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Original Article

Classification of some Boesenbergia and Alpinia extracts and their medicinal products based on chemical composition, antioxidant activity, and concentration of some heavy metals

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Abstract

The result showed total phenolic content, total flavonoid content and radical scavenging activity in ethanol extracts higher than oil extracts and massage products of *Boesenbergia rotunda*, *Alpinia conchigera*, *Alpinia galangal*, and *Alpinia siamensis*. Fourier-transform infrared spectroscopy of the ethanol extracts provided specific peaks in a range of 4,000 to 550 cm^{-1}. Chemical differentiation between Alpinia sp. and Boesenbergia sp. was indicated by main peaks that corresponded to aromatic and N-H in amino acids, O-H in phenyl group, and C-O in carbohydrates and glycoprotein. Chemical differentiation among three Alpinia species were wavenumber ranges of C-O in acid or ester, C-O in carbohydrates and glycoprotein, and C-H in isoprenoids. The cluster and PCA analysis showed good separation of chemical compositions and antioxidant activity among the ethanol extract, massage solution and oil extract. Additionally, Ni, and Cd were found in all massage products, especially Ni above its maximum permission level.

Keywords: lignocellulosic biorefinery, phenolic compounds, autohydrolysis, aqueous two-phase systems, eucalyptus residues

1. Introduction

Medicinal plants in the Zingiberaceae family are widely throughout tropical and subtropical areas, and are abundant sources of antioxidants, nutrients and fiber that are often used as local food and folk medicine (Rachkeeree *et al.*, 2018). Specially, their rhizomes are well known as major ingredient in local food, spices, medicines and cosmetic due to their high therapeutic and nutritional values (Saensouk, Saensouk, Pasorn, & Chanshotikul, 2018). Nowadays, healthy food and organic medicinal products have become worldwide increasingly popular. Several bioactive agents (i.e. flavonoids) can act as antioxidants that are linked to prevent several diseases, such as anti-oxidative activity and inflammation (Panche, Diwan, & Chandra, 2016).

In Thailand, the Zingiberaceae family has been classified into 30 genera and 300 species (Larsen & Larsen, 2006). Of these, *Boesenbergia rotunda* is one of local edible herbs that there are several medicinal properties (i.e. antioxidant activity and antibacterial activity) and used as natural ingredient in Thai food (Ongwisespaiboon & Jiraung koorskul, 2017). Moreover, other edible herbs in Zingi beraceae family, namely *Alpinia conchigera*, *Alpinia galanga* and *Alpinia siamensis*, are also commonly used as food and spices (Saensouk *et al.*, 2018). Alpinia species contain several bioactive agents (i.e. flavonoids, terpenoids and kavalactones) with antioxidant activities involving arterial hypertension and inflammation (Victório, 2011).

Nowadays, trend of consumer awareness and concern involving safety and quality of foods and cosmetics is growing that lead to increase consumer demand for natural

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foods and products. To reduce side effects from synthetic chemicals, native herb is become popular and applied as medicinal products due to its high medical value, safety, friendly to human, and cheap. Massage ointment is one of herbal medicinal products that is used as biologically based therapy and recognized as complementary and alternative medicine.

However, massage products consist of several components (i.e. vegetable oil, essential oil, and phytochemicals) that may show neither synergistic or antagonistic interactions, including plant substance (i.e. solvent extract and oil) and artificial ingredients may be contaminated with some toxic elements, such as pesticides and heavy metals that may effect on skin or health of consumers (Andersen, Holmberg, Larsen, Søborg, & Cohr, 2006). Nowadays, heavy metal contamination and biological properties of massage products from extracts of *B. rotunda*, *A. conchigera*, *A. galanga* and *A. siamensis* is still unknown. Specially, phytochemicals and medical properties of *A. siamensis* are not well known. Combination of Fourier transform infrared spectra (FTIR) fingerprint and bioactivity assessment of this plant extracts is novel knowledge and big challenge for future health care, such as development of medicinal products.

Therefore, our major objective was to characterize ethanol extracts, oil extracts, and massage products of *B. rotunda*, *A. conchigera*, *A. galanga* and *A. siamensis* by total phenlic contents, total flavonoid contents, antioxidant activity, and Fourier-transform infrared (FTIR) spectra, and to monitor heavy metal contamination in massage products from the local herbs. These data was useful for improvement of their pharmaceutical activities, safety and quality as healthy natural products for consumers, especially children and women.

2. Method and Materials

2.1 Sample collection

Boesenbergia rotunda, *Alpinia conchigera*, *Alpinia galanga* and *Alpinia siamensis* were collected from an organic family farm in Nong Saeng sub-district, Pakphli district, Nakhon Nayok province (14.197479 °N, 101.303588 °E). The external morphology of each plant sample was then identified by comparing with the samples from BGO plant databases of The Botanical Garden Organization (2011). Some specimens were kept at Faculty of Environmental Culture and Eco-tourism, Srinakharinwirot University. After that, each sample was cleaned, and left for 24 hours at room temperature, followed by cutting and homogenizing into small pieces.

2.2 Sample extraction by coconut oil

Each ground sample was fried in coconut oil on 1:1 ratio at 80 ^{o}C for 1 hour, and left it cool at room temperature, then filtrated through a fine sieve, and kept at 4 ^{o}C. Each sample was processed in duplicate.

2.3 Sample extraction by solvent extraction

Each ground sample was mixed with 95% ethanol solvent in 1:2 ratio, and incubated at 37 ^{o}C for 16 hours. After that, the solvent was evaporated by a vacuum evaporator (IKAa RV10) for 2-3 hours, and kept at -20 ^{o}C (Thummajit sakul, Kaewsri, & Deetae, 2016).

2.4 Production of massage solution and ointment

Massage ointment is a medicinal product that consists of borneol, menthol, camphor, basic oil, petroleum jelly and wax, but massage solution is a form without petroleum jelly and wax and suitable for determining biological activities. A stock solution was prepared by mixing camphor, borneol and menthol in the ratio of 5: 4.5: 1.25, and melted at approximately 80 ^{o}C temperature. After letting it to cool down, oil extract (20 ml) from each plant sample was added and stirred, and poured into a glass bottle. For 95% ethanol extract, each sample extract was used for 2.5 ml, and coconut oil for 17.5 ml was added in the mixed solution. Coconut oil was used as basic oil or non-volatile oils.

For ointment preparation, petroleum jelly and wax were mixed in the ratio of 15.6: 3.4, and melted at approximately 90 ^{o}C temperature. After that, the stock solution was added, and left to cool down. Each extract was added, stirred, poured into a glass bottle, and left at room temperature.

2.5 Total phenolic contents

The Folin–Ciocalteu colorimetric technique was used to estimate total phenolic contents (Deetae, Parichanon, Trakunleewatthana, Chanseetis, & Lertsiri, 2012; Thummajit sakul *et al.*, 2016). Each sample (300 µl) was reacted with Folin-Ciocalteu reagent (1.5 ml) at room temperature for 5 min, and then reacted with sodium carbonate (7.5% w/v) (1.2 ml) for 30 min at room temperature, followed by measuring the absorbance at 765 nm using a spectrophotometer (Model T60UV). Each reaction was performed in duplicate. Gallic acid at concentration 0 to 200 µg/ml was used as positive control to produce a calibration curve ($y=0.0114x-0.1699$, $R^2 = 0.9518$). The total phenolic content was measured from the calibration curve and expressed as mg gallic acid per g extract for the ethanol extract, mg gallic acid per g fresh weight for the oil extract, or mg gallic acid per ml solution for the massage solution.

2.6 Total flavonoid contents

Total flavonoid content was measured using aluminum chloride colorimetric method (Chang, Yang, Wen, & Chern, 2002). Rutin was prepared in 80% ethanol solvent at concentration 25, 50 and 100 µg/ml and was used to generate a calibration curve ($y=0.0019x-0.0078$, $R^2 = 0.9918$). Each sample (500 µl) was reacted with 95% ethanol (1.5 ml), 10% aluminum chloride (0.1 ml), 1M potassium acetate (0.1 ml), and distilled water (2.8 ml). After that, each reaction was incubated for 30 min at room temperature, and absorbance at 415 nm was determined using a spectrophotometer (Model T60UV). Distilled water was used as blank. Each sample was performed in duplicate, and the total flavonoid content was measured from the calibration curve and expressed as mg rutin equivalent per g extract for the ethanol extract, mg rutin equivalent per g fresh weight for the oil extract, or mg rutin equivalent per ml solution for the massage solution.

2.7 Antioxidant activity

Antioxidant activity was measured by ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium] method (Deetae *et al.* 2012). Briefly, ABTS radical solution was prepared from 7 mM ABTS solution (10 ml) and 140 mM potassium persulfate (179 µl) by incubation under dark at room temperature for 12-16 hours. The ABTS radical solution (3.9 ml) with absorbance 0.700±0.005 at 734 nm was reacted with each extract or massage solution (20 µl) in the dark at room temperature for 6 min, and the absorbance at 734 nm was measured. Each reaction was carried out in duplicate. The percentage of antioxidant capacity was calculated according to Deetae *et al.* (2012) and Thummajitsakul *et al.* (2016).

The radical scavenging capacity was demonstrated as 50% effective concentration (EC_{50}) that was sample concentration needed to reduce ABTS radicals by 50% and was calculated from each simple linear regression (R^2 =0.97-1.00).

2.8 Fourier-transform infrared spectroscopy (FTIR)

The powdered sample (5 mg) from each ethanol extract of the plant samples was directly placed on the center of the crystal plate in FTIR spectroscope (Spectrum Two™, Perkin Elmer, USA). Each sample was pressed using an identical mechanical pressure to provide FTIR spectra in a range of 550 to 4,000 cm^{-1} with a resolution of 4 cm^{-1}. Duplicate sample was run for five times. Each FTIR spectra was analyzed with PerkinElmer spectrum IR version 10.6.0, and compared with the reports of Coates (2006), Caunii, Pribac, Grozea, Gaitin, and Samfira (2012), Cao, Wang, Shang, and Zhao (2017), and Topalăa, Tătarua, and Ducu (2017).

2.9 Determination of heavy metals

Each massage ointment (0.5 g) or massage solution (15 ml) were digested with 65% HNO_3 (10 ml) at 100° C until completely digestion, then added 1% HNO_3, and filtrated by Whatman No. 1 paper. The volume of each digested sample was then adjusted with 1% HNO_3 to 50 ml. Each digestion was carried out in duplicate. Heavy metals namely Cu, Pb, Cd, Ni, Hg, Mn and Zn in the digested samples were determined using an atomic absorption spectrometry (Model 200 Series AA, Agilent Technologies, U.S.A.). External standard method was used to generate a standard curve (R^2 = 1), which was used to compare concentration of each heavy metal (Thumma jitsakul *et al.*, 2018).

2.10 Statistical analysis

Descriptive statistics (i.e. mean, SD and percentage) were used to express heavy metal concentration, total phenolic content, total flavonoid content, and antioxidant activity by using PSPP program version 0.10.5 (Pfaff *et al.*, 2013). In addition, the principal component analysis (PCA) and unweighted pair group method (UPGMA) were performed by Paleontological statistic program version 3.16 (Hammer, Harper, & Ryan, 2001).

3. Results and Discussion

In our result, total phenolic contents in the ethanol extracts and oil extracts were found in order of *A. galanga* > *A. siamensis* > *A. conchigera* > *B. rotunda* ranging from 23.62 to 31.43 mg gallic/ g extract and from 2.39 to 2.74 mg gallic/g fresh weight, respectively. Additionally, total flavonoid contents in the ethanol extracts were found in order of *A. galanga* > *B. rotunda* > *A. conchigera* > *A. siamensis* ranging from 19.36 to 77.62 mg rutin equivalent per g extract. However, the order of total flavonoid content in oil extracts was *B. rotunda* > *A. galanga* > *A. conchigera* > *A. siamensis* ranging from 1.73 to 2.75 mg rutin equivalent per g fresh weight. Moreover, radical scavenging activities were found in the ethanol extracts of *A. galanga*, *A. siamensis*, *A. conchigera*, and *B. rotunda* that were expressed as $1/EC_{50}$ values for 108.70, 96.15, 81.97 and 39.84, respectively. However, the order of radical scavenging activities in the oil extracts were *B. rotunda* > *A. conchigera* > *A. galanga* > *A. siamensis* ranging from 0.12 to 0.35 (Table 1).

Moreover, the result showed that the order of total phenolic content in the massage solution from the ethanol extract was *A. siamensis*> *A. conchigera* > *A. galanga* > *B. rotunda* in ranging of 0.0541 to 0.0657 mg gallic/ ml solution, while the order of total phenolic content of massage solution from oil extract was *A. conchigera* > *A. siamensis* > *A. galanga* > *B. rotunda* in ranging of 0.0711 to 0.854 mg gallic/ ml solution (Table 2).

Table 1. Total phenolic contents, total flavonoid contents, and antioxidant activities of 95% ethanol extracts and oil extracts of *B. rotunda*, *A. conchigera*, *A. galanga* and *A.siamensis*

Plant types	Samples	Total phenolic contents (mg gallic/ g extract)	Total flavonoid contents (mg rutin equivalent/ g extract)	Antioxidant activities	
				EC50	1/EC
B. rotunda	95% ethanol extract	23.62±0.92	74.87±3.30	0.0251±0.0009	39.84
	Oil extract	2.39±0.36[a]	2.75±0.12[b]	2.8440±1.6866	0.35
A. conchigera	95% ethanol extract	24.73±4.23	48.36±1.49	0.0122±0.0006	81.97
	Oil extract	2.51±0.22[a]	1.82±0.02[b]	3.6734±1.1078	0.27
A. galanga	95% ethanol extract	31.43±5.96	77.62±2.38	0.0092±0.0021	108.70
	Oil extract	2.74±0.20[a]	2.21±0.10[b]	5.6292±4.3209	0.18
A.siamensis	95% ethanol extract	25.99±1.64	19.36±2.13	0.0104±0.0014	96.15
	Oil extract	2.58±0.25[a]	1.73±0.08[b]	8.0980±1.7095	0.12

[a] Total phenolic content was expressed as mg gallic/ g fresh weight
[b] Total flavonoid contents was expressed as mg rutin equivalent/ g fresh weight

Table 2. Total phenolic contents, total flavonoid contents, and antioxidant activities of massage solution of *B. rotunda*, *A. conchigera*, *A. galanga* and *A.siamensis*

Plant types	Samples	Total phenolic contents (mg gallic/ ml solution)	Total flavonoid contents (mg rutin equivalent/ ml solution)
B. rotunda	Massage solution 1[1]	0.054±0.002	0.29±0.04
	Massage solution 2[2]	0.071±0.018	0.15±0.04
A. conchigera	Massage solution 1[1]	0.059±0.007	0.81±0.34
	Massage solution 2[2]	0.085±0.012	0.02±0.01
A. galanga	Massage solution 1[1]	0.058±0.003	0.72±0.13
	Massage solution 2[2]	0.075±0.003	0.20±0.04
A. siamensis	Massage solution 1[1]	0.066±0.014	0.70±0.49
	Massage solution 2[2]	0.080±0.014	0.04±0.03
Massage solution without sample[3]		-	1.92±0.02

[1] Massage solution 1 was produced from 95% ethanol extract.
[2] Massage solution 2 was produced from oil extract.
[3] Massage solution was produced from coconut oil without sample.

Additionally, our result showed that total flavonoid contents in the massage solution from ethanol extract of *A. conchigera*, *A. galanga*, *A. siamensis* and *B. rotunda* were in ranging of 0.29 to 0.81 mg rutin equivalent per ml solution, while the order of total flavonoid contents of massage solution from oil extract was *A. galanga*> *B. rotunda*> *A. siamensis* > *A. conchigera* in ranging of 0.02 to 0.20 mg rutin equivalent per ml solution. However, the radical scavenging activities were not found in the massage solutions. It is possible that the massage solution consists of components that are not associated with radical scavenging activity, or its solute and solvent capacities in solubility effect on radical scavenging activity. Complicated solvation and surface characteristics in a multiphase system may disturb ingredient interaction and antioxidant capacity (Mcclements & Decker, 2000), such as antioxidant partition among hydrophobic phase, hydrophilic phase and the interfacial region (Yin, Becker, Andersen, Leif, & Skibsted, 2012).

Consequently, FTIR tool was applied in this study to classify *A. siamensis*, *A. conchigera*, *A. galanga*, and *B. rotunda*. The FTIR spectra of their ethanol extracts was performed by a wavenumber range of 4000-550 cm^{-1} (Figure 1). Most peaks corresponded to the absorbance of C-O, C-H, N-H, C=O and O-H bands, respectively (Table 3) that indicated the presence of carbohydrates, isoprenoids, phenyl groups, amino acids, fatty acids, ester, alcohols and phenols. The FTIR spectra showed some peaks that specific to *A. conchigera*, *A. siamensis*, *A. galanga*, and *B. rotunda* at different wavenumbers that confirmed the presence of different chemical components in the ethanol extracts (Figure 1).

Additionally, cluster analysis from data of total phenolic content, total flavonoid content, and radical scavenging activity provided good separation among ethanol extract, massage solution, and oil extract of the studied plants (Figure 2A). The phylogenetic tree classified them into two clusters that the first cluster was ethanol extracts, and the second cluster consisted of massage solution and oil extracts of each plant species. The phylogenetic tree of *A. siamensis*, *A. conchigera*, and *A. galanga* showed close relationship in sub-group of the first cluster, especially between *A. siamensis* and *A. conchigera*. Corresponding to the result of the FTIR spectra, cluster analysis was also able to recognize the studied plants into two clusters (Figure 2B). The first group consisted of sub-clusters namely *A. siamensis*, *A. conchigera* and *A. galanga*, and another group consisted of *B. rotunda*.

Table 3. FTIR spectral peak values and functional groups of ethanol extracts of each sample

Wavenumber range (detected in this studied, cm^{-1})	Wavenumber range (reference, cm^{-1})	Assignment	Function groups	References
3281.7 - 3336.71[1]	3000–3600 3500-3000	O-H and N–H stretch	water, alcohols, phenols, carbohydrates, peroxides	Caunii *et al.* (2012) Cao *et al.* (2017)
2923.45 - 2926.35[2]	2920	C-H stretch	polysaccharides, lipids, and carbohydrates	Cao *et al.* (2017)
2853.24 - 2854.3[3]	2800-2900 2850	C-H stretch	polysaccharides, lipids, and carbohydrates	Cao *et al.* (2017) Topalăa *et al.* (2017)
1607.53 - 1739.81[4]	1600-1760	N-H bending vibrations, C=O bending vibrations	amino acids, fatty acids, ester	Topalăa *et al.* (2017)
1513.11- 1513.69[5]	1500–1600	aromatic and N-H bending vibrations	amino acids	Caunii *et al.* (2012) Topalăa *et al.* (2017)
1301.59-1443.99[6]	1300-1450	Primary or secondary O-H bending (in-plane), and phenol or tertiary alcohol (O-H bend)	phenyl groups	Coates (2006) Caunii *et al.* (2012)
1153.79-1230.98[7]	1150-1270	C-O stretching vibrations	acid or ester	Caunii *et al.* (2012) Topalăa *et al.* (2017)]
1016.54-1052.68[8]	997–1130 997-1140	C-O stretching vibrations	mono–, oligo– carbohydrates, oligosaccharides, glycoprotein	Caunii *et al.* (2012) Topalăa *et al.* (2017)
570.75- 921.65[9]	< 1000	C-H bending vibrations	Isoprenoids	Caunii *et al.* (2012) Topalăa *et al.* (2017)

[1-9] codes of wavenumber ranges.

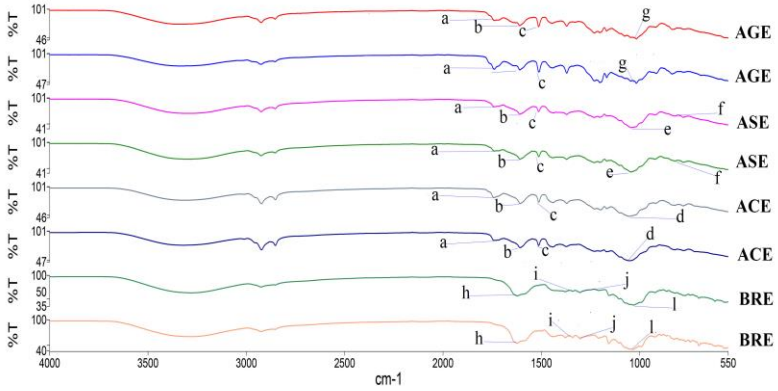

Figure 1. The FTIR Spectra at wavenumber 4000-550 cm^{-1} of the ethanol extracts of *B. rotunda* (BRE), *A. conchigera* (ACE), *A. siamensis* (ASE) and *A. galanga* (AGE). Specific peaks of each sample were indicated at a wavenumber of 1736.81 - 1739.31 cm^{-1} (a), 1607.53-1608.92 cm^{-1} (b), 1513.11-1513.85 cm^{-1} (c), 1051.12-1052.68 cm^{-1} (d), 1043.91-1043.78 cm^{-1}(e), 819.18-819.15 cm^{-1}(f), 1016.54-1016.75 cm^{-1} (g), 1621.97-1622.15 cm^{-1} (h), 1339.36-1339.35 cm^{-1} (i), 1303.03-1301.59 cm^{-1} (j), and 1030.62-1031.34 cm^{-1}(l)

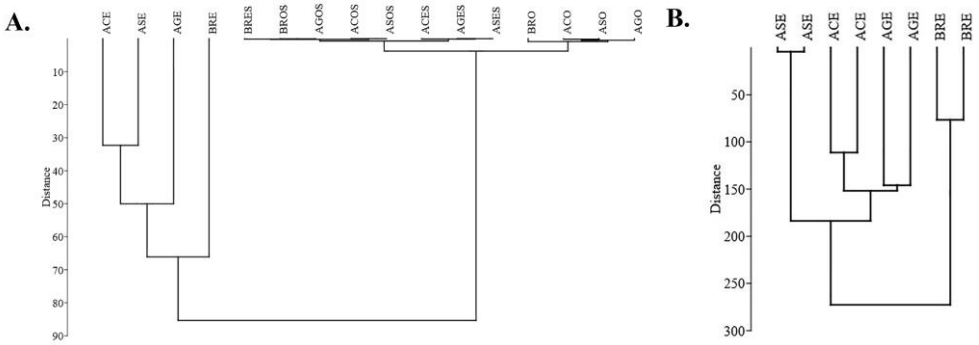

Figure 2. An unweighted pair group method (UPGMA) tree of *B. rotunda* (BR), *A. conchigera* (AC), *A. siamensis* (AS) and *A. galanga* (AG). (A) was UPGMA tree of relationship among ethanol extracts, massage solution from 95% ethanol extracts (BRES, ACES, ASES, and AGES), massage solutions from oil extract (BROS, ACOS, ASOS, and AGOS), and oil extracts (BRO, ACO, ASO, and AGO) based on total phenolic contents, total flavonoid contents, and antioxidant activity. (B) was UPGMA tree of relationship among absorption values of the FTIR spectra from ethanol extracts (BRE, ACE, ASES, and AGE) of the studied plants.

As the result, the composition of ethanol extracts, massage solution, and oil extracts of the studied plants was also obvious from PCA analysis. The values of total phenolic content, total flavonoid content, and radical scavenging activity represented good segregation among ethanol extracts, massage solution, and oil extracts of the studied plants, and among ethanol extracts of plant species (Figure 3A). Additionally, the PCA result showed that ethanol extracts of *B. rotunda*, *A. conchigera*, *A. siamensis*, and *A. galanga* showed more total phenolic content, total flavonoid content, and radical scavenging activity than those of massage solution and oil extracts (Figure 3A).

Similarly, PCA analysis from absorption values of the FTIR spectra show also good separation among *B. rotunda*, *A. conchigera*, *A. siamensis*, and *A. galanga* (Figure 3B). The major peaks discriminating of the ethanol extracts of the three *Alpinia* sp. from *B. rotunda* were vibration 5 (aromatic and N-H in amino acids), vibration 6 (primary or secondary, O-H in-plane bend) and phenol or tertiary alcohol, O-H bend), and vibration 8 (C-O in mono–, oligo– carbohydrates and glycoprotein). However, the three Alpinia sp. was differentiated by vibration 7 (C-O in acid or ester), 8 (C-O in mono–, oligo– carbohydrates and glycoprotein), and 9 (C-H in isoprenoids). Therefore, the cluster and PCA analysis can help to discriminate types of the plant samples and their massage products based on data of bioactive activity and the FTIR absorbance. Interestingly, combination of Fourier transform infrared spectra (FTIR) and bioactivity analysis indicated that *A. siamensis* had FTIR fingerprint, bioactive contents and biological activity similarly to *A. conchigera*. Corresponding to previous studies, *A. siamensis* has close relationship with *A. conchigera*, indicated by a key to the Zingiberaceae genus in Thailand (Larsen, 1980).

The FTIR technique has been used for classifying several plants, such as *Medicago sativa* and *Cucumis* sp. (Caunii *et al.*, 2012; Kukula-Koch, Grzybek, Strachecka, Jaworska, & Ludwiczuk, 2018). In addition, it has been reported that this technique was used for quality evaluation and identification of several plants from various locations and plant products such as *Rhodobryum roseum* Limpr. and edible vegetable oils (Cao *et al.*, 2017; Jiménez-Carvelo, Osorio, Koidis, González-Casado, & Cuadros-Rodríguez, 2017).

Several studies have been reported that ethanol extract from *B. rotunda* rhizome consists of three flavanones namely 2',4'-dihydroxy-6-methoxychalcone, 5-hydroxy-7-methoxyflavanone and 5, 7-dihydroxyflavanone, which relate

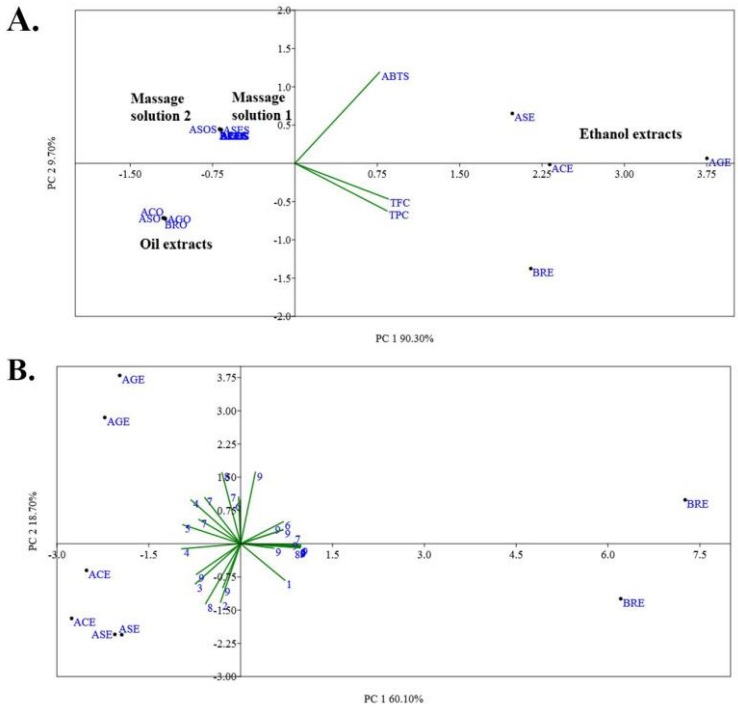

Figure 3. Principal component analysis (PCA). (A) PCA of total phenolic contents, total flavaniod contents, and antioxidant activity of ethanol extracts, massage solution from ethanol extracts, massage solutions from oil extract, and oil extracts. (B) PCA of absorption values of the FTIR spectra of ethanol extracts

to antioxidant and antibacterial activities (Atun, Handayani, & Rakhmawati, 2018). Similarly, the rhizome of *A. conchigera* has been used as folk medicine in treatment of fungal or skin infection (Ibrahim *et al.*, 2000). Moreover, its extract and bioactive agents (i.e. caryophyllene oxide, chavicol acetate, and 1'S-1'-acetoxychavicol acetate) show antimicrobial, anticandidal and antidermatophyte activities (Aziz *et al.*, 2013). For *A. galanga*, it possess several bioactive agents that are many useful in treatment of several diseases, such as antifungal activity and antioxidant activity (Wong, Lim, & Omar, 2009).

Although the local herbs have several effective biological properties, the safety concern of the pharmaceutical products must also be considered. Thus, monitoring of heavy metal contamination in massage products from the local herbs was also carried out in this study. Recently, several reports show that Cd, Ni and Mn are commonly found in several cosmetics, such as facial cosmetics, moisturizing creams, and skin-lightening creams (Iwegbue *et al.*, 2015; Iwegbue, Bassey, Obi, Tesi, & Martincigh, 2016). In our current study, the seven heavy metals namely Cu, Pb, Cd, Ni, Hg, Mn and Zn were investigated in all massage products. The result showed that Cd and Ni were found in all massage products, while Mn was only found for 2.68±2.27 mg/kg in massage solution from oil extract of *A. conchigera* (Table 4). Cd was found in the massage ointment from oil extract of each plant sample in a range of 2.10±1.06 to 3.02±0.79 mg/kg higher than those of massage solution from 95% ethanol and oil extract (ranged from 1.30±0.47 to 1.92 ± 0.58 mg/kg). Similarly, Ni was found in the massage ointment products from oil extract in a range of 23.14±3.72 to 26.83±4.78 mg/kg higher than those of the massage solutions (ranged from 15.53±3.63 to 16.78±3.42 mg/kg). Interestingly, massage ointment without a sample was also contaminated with Cd and Ni for 1.81±0.88 and 26.72±3.77, respectively.

Although, Cd was found in all massage products, it was below its maximum permission level in comparison with a 3 mg/kg criteria value (Health Canada, 2011), except for massage ointment from oil extract of *A. conchigera* (3.02± 0.79). The presence Cd in the samples can effect on human body through skin and may lead to damage organ in the body, if cadmium blood concentration is higher its maximum permissible value (Godt *et al.*, 2006). More importantly, Ni showed higher mean concentration in all massage products than its safe maximum permissible limit (5 ppm) from the report of Basketter, Angelini, Ingber, Kern, and Menné (2003). It has been reported that the contamination of Ni is cause of contact dermatitis (Torres, das Graças, Melo, & Tosti, 2009) that its threshold values are 0.2 µg/cm^2/week for direct and prolonged contact with human skin, and 0.5 µg/cm^2/week for short-term contact (Ma'or *et al.*, 2015; Menné *et al.*, 1987). Additionally, Mn may also effect on human body including brain, liver, and the cardiovascular system if they are overexposed (O'Neal & Zheng, 2015), which Mn concentration in human blood should be ranged from 4 to 15 µg/L for normal level (Agency for Toxic Substances and Disease Registry, 2012).

However, Cd and Ni were higher found in each massage ointment with and without a sample than each massage solution product. The massage ointment consisted of petroleum jelly as an ointment base to protect moisture loss from skin, and paraffin wax to make soft-solid texture of the ointment. Petroleum jelly is recognized as a byproduct of the oil from industry, and commonly used in cosmetics and

Table 4. The concentrations of heavy metals in massage products

Plant types	Samples	Heavy metal content (mg/kg for solid and mg/L for liquid)		
		Cd	Ni	Mn
B. rotunda	Massage solution 1[1]	1.42±0.96	15.83±2.95	ND**
	Massage solution 2[2]	1.57±0.56	16.78±3.42	ND**
	Massage ointment from oil extract	2.62±0.90	25.01±3.79	ND**
A. conchigera	Massage solution 1[1]	1.59±0.35	15.68±2.29	ND**
	Massage solution 2[2]	1.60±0.46	15.68±2.68	2.68±2.27
	Massage ointment from oil extract	3.02±0.79	23.14±3.72	ND**
A. galanga	Massage solution 1[1]	1.92±0.58	16.05±2.06	ND**
	Massage solution 2[2]	1.30±0.47	15.53±3.63	ND**
	Massage ointment from oil extract	2.94±0.64	23.69±4.27	ND**
A. siamensis	Massage solution 1[1]	1.41±0.60	16.23±2.67	ND**
	Massage solution 2[2]	1.51±0.56	15.53±2.69	ND**
	Massage ointment from oil extract	2.10±1.06	26.83±4.78	ND**
Massage ointment without a sample		1.81±0.88	26.72±3.77	ND**
*$MPL_{cosmetics}$		3[a]	5[b]	NV***

*MPL = Maximum permitted levels (mg/kg for solid samples and mg/L for liquid samples). **ND = Not detected. ***NV= No value. [1] Massage solution 1 was produced from 95% ethanol extract. [2]Massage solution 2 was produced from oil extract. [3] Massage solution was produced from coconut oil without sample. [a] Environmental Defense Canada (2011). [b] Data from Basketter *et al.* (2003).

pharmaceutical products (i.e. skin cream, pain plam, lipsticks, and skin ornaments), and it should not content heavy metal over 20 ppm (Unicorn, 2008). Heavy metals in cosmetics that consist of petroleum jelly as major intergradient, such as lipsticks, moisturizing creams, and skin-lightening creams, have been reported in several studies (Iwegbue *et al.*, 2015). Paraffin wax is a byproduct that is also generated from oil with saturated long-chain hydrocarbons (C18 to C60), and is commonly used for several applications, such as cosmetics and pharmaceuticals (Cottom, 2000). These ingredients from petroleum are frequently contaminated with impurities that lead to skin irritation. Therefore, healthy natural ingredients (i.e. plant butter and beeswax) are good alternative that should be used to replace petrolatum ingredients in the pharmaceutical products. This finding can help to more understand of chemical composition of the plants with radical scavenging activity, to evaluate quality of the pharmaceutical products, to develop the pharmaceutical products, and to avoid heavy metal hazard through dermal exposure to humans

4. Conclusions

In this study, the ethanol extract showed the highest total phenolic content, total flavonoid content, and radical scavenging activity, while radical scavenging activities was not found in the massage solution. Furthermore, the FTIR result showed specific chemical fingerprint that helped to separate different types of plant samples namely *B. rotunda*, *A. conchigera*., *A. siamensis*, and *A. galanga*. Cluster and PCA analysis from the FTIR data corresponded to the result from total phenolic and flavonoid contents and biological activity of the ethanol extract. This result confirmed that the FTIR method can predict components in the ethanol extract of the plant samples. However, the result showed that Ni and Cd showed the highest concentration in massage ointment without any sample extracts, followed by massage ointment from the oil extracts, massage solution from the oil extracts, and massage solution from the ethanol extracts. Importantly, Ni in all tested samples exceeded its maximum permission level. This research therefore emphasizes monitoring chemical compounds, biological activities, and heavy metals in massage products commonly used in Thailand.

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