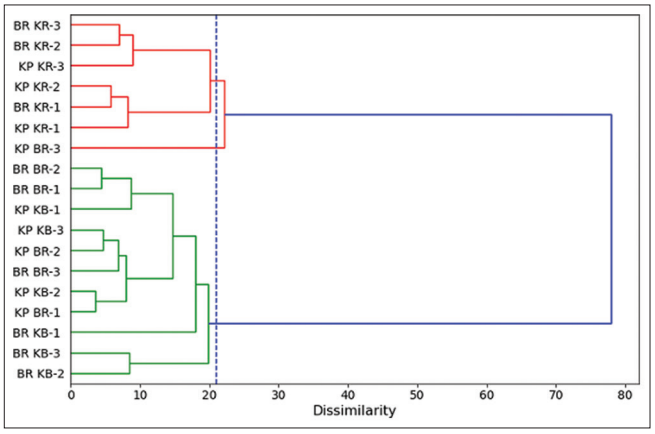

Figure 2: Dendrogram of the hierarchical cluster analysis among *M. alba* leaves using Ward's method based on squared Euclidean distance. (BRKR = var. BR60 from Nakhon-Ratchasima, KPKR = var. KP from Nakhon-Ratchasima, KPBR = var. KP from Buriram, BRBR = var. BR60 from Buriram, KPKB = var. KP from Kanchanaburi, BRKB = var. BR60 from Kanchanaburi provinces)

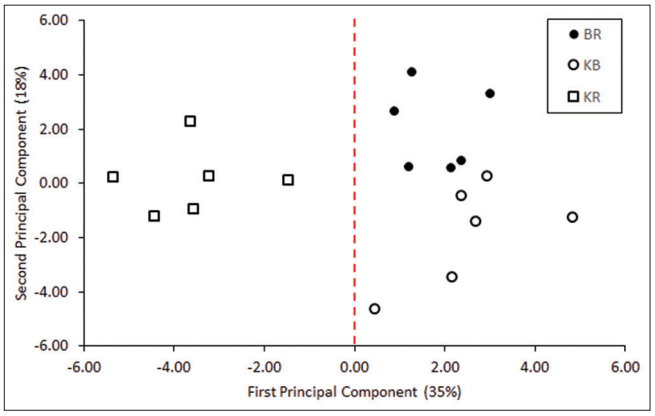

Figure 3: Scores plots obtained from the PCA of *M. alba* leaves

[Figure 2]. The dendrogram was examined at point 22 (dissimilarities distance), and three groups were found based on existing similarities with respect to variables analyzed. The first group comprised all six samples from both varieties obtained from KR province (BRKR 1–3 and KPKR 1–3). The

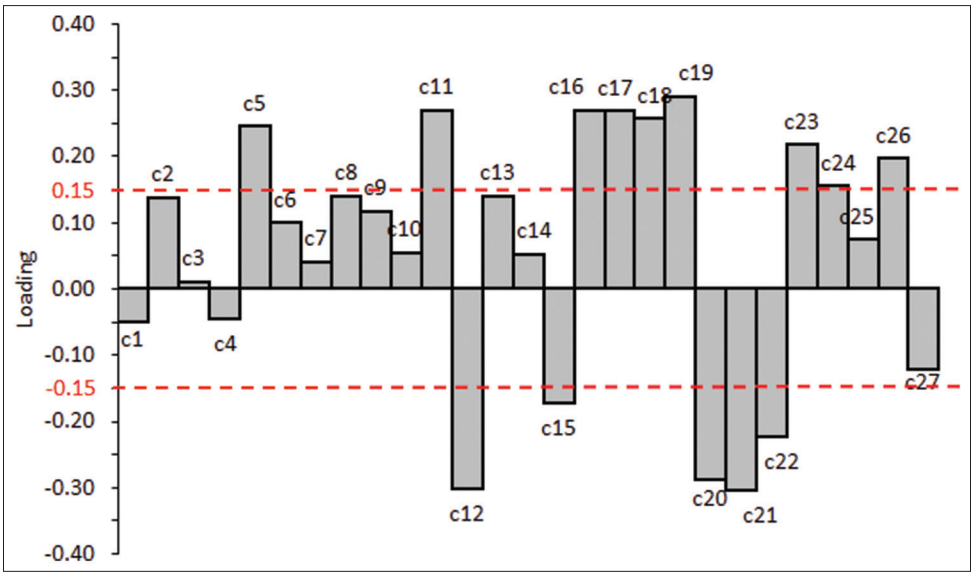

Figure 4: Loadings bar plots obtained from the PCA of *M. alba* leaves

Table 4: Pearson's correlation coefficient of the chemical constituents among *M. alba* leaves and tea products

	Tea1	Tea2	Tea3	Tea4	Tea5
var. Buriram 60	0.859	0.842	0.881	0.843	0.859
var. Khun Pai	0.868	0.884	0.848	0.876	0.901

other two included one sample from BR province (KPBR 3) and the rest of the samples.

The cluster analysis results were confirmed by PCA as shown in the score and loading plot of Figures 3 and 4. PCA was an unsupervised machine learning method that transformed a larger number of original variables which had correlations into a smaller number of uncorrelated variables called factors or principal components. In general, PCA interpretation was displayed as a two-dimensional graph where the principal axis represented the directions of the first two main principal components (First principal component, PC1 vs. Second principal component, PC2): 1) scores plot and 2) loadings plot. PC1 in the scores plot of Figure 3 presented differentiation among two groups. PC1 and PC2 collectively were able to explain the highest variance of 53% information and the first seven uncorrelated principal components explained 88% of the total variation.

Figure 3 showed that all leaf samples from KR province strongly negatively correlated with PC1, whereas samples from BR and KB Provinces positively correlated with PC1. The loading bar plot in Figure 4 illustrated that 14 marker peaks out of 27 peaks (with loadings <-0.15 or >0.15) at retention time 40.18 (c5, myristaldehyde), 43.76 (c11, methyl palmitate), 44.99 (c12, palmitic acid), 45.97 (c15, ethyl palmitate), 48.99 (c16, methyl linoleate), 49.19 (c17, methyl linolenate), 49.52 (c18, phytol), 50.05 (c19, methyl stearate), 50.18 (c20, linoleic acid), 50.39 (c21, oleic acid), 51.20 (c22, stearic acid), 52.57 (c23, unknown), 55.59 (c24, *trans*-ferulic acid), and 60.74 (c26, monopalmitin) were able to discriminate among the leaves from KB, KR, and BR provinces. With negative PC1-loadings, 5 marker peaks at retention time 44.99 (c12), 45.97 (c15), 50.18 (c20), 50.39 (c21), and 51.20 (c22) corresponded to samples from KR. On the other hand, with positive PC1-loadings, nine marker peaks at retention time 40.18 (c5), 43.76 (c11), 48.99 (c16), 49.19 (c17), 49.52 (c18), 50.05 (c19), 52.57 (c23), 55.59 (c24), and 60.74 (c26) corresponded to samples from KB and BR provinces.

From the chemometric analysis, for both varieties, samples from KR province could be characterized by five chemical constituents, that is, palmitic acid, ethyl palmitate, linoleic acid, oleic acid, and stearic acid. However, the GC-MS fingerprints showed that the % peak area of palmitic acid, oleic acid and linoleic acid were much higher in leaves from KR than KB and BR provinces.

CONCLUSIONS

The GC-MS fingerprints from the ethanolic extracts of *M. alba* leaves var. BR60 and var. Khun Pai grown in KB, KR, and BR provinces presented 14 metabolites in common, with five main compounds: Phytol, palmitic acid, stearic acid, methyl linolenate, and oleic acid. With the samples studied, profiling the chemical constituents using GC-MS chromatograms could authenticate two varieties of *M. alba* leaves, and associate their tea products with the leaves. Moreover, the GC-MS fingerprints combining with chemometric analysis were preferred approaches for the authentication and characterization of *M. alba* leaves from various sources and varieties. In addition, with further studies of the chemical constituents from other chromatographic and spectroscopic analyses, a complete metabolomic fingerprint for *M. alba* leaves from Thailand will be established.

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