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Analysis of FTIR Spectra, Flavonoid Content and Anti-Tyrosinase Activity of Extracts and Lotion from *Garcinia schomburgkiana* by Multivariate Method

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Abstract

In this study, fourier transform infrared (FTIR) study, total flavonoid content and anti-tyrosinase activity of leaf, seed and flesh extracts of *Garcinia schomburgkiana* and lotion formulation from the extracts were carried out. Additionally, a relationship among FTIR spectra, total flavonoid content and anti-tyrosinase activity was analysed by partial least squares structural equation modelling (PLS-SEM) and principal component analysis (PCA) analysis. The result indicated that the highest total flavonoid content was found in leaf ethanol extract (214.00 ± 7.32 mg rutin equivalent per mg extract). For lotion samples, lotion containing leaf ethanol extract revealed the strongest level of total flavonoid content (5.51 ± 0.04 mg rutin equivalent per mL lotion). Moreover, anti-tyrosinase activity was observed in lotion containing the extracts ($1/EC_{50}$ ranged from 0.0063 to 7.2334) higher than that of aqueous, ethanol and propanediol extracts ($1/EC_{50}$ ranged from 0.0099 to 0.5319). The FTIR result confirmed the presence of various function groups in different parts of *G. schomburgkiana* and lotion samples, which involved the structure of flavonoids namely O-H stretch, C=O bending vibrations, unsaturation bonds, phenol or tertiary alcohol (O-H bend), C-O-H deformation and C-O stretching vibrations of aromatic ethers. The PLS-SEM analysis indicated a moderate positive relationship between the total flavonoid content and FTIR data, significantly ($\beta = 0.502$, $t = 2.239$, p-value < 0.05). The FTIR and subsequent PCA analysis could differentiate of aqueous, ethanol and propanediol extracts and lotion samples.

Keywords: Total flavonoid content, Anti-tyrosinase activity, Extract, Lotion, FTIR, PCA, PLS-SEM, *Garcinia schomburgkiana*

Introduction

Garcinia schomburgkiana Pierre, Madan in Thai name, is a sour fruit tree and commonly found growing near cannel in South-East Asia [1]. Its leaves and fruit are well known, and have commonly used as plant based foods for cooking such as soup and chili sauce or eating fresh leaves as vegetable side dishes. Moreover, its leaves, root and fruit have medicinal properties such as anti-diabetic, antioxidant and laxative properties [1,2]. Previously, several studies revealed the presence of several phytochemical compounds in *G. schomburgkiana* branches such as (-)-5,7,3′,5′-tetrahydroxyflavanone, kaempferol, (-)-dihydrokaempferol, euxanthone, gentisein, norathyriol and dihydroosajaxanthone [3,4]. Generally, *G. schomburgkiana* is applied as foods, such as healthy drinks [5]. However, product development of this plant as healthy cosmetics is still unknown.

Lotion is one of skin care products which are used to protect skin dryness. Several studies report about biological activities of lotion from plant extracts (i.e. *Mimusops elengi*, *Tithonia diversifolia* Helms, *Aloe secundiflora* and *Azadirachta indica*) [6,7]. Generally, several medicinal plants are applied in cosmetics, such as in the form of lotion with antimicrobial properties and anti-tyrosinase activity [7-9].

Nowadays, hyperpigmentation is a common skin problem found in Thai people. Accumulation of melanin, which is a skin pigment synthesized by tyrosinase enzyme, leads to the occurrence of hyperpigmentation [10]. It therefore appear that skin-whitening products are increasingly popular in the market. Especially, the prevalence of the products from plant extracts has commercially increased

because there are fewer side effects. Several plant extracts (i.e. citrus essential oils and citrus peel crude extracts) contain many flavonoids (i.e. nobiletin and hesperidin) which can act as tyrosinase inhibitors, involving melanin production in the skin [11,12].

Previously, several researches afford to find bioactive compounds in plant extracts. FTIR is one of effective techniques, which is popularly used for identification of functional groups in plant extracts [6]. However, biological activities in plants represent complex system, which involve several components in metabolic pathways. Multivariate analysis can help to represent the correlation between variables of a biological system, such as PCA [6], and to elucidate the effects of multiple independent factors on a measured factor, such as partial least squares structural equation (PLS-SEM) [13].

However, there is a little knowledge about application of *G. schomburgkiana* in cosmetics, and chemical fingerprint from extracts and its product. Although several bioactive compounds, specifically to flavonoids, had been discovered as tyrosinase inhibitor in many plants, the relationship study of flavonoid content and anti-tyrosinase activity using multivariate methods was still unknown. Therefore, the current study was designed to assess total flavonoid content and anti-tyrosinase activity of extracts from different parts of *G. schomburgkiana*. Additionally, FTIR study was conducted and lotion formulation from the extracts of *G. schomburgkiana* was developed. Then, total flavonoid content and anti-tyrosinase activity of the lotion was determined. Moreover, analysis of a relationship among FTIR spectra, total flavonoid content and anti-tyrosinase activity using PCA and PLS-SEM analysis was carried out.

Materials and methods

Chemicals

Tyrosinase from mushroom, 3,4-dihydroxy-L-phenylalanine, 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), gallic acid, absolute ethanol, 1-hexadecanol, potassium sorbate, emulsifying wax, propylene glycol, kojic acid and glycerol were purchased from Sigma-Aldrich. Folin-Ciocalteu's phenol reagent was purchased from Merck. Potassium persulfate was purchased from Ajax Finechem.

Preparation and extraction of samples

Leaves and fruits of *G. schomburgkiana* were obtained from Nakorn Nayok province. Leaves and fruits were cleaned with water. Then, its fruits were separated into seed and flesh. The seed and flesh samples were dried at 65 ºC for 48 h, while its leaves was dried at 45 ºC for 48 h. After that, each sample was pulverized into fine powder. Accurately weigh 10 g powder sample was extracted in 250 mL of each solvent namely distilled water, ethanol and propanediol at 45 ºC for 48 h. The solvent extraction was carried out in duplicate for each sample (leaves, seed and flesh). Therefore, the total number of extract samples were 18 extracts. Each extract was filtered through a filter cloth, then it was evaporated at 45 ºC for 19 min (for ethanol and propanediol extracts) and 30 min (for aqueous extract) by using a rotary evaporator (IKA, RV10 D S99, Germany). Each sample was extracted in duplication [2]. Each extract was adjusted into a final concentration (10 mg/mL) with each solvent.

Preparation of lotion

Each lotion base was prepared by mixing 3 % 1-hexadecanol, 3 % emulsifying wax, 4 % coconut oil, and 2 % polysorbate 80 at 70 ºC until the mixture became clear. Then, 2 % glycerol was mixed, and left to 40 ºC. The lotion base was then mixed with each extract in ratio 4:1 by volume of lotion base to extract, and allowed to cool down to room temperature. Because the lotion samples were in viscous form, each lotion sample was prepared to a 1:2, 1:4 and 1:8 dilution of a stock lotion for providing better measurement.

Determination of flavonoid content

Total flavonoid content was determined according to the method of aluminium chloride colorimetric method [14]. Each sample was prepared to a 1:4 and 1:8 dilution of a stock solution or lotion. Each diluted sample (500 µL) was reacted with ethanol (1.5 mL), 10 % aluminum chloride (0.1 mL), 1 M potassium acetate (0.1 mL), and distilled water (2.8 mL), respectively. Each diluted sample was performed in duplicate and incubated for 30 min at room temperature. The absorbance was then determined at 415 nm by an UV-VIS spectrophotometer (PG Instruments Limited, Model T60UV, United Kingdom). A positive control was rutin (0 - 500 µg/mL), which was used to generate a standard curve ($y = 0.0022\times - 0.019$, $R2 = 0.9938$). Total flavonoid content was expressed as mg rutin equivalent per mg extract for aqueous and ethanol extracts, mg rutin equivalent per g powder sample for propanediol extract, and mg rutin equivalent per mL lotion for lotion samples.

Estimation of tyrosinase inhibition

Tyrosinase inhibitory activity was detected according to the method of Liang *et al*. [15]. Each sample was prepared to a 1:2, 1:4 and 1:8 dilution of a stock solution or lotion. Each diluted sample (40 µL) was mixed with 5 mM L-DOPA (100 µL) and 0.1 M sodium phosphate buffer pH 6.8 (20 µL). The mixture was then reacted with 200 units/mL of mushroom tyrosinase (40 µL) for 20 min at 37 ºC. Each diluted sample was carried out in duplicate. The absorption was measured at 450 nm by the UV-VIS spectrophotometer (PG Instruments Limited, Model T60UV, United Kingdom). The percentage of tyrosinase inhibition was carried out by the below formula:

$$\% \text{ Tyrosinase inhibition} = (OD_{blank} - OD_{sample})/OD_{blank} \times 100 \quad (1)$$

OD_{blank} was the absorbance of each mixture without each sample, while OD_{sample} was the absorbance of each mixture with each sample. A simple linear regression was then generated to calculate an effective concentration that expressed 50 % tyrosinase activity inhibition (EC_{50}), then $1/EC_{50}$ was calculated and analysed by PCA and PLS-SEM.

Fourier transform infrared spectra (FTIR)

Functional groups in extracts and lotion samples were detected under 550 to 4000 cm^{-1} with a 4 cm^{-1} resolution via FTIR spectroscopy (Spectrum Two™, Perkin Elmer, USA). A small quantity of each sample was directly placed on the top of the crystal plate of the FTIR spectroscope, and gently squeezed until a thin film of sample was obtained. Duplicate measurements were carried out on each sample. Functional groups were indicated according to the studies of Manrique and Lajolo [16], Coates [17], Caunii *et al*. [18], Baker *et al.* [19], Hands *et al.* [20], Cao *et al*. [21], Topalăa and Tătarua [22], Hemmalakshmi *et al.* [23], Duraka and Depciuch [24], Noh *et al*. [40] and Abbas *et al*. [41].

Statistical analysis

Data were analyzed as mean ± SD to express flavonoid content and tyrosinase inhibitory activity, and one-way analysis of variance (one-way ANOVA) to perform differentiation of the flavonoid content and tyrosinase inhibition among sample groups. The statistics were performed by the PSPP program version 0.10.5 [25]. Moreover, PCA, implemented in the paleontological statistic program version 3.16 [26], were used to analyse the similarity of sample groups. Additionally, the PLS-SEM from SmartPLS version 3 was used to performed direct association among FTIR spectra, chemical content, anti-tyrosinase activity, and sample forms [27].

Results and discussion

In current study, total flavonoid content was observed in the aqueous, ethanol and propanediol extracts of leaf, seed and flesh of *G. schomburgkiana* and lotions containing the extracts. The strongest total flavonoid content was observed in *G. schomburgkiana* leaves which was extracted with ethanol solvent (214.00 ± 7.32 mg rutin equivalence/g extract), with aqueous solvent (32.12 ± 0.73 mg rutin equivalence/g extract), and with propanediol solvent (28.97 ± 14.31 mg rutin equivalence/ g powder sample) (**Table 1**). Corresponding with the result, the strongest total flavonoid content was observed in lotions from the leaf ethanol extracts (5.51 ± 0.04 mg rutin equivalence/mL lotion), in lotions from the leaf aqueous extracts (4.93 ± 0.36 mg rutin equivalence/mL lotion), and in lotions from the leaf propanediol extracts (3.74 ± 0.41 mg rutin equivalence/mL lotion) (**Table 2**).

Table 1 Total flavonoid content and tyrosinase inhibitory activity of aqueous, ethanol and propanediol extracts of leaves, seeds and flesh of *G. schomburgkiana.*

Samples	Plant parts	Total flavonoid content (N = 8) (mg rutin equivalent per mg extract)	Anti-tyrosinase activity (N = 4)	
			EC_{50} (mg/mL)	$1/EC_{50}$
Ethanol extracts	seed	66.43 ± 2.37	55.83 ± 16.28	0.0179
	flesh	38.53 ± 1.01	28.62 ± 15.85	0.0349
	leaves	214.00 ± 7.32	35.84 ± 23.92	0.0279
***p*-value**		**0.000****	**0.258**	
Aqueous	seed	1.27 ± 0.09	73.15 ± 1.32	0.0137

Samples	Plant parts	Total flavonoid content (N = 8) (mg rutin equivalent per mg extract)	Anti-tyrosinase activity (N = 4)	
			EC_{50} (mg/mL)	$1/EC_{50}$
extracts	flesh	1.72 ± 0.67	78.47 ± 6.85	0.0127
	leaves	32.12 ± 0.73	101.19 ± 6.80	0.0099
***p*-value**		**0.000****	**0.001****	
Propanediol	seed	14.75 ± 3.10*	34.39 ± 4.13	0.0291
extracts	flesh	18.28 ± 3.28*	8.53 ± 5.56	0.1172
	leaves	28.97 ± 14.31*	1.88 ± 0.59	0.5319
***p*-value**		**0.105**	**0.002**	

Note: N was the number of samples for total flavonoid content or the number of EC_{50} values for anti-tyrosinase activity, which were used to compute mean and standard deviation (mean ± SD).
*mg rutin equivalent per g powder sample.
***p*-value < 0.05 indicated a significant level.

Table 2 Total flavonoid content and tyrosinase inhibitory activity of lotion mixed with each extract namely aqueous, ethanol and propanediol extracts of leaves, seeds and flesh of *G. schomburgkiana*.

Samples	Madan parts	Total flavonoid content (N = 8) (mg rutin equivalent per mL lotion)	Anti-tyrosinase activity (N = 4)	
			EC_{50} (mg/mL)	$1/EC_{50}$
Lotion containing ethanol	seed	5.04 ± 0.13	7.23 ± 1.47	0.1384
extracts	flesh	4.70 ± 0.15	21.51 ± 7.12	0.0465
	leaves	5.51 ± 0.04	157.94 ± 120.83	0.0063
***p*-value**		**0.000***	**0.113**	
Lotion containing aqueous	seed	4.39 ± 0.18	0.23 ± 0.06	4.3992
extracts	flesh	4.50 ± 0.11	0.15 ± 0.02	6.7718
	leaves	4.93 ± 0.36	2.41 ± 3.92	0.4157
***p*-value**		**0.026***	**0.364**	
Lotion containing propanediol	seed	2.83 ± 0.35	0.9 ± 0.68	1.0870
extracts	flesh	3.51 ± 0.64	0.14 ± 0.08	7.2334
	leaves	3.74 ± 0.41	0.23 ± 0.19	4.3390
Lotion containing kojic acid		4.48 ± 0.15	0.05 ± 0.02	18.8709
Lotion base		3.97 ± 0.14	0.08 ± 0.05	12.5085
Kojic acid	-	4.48 ± 0.15	0.4152 ± 0.00	2.4082
***p*-value**		**0.011***	**0.065**	

Note: N was the number of samples for total flavonoid content or the number of EC_{50} values for anti-tyrosinase activity, which were used to compute mean and standard deviation (mean ± SD).
**p*-value < 0.05 indicated a significant level.

Interestingly, the result showed the highest level of total flavonoid content in leaf extracts and lotions containing the leaf extracts. Corresponding previous reports of Singsai *et al*. [28] and Yahia *et al*. [29], flavonoids showed the highest level in leaf extract comparing with shoots, flesh and seeds, found in other plant families (i.e. *Leucaena leucocephala* and *Ziziphus lotus*) [28,29]. However, leaves of Madan are abundant sources of bioactive compounds and nutrients for human body, such as vanillin, fatty acids, mannose, fructose and glucose [30].

Flavonoids are phenolic compounds namely flavones, flavanols, flavanones, chalcones, isoflavones, and anthocyanins, which are commonly found in fruits and vegetables. These compounds have medicinal properties, such as antioxidant and anti-tyrosinase activities [31,32]. Previously, it has been reported that several flavonoids (i.e. kuwanon C, papyriflavonol A, sanggenon D and sophoflavescenol, and sanggenon D) in plants showed tyrosinase inhibitory potentials [33,34].

In the current result, it showed that tyrosinase inhibitory activity was found in all extracts and lotion samples. The high $1/EC_{50}$ values were observed in the leaf propanediol extracts ($1/EC_{50}$ = 0.5319), flesh ethanol extracts ($1/EC_{50}$ = 0.0349), and seed aqueous extracts ($1/EC_{50}$ = 0.0137), while the high

$1/EC_{50}$ values of lotion samples were found in lotion containing flesh propanediol extracts ($1/EC_{50}$ = 7.2334), lotion containing flesh aqueous extracts ($1/EC_{50}$ = 6.7718), and lotion containing seed ethanol extracts ($1/EC_{50}$ = 0.1384). For positive control , lotion containing kojic acid showed the strongest anti-tyrosinase activity ($1/EC_{50}$ = 18.8709), and kojic acid showed high anti-tyrosinase activity ($1/EC_{50}$ = 2.4082) (**Tables 1** and **2**).

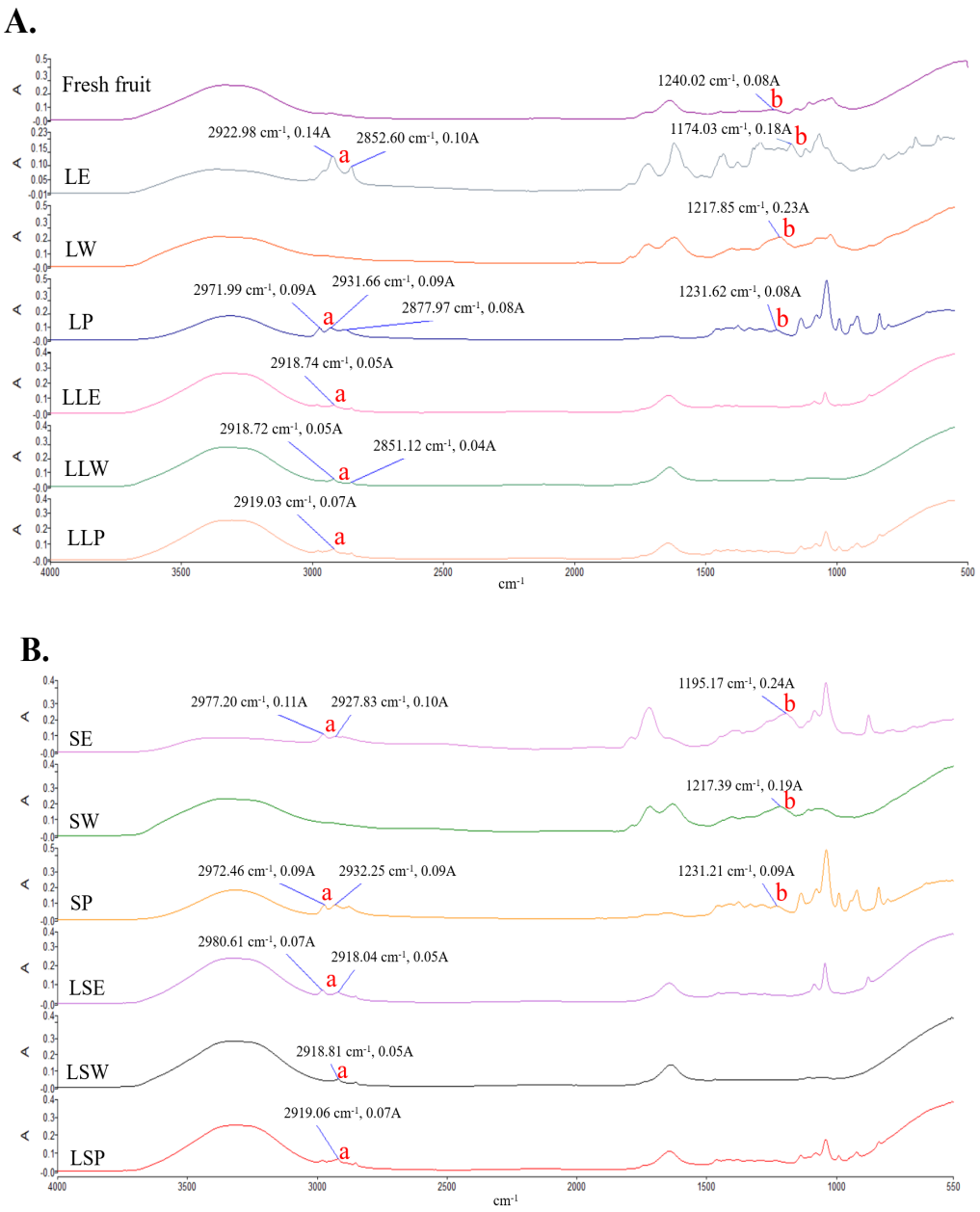
However, the FTIR spectra of fresh fruit, the aqueous extracts, ethanol extracts and propanediol extracts from leaves, seeds and flesh of *G. schomburgkiana* including lotion containing each extract were performed under a 550 to 4000 cm^{-1} wavelength with a 4 cm^{-1} resolution (**Figure 1** and **Table 3**). FTIR has been reported as an effective tool for the characterization and identification of functional groups in aqueous and ethanol extracts including tea products from *G. schomburgkiana* leaves [5].

Table 3 Wavenumber range of FTIR peaks found in fresh fruit, lotion samples, aqueous, ethanol and propanediol extracts of leaves, seeds and flesh of *G. schomburgkiana.*

Wavenumber range of FTIR peaks found in samples (cm^{-1})	Function group assignment	Predicted phytochemicals	References
3306.81 - 3424.62	H-boned, O-H stretch	water, alcohols, phenols, flavonoids	[18,21,40]
2850.72 - 2980.65	CH_2 and CH_3 stretching vibrations	lipid acyl chain	[16]
1626.69 - 1733.13	N-H bending vibrations, C=O bending vibrations	amino acids, fatty acids, ester, flavonoids	[22,40]
1715.97 - 1790.37	C=O	lipids	[20]
1621.83 - 1658.29	Unsaturation bonds	flavonoids	[40]
1380.21 - 1456	CH_3 and COO^-	lipids, proteins, amino acids	[20]
1467.31	Amide II of proteins (α-helix structures, β-pleated sheet structures, turns, random coils)	proteins	[20]
1328.14 - 1443.27	Primary or secondary O-H bending (in-plane), and phenol or tertiary alcohol (O-H bend)	phenyl groups	[17, 18]
1328.14 - 1379.73	CH_3 bending, C-O-H deformation	lipids, phenolic compound	[19,41]
1283.8 -1289.41	C-O stretching vibrations of aromatic ethers	acid, flavonoids	[23,41]
1192.18 - 1241.6	C-O stretching vibrations of aromatic ethers	acid, ester, flavonoids	[18,22,41]
1041.2 - 1136.85	C-O stretching vibrations	mono-carbohydrates, oligosaccharides, glycoprotein, phenyl group	[18,22,41]
1019.04 - 1079.73	glycosidic	carbohydrates	[24]
502.94 - 990.96	C-H bending vibrations	isoprenoids	[18]

The functional groups of each sample were identified in position of a wavenumber range of their FTIR peaks (**Figure 1**). The FTIR result showed total 14 FTIR peaks of the fresh fruit, aqueous extracts, ethanol extracts and propanediol extracts from leaf, seed and flesh of *G. schomburgkiana* and their lotions, which confirmed the presence of various function groups, which involved the structure of flavonoids namely O-H stretch, C=O bending vibrations, unsaturation bonds, phenol or tertiary alcohol (O-H bend), C-O-H deformation and C-O stretching vibrations of aromatic ethers. Thus, the result

confirmed the presence of several constituents in leaf, seed and flesh of *G. schomburgkiana* and their lotions.



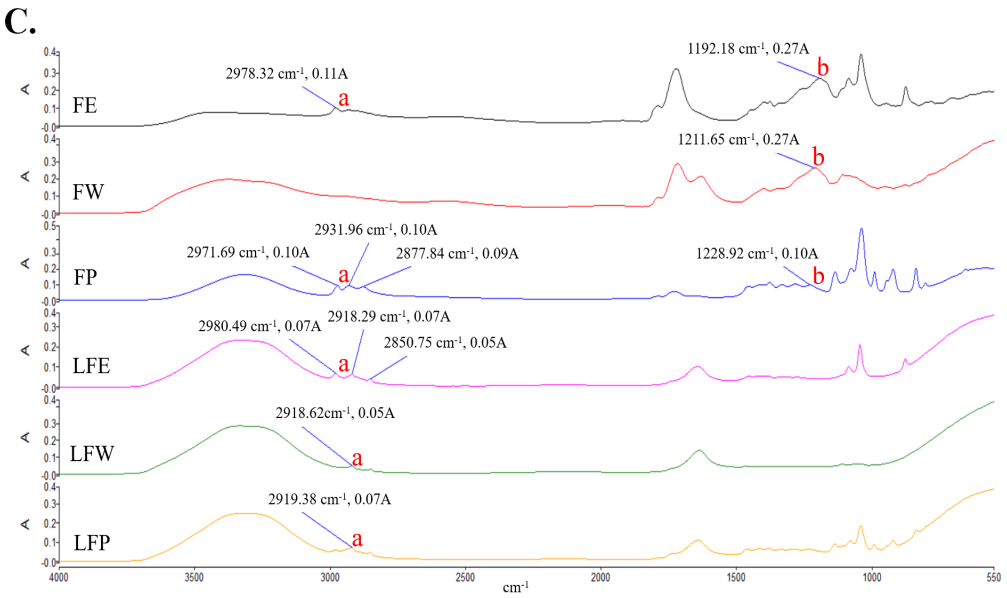


Figure 1 Examples of FTIR spectra, which were obtained from a wavelength under 550 to 4000 cm^{-1} with a 4 cm^{-1} resolution. Note: (A) showed FTIR spectra of fresh fruit, leaf ethanol extract, leaf aqueous extract and leaf propanediol extract, and lotion mixed with each extract, (B) showed seed ethanol extract, seed aqueous extract and seed propanediol extract, and lotion mixed with each extract and (C) showed flesh ethanol extract, flesh aqueous extract and flesh propanediol extract, and lotion mixed with each extract. A name list of abbreviations was provided namely LE = leaf ethanol extract, SE = seed ethanol extract, FE = flesh ethanol extract, LW = leaf aqueous extract, SW = seed aqueous extract, FW = flesh aqueous extract, LP = leaf propanediol extract, SP = seed propanediol extract, FP = flesh propanediol extract, LLE = lotion from leaf ethanol extract, LSE = lotion from seed ethanol extract, LFE = lotion from flesh ethanol extract, LLW = lotion from leaf aqueous extract, LSW = lotion from seed aqueous extract, LFW = lotion from flesh aqueous extract, LLP = lotion from leaf propanediol extract, LSP = lotion from seed propanediol extract, and LFP = lotion from flesh propanediol extract. Symbol a and b indicated specific bands.

Moreover, differentiation of the absorbance values and band width of the FTIR spectra were observed among 5 sample groups (fresh fruit, aqueous extracts, ethanol extracts, propanediol extracts and lotion samples). The specific FTIR peak was found in ethanol extracts, propanediol extracts and lotions containing the extracts at 2850.72 - 2980.65 cm^{-1} which was assigned to CH_2 and CH_3 stretching vibrations (lipid acyl chain). Moreover, the specific FTIR peak was found in fresh fruit, aqueous extracts, ethanol extracts and propanediol extracts at 1192.18 - 1241.6 cm^{-1} which are assigned to C-O stretching vibrations of aromatic ethers (acid, ester and flavonoids compounds). Interestingly, leaf ethanol extracts provided the most number of FTIR peaks of 12 wavenumber ranges, followed by the propanediol extracts from leaf, seed and flesh (11 wavenumber ranges), and the flesh ethanol extracts (10 wavenumber ranges).

Interestingly, the FTIR spectral data showed moderate positively correlation with total flavonoid content ($r = 0.502$, p-value < 0.05), while correlation between the spectral and anti-tyrosinase activity ($r = -0.310$, p-value > 0.05) and between total flavonoid content and anti-tyrosinase activity ($r = -0.228$, p-value > 0.05) were not observed. The result indicated that other phytochemicals, apart from flavonoids, may be responsible for the anti-tyrosinase activity. Other phytochemicals such as those predicted to be present in **Table 1** may also be responsible for the anti-tyrosinase activity. In coressonding to the result, a causal relatioship between the FTIR spectra and chemical content, between the FTIR spectra and biological activity, and between the chemcial content and biological activity were performed by using partial least squares structural equation modeling.

Path coefficient, known as standardised beta (β) was caculated to demonstrate the relationship between the variables. In this current study, moderate positively relationship between total flavonoid content and the FTIR spectra was significantly found ($\beta = 0.502$, $t = 2.239$, p-value < 0.05). However, a

causal relatioship between the FTIR spectra and biological activity (β = -0.262, t = 1.458, *p*-value > 0.05), and between the chemcial content and biological activity (β = -0.097, t = 0.672, *p*-value > 0.05) was observed, insignificantly (**Figure 2**).

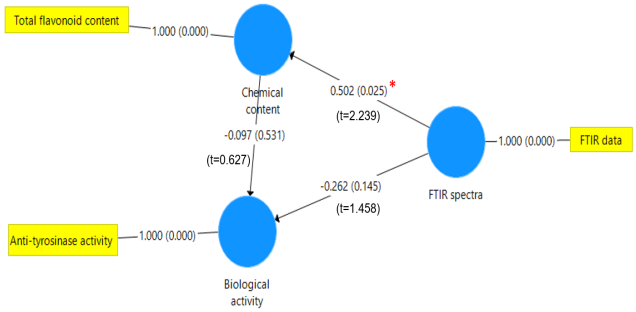


Figure 2 The path diagram analyzed from the partial least squares structural equation. The arrows showed a causal relationship between chemical content and biological activity, between FTIR spectra and biological activity, and between FTIR spectra and chemical content. All *p*-values, shown in parentheses, were obtained from 10000 bootstrapping. A *p*-value of less than 0.05 was regarded as statistically significant, and given by an asterisk symbol.

Generally, kojic acid is a natural compound from fungi that shows tyrosinase inhibitory activity involving melanin synthesis, therefore, it is commonly applied in cosmetic products (i.e. cream, lotion and soaps) [35]. Moreover, lotion base consisted of cetyl alcohol, emulsifying wax, coconut oil, and polysorbate 80. Of these, it has been reported that coconut oil has antioxidant activity and anti-tyrosinase activity, which is useful as a function component in skin-whitening products [36]. Moreover, polysorbate 80 or tween 80 is a surfactant and emulsifier that is commonly used to generate emulsion, to stabilize protein formulation [37,38]. Surfactants consist of both hydrophobic and hydrophilic regions that help to improve the extraction efficiency of bioactive compounds [39]. Thus, it is possible that lotion ingredients may improve tyrosinase inhibitory activity of lotion samples. As the result, the lotion base provided higher anti-tyrosinase activity than all extracts and the lotion samples mixed with each extract. It implied that specific phytochemicals, presented in different extracts of different plant parts, may produce antagonistic effect between compounds in the lotion mixed with each extract. The interaction of multiple agents in the mixture of plant extract and lotion can affect on biological activity. However, all extracts and the lotion samples mixed with each extract provided higher total flavonoid content than the lotion base. Flavonoids have been proven to demonstrate a variety of pharmacological properties, such as antimicrobial, antioxidant, anti-inflammatory, and anticarcinogenic activities [31]. Therefore, the combination of each extract to the lotion may provide other biological activities, which have positive impact on the stability and efficacy of the formulation. However, the analysis of skin-whitening components in lotion and plant extracts will provide new data leading to the improvement of natural skin-whitening products.

In current study, PCA, based on the FTIR data, total flavonoid content, and tyrosinase inhibitory activity, was used to demonstrate relationship between FTIR spectra and sample preparation from leaf, seed and flesh of *G. schomburgkiana*. The result indicated that 2 principal components were enough to explain differences among samples.

The PCA graph from the FTIR data showed the 1st principal component (PC1) for 34.50 % of total variation, while the 2nd principal component (PC2) was 25.44 % (**Figure 3**(**B**)). The PCA result indicated that the wavenumber ranges of 1328.14 - 1443.27, 1328.14 - 1379.73, 1283.8 - 1289.41 and 502.94 - 990.96 cm^{-1} were respectively related to phenyl groups, lipid, acid and isoprenoids, which were mainly found in propanediol extracts (seed, leaf and flesh) and fresh fruit, and flesh ethanol extract. The wavenumber ranges of 1715.97 - 1790.37 and 1192.18 - 1241.6 cm^{-1} were respectively related to lipids, acid, ester and flavonoids which were mainly found in leaf ethanol extracts.

The wavenumber range of 1380.21 - 1456 cm^{-1} were respectively related to lipids, proteins, amino acids, which mainly found in seed, leaf and flesh aqueous extracts, and seed ethanol extract. Moreover, the wavenumber range of 1621.83 - 1658.29, 2850.72 - 2980.65, 1626.69 - 1733.13, 1467.31, 1041.2 - 1136.85 and 1019.04 - 1079.73 cm^{-1} were respectively related to flavonoids, proteins, lipid acyl chain,

amino acids, fatty acids, ester, proteins, mono-carbohydrates, oligosaccharides, glycoprotein, phenyl group, carbohydrates, which were mainly found in lotion samples. Interestingly, the aqueous extracts, propanediol extracts, ethanol extracts and lotion samples were far to each other on the PCA scatter plot based on the FTIR data which provide sample differentiation could be identified.

However, the PCA result from total flavonoids content and tyrosinase inhibitory activity of the samples showed that PC1 was 61.40 % of total variation, while PC2 was 38.60 % (**Figure 3**(**A**)). The result confirmed that total flavonoids content showed the highest value in leaf, seed and flesh ethanol extract, while the most tyrosinase inhibitory activity was found in lotion samples, followed by propanediol extracts, aqueous extracts, and ethanol extracts. Moreover, the aqueous extracts, propanediol extracts, and lotion containing the extracts were closed to each other on the PCA scatter plot based on total flavonoids content and tyrosinase inhibitory activity, which showed low ability to identify differentiation between extracts and lotion.

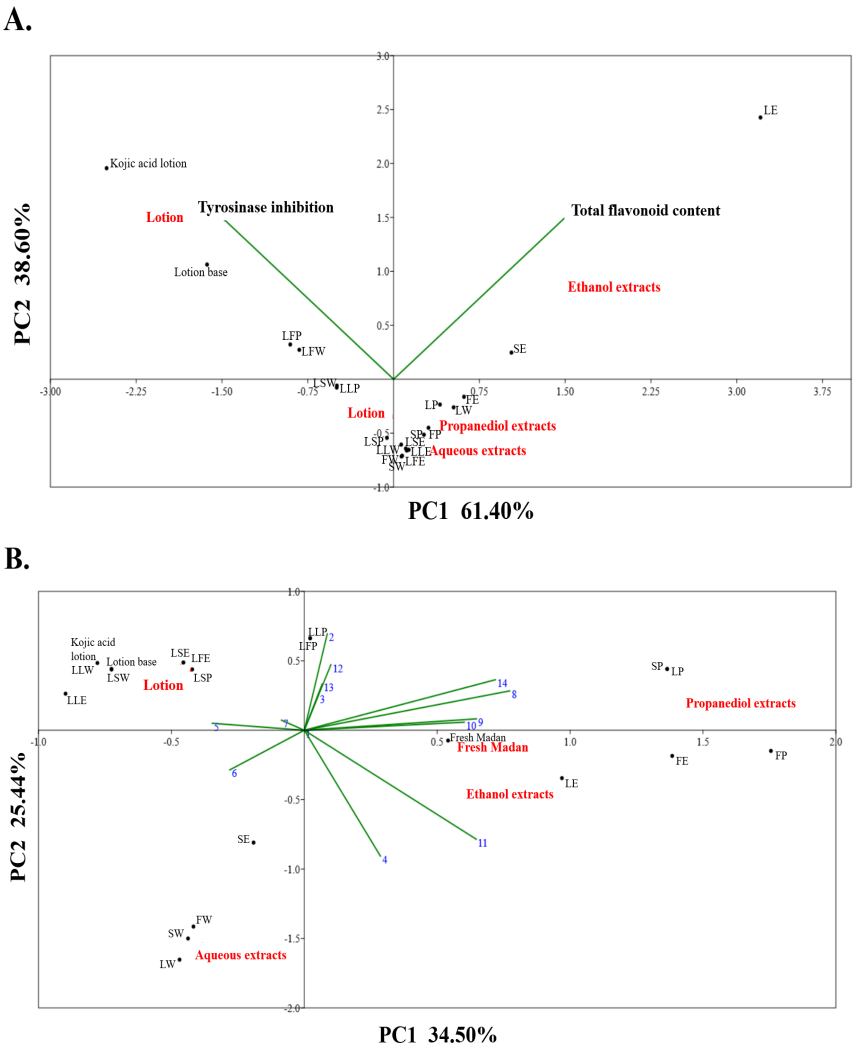


Figure 3 (PCA) from total flavonoid content and biological activities (A) and FTIR data (B) of ethanol, aqueous, and propanediol extracts, and lotion samples of *G. schomburgkiana* A name list of abbreviations was provided namely LE = leaf ethanol extract, SE = seed ethanol extract, FE = flesh ethanol extract, LW = leaf aqueous extract, SW = seed aqueous extract, FW = flesh aqueous extract, LP = leaf propanediol extract, SP = seed propanediol extract, FP = flesh propanediol extract, LLE = lotion from leaf ethanol extract, LSE = lotion from seed ethanol extract, LFE = lotion from flesh ethanol extract, LLW = lotion from leaf aqueous extract, LSW = lotion from seed aqueous extract, LFW = lotion from flesh aqueous extract, LLP = lotion from leaf propanediol extract, LSP = lotion from seed propanediol extract, and LFP = lotion from flesh propanediol extract.

Therefore, the PCA analysis of FTIR data was used to perform differences between extract and lotion samples, while the PCA analysis from total flavonoid content, and tyrosinase inhibitory activity was not used to differentiate the samples. The knowledge of differentiation between extract and lotion forms can be applied in selecting plant raw materials and other lotion ingredients to develop herbal formulation, providing better chemical content and biological activity.

Conclusions

The current studies indicated that total flavonoid content and tyrosinase inhibitory activity were found in leaf, seed and flesh of *G. schomburgkiana*, which were extracted by distilled water, ethanol and propanediol solvent, and found in lotion containing the extracts. Moreover, the 2 specific FTIR peaks observed, which there was 1 peak specific to ethanol extracts, propanediol extracts and lotions containing the extracts at CH_2 and CH_3 stretching vibrations (lipid acyl chain), while another was found in fresh fruit, aqueous extracts, ethanol extracts and propanediol extracts at C-O stretching vibrations (acid or ester compounds). Interestingly, the PLS-SEM analysis showed moderate positively relationship between total flavonoid content and the FTIR spectra, significantly. In corresponding to Pearson's correlation, the FTIR spectral data revealed moderate positively correlation with total flavonoid content. Moreover, the result suggested that the PCA analysis from FTIR data was able to differentiate extracts from lotion samples, while the PCA analysis from total flavonoid content, and tyrosinase inhibitory activity was not able to differentiate the samples. Therefore, the FTIR technique and subsequent PCA and PLS-SEM analysis could be used as a reliable and fast method to monitor the occurrence of functional groups of crude plant extracts and chemical changes that may occur after plant processing which created more complex components.

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