

Analysis of flower extract and natural dye solution from *Sesbania javanica* using Fourier-transform infrared spectroscopy (FTIR) chemometrics, and determination of its antioxidant and anti-glucosidase activities

¹*Thummajitsakul, S., ²Boonburapong, B. and ³Silprasit, K.

¹Faculty of Physical Therapy, Srinakharinwirot University, Ongkharak, 26120 Nakhon-Nayok, Thailand

²Faculty of Science, Srinakharinwirot University, 10110 Bangkok, Thailand

³Faculty of Environmental Culture and Ecotourism, Srinakharinwirot University, 10110 Bangkok, Thailand

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Abstract

Fourier-transform infrared spectroscopy (FTIR) chemometrics and determination of antioxidant and anti-glucosidase activities of flower extract and natural dye solution from *Sesbania javanica* were performed. Total phenolic content and total flavonoid content were the highest in ethanolic flower extract, while antioxidant activity ($1/EC_{50} = 0.4935$) and α -glucosidase inhibition ($1/EC_{50} = 2.9671$) were the highest in dye solution, obtained from mixing white clay filler with aqueous dye extract. The correlations of FTIR data, bioactive compound contents, and biological activities were assessed by partial least squares structural equation modelling. Results showed that a strongly positive relationship was significantly demonstrated between FTIR fingerprints and bioactive compound contents ($\beta = 0.825$, $t = 17.037$, p value < 0.05), and between bioactive compound contents and biological activities ($\beta = 0.563$, $t = 3.000$, p value < 0.05). However, a strongly negative relationship was significantly found between FTIR fingerprints and biological activities ($\beta = -1.204$, $t = 6.065$, p value < 0.05). Moreover, the PCA and cluster analysis from FTIR data led to well grouping in PC plots and phylogenetic trees. Interestingly, the PCA and cluster analysis from FTIR data of plant extracts in powdered form provided better separation in PC plots and phylogenetic trees than in liquid form.

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Introduction

Prevalence of type 2 diabetes is continuously increasing worldwide, and it has been reported that approximately 700 million people (aged 20 - 79 years) will have type 2 diabetes by 2045 (Saedi *et al.*, 2019). In Thailand, it has been reported that several factors (*i.e.* sociodemographic, lifestyle, and economic development) are associated with type 2 diabetes of Thai adults (Papier *et al.*, 2016). In patients with diabetes, hyperglycaemia can induce the generation of reactive oxygen species via several pathways, such as production of advanced-glycation end-products and activation of protein kinase C, thus leading to the development of diabetic complications (Ogutibeju, 2019).

Several studies have reported that phytochemicals in fruits and vegetables are capable of decreasing type 2 diabetes incidences. Some phytochemicals (*i.e.* polyphenols, carotenoids, vitamin E, and vitamin C) play an important role as

antioxidants, which help to prevent type 2 diabetes complications (Rahimi-Madiseh *et al.*, 2016). Antioxidants in several plants (*i.e.* vegetables, fruits, grains, and herbs) have potential against the development and progression of type 2 diabetes complications (*i.e.* retinopathy, nephropathy, and neuropathy) (Rahimi-Madiseh *et al.*, 2016). Moreover, certain phenolic compounds from plants (*i.e.* resveratrol, curcumin, and chlorogenic acid) can act as anti-diabetic agents, such as stimulating glucose uptake in the condition without insulin, reducing insulin resistance, and inhibiting α -glucosidase enzyme (Ahangarpour *et al.*, 2018).

The management of blood glucose level after meals is an important part of type 2 diabetes treatment. α -glucosidase is an intestinal enzyme that catalyses the hydrolysis of oligosaccharide to glucose (Kumar *et al.*, 2011). Therefore, the inhibition of α -glucosidase in the small intestine can help inhibit the digestion of carbohydrates, thus causing lower postprandial plasma glucose. Bioactive compounds

*Corresponding author.
 Email: sirikul.thum@gmail.com

with α -glucosidase inhibitory potential (*i.e.* terpenes, alkaloids, quinine, flavonoids, phenol, and phenylpropanoid) are abundant in plants (Yin *et al.*, 2014). It has been confirmed that increased fruit and vegetable consumption can reduce the risk of type 2 diabetes (Wang *et al.*, 2016).

Sesbania javanica flower, known as *sano* in Thai, is a member of the Fabaceae family (BGO, 2017), and widely distributed in canals throughout Thailand, especially in the rainy season. *Sesbania javanica* flower has a high content of β -carotene, and can be used as a pigment source to enhance the colour of egg yolk (Kijparkorn *et al.*, 2010); thus it is often used in Thai cooking. It has been reported that *S. javanica* flower contains high quantities of flavonoids (*i.e.* flavonol, glycosides, and quercetin 3-2(G)-rhamnosylrutinoside) which have anti-mutagenic activity (Tangvarasittichai *et al.*, 2005). The flowers and leaves of *S. javanica* also contain several bioactive compounds, such as β -sitosterol, prunetin, genistein, 4-hydroxycinnamic acid, and stioesterol-3-O- β -D-glucopyranoside, some of which (*i.e.* prunetin, genistein, and 4-hydroxycinnamic acid) have antioxidant activities (Loedsakasesakul, 2007).

Nevertheless, the biological activity using bioactive compound fingerprinting of flowers and processed products of *S. javanica* is still largely unstudied. Preliminary phytochemical screening is a rapid, easy, and low-price method, and appropriate to analyse multiple phytochemicals in a sample mixture to screen for the presence of phytochemicals. Fourier-transform infrared spectroscopy (FTIR) is an effective technique used for identifying any functional groups in plant extracts, and expressed as FTIR spectra fingerprint (Baker *et al.*, 2014). Therefore, the objectives of the present work were to evaluate total phenolic contents, total flavonoid contents, antioxidant activity, and α -glucosidase inhibitory activity by combining FTIR spectra analysis of ethanolic and water extracts, and dye powder of *S. javanica* flower obtained from Nakhon Nayok province. Nakhon Nayok is an agricultural province which contains many small canals. At present, there have not been any reports about product development of this plant as a cosmetic and food ingredient by using the FTIR spectra fingerprint. Therefore, the present work could provide information which may be used to develop new techniques for detecting important ingredients in health foods and pharmaceutical cosmetics, which is of value to consumers.

Materials and methods

Chemicals

3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS), gallic acid, absolute ethanol, L-glutathione reduced, α -glucosidase from *Saccharomyces cerevisiae*, 4-Nitrophenyl-alpha-D-glucopyranoside (PNPG), rutin, aluminium chloride, and acarbose were purchased from Sigma-Aldrich. Folin-Ciocalteu's phenol reagent was purchased from Merck. Potassium persulfate, sodium carbonate, and ascorbic acid were purchased from Ajax Finechem. Potassium acetate was purchased from KemAus. Potassium phosphate was purchased from Bio Basic Canada Incorporated.

Sample preparation

Sesbania javanica flower samples were collected from Nakhon Nayok province, and identified by comparing with data source from BGO Plant Databases (BGO, 2017). The flower samples were then cleaned by water, and dried in an incubator at 45°C for 48 h. The dried sample was then finely ground by a homogeniser, and kept at 4°C.

Plant extraction

Dried flower powder of *S. javanica* was extracted by each solvent (95% ethanol and distilled water) at a ratio of 1 g to 25 mL of solvent. Optimisation of incubation was carried out at two different temperatures and durations, which were classified into general conditions for flower extraction (45°C for 48 h) (Thummajitsakul *et al.*, 2019), and for dye extraction (80°C for 1 h) (Mansour, 2018). Each extracted flower sample was then filtered through filter cloth. After that, ethanol solvent was removed by a rotary evaporator at 45°C for 30 min, while water solvent was removed at 45°C for 50 min. Each sample was extracted in duplicate. The dried crude extract was dissolved by its extracting solvent, adjusted to a final concentration of 50 mg/mL, and the percentage of yield crude extract was calculated using Eq. 1:

$$\% \text{ yield crude extract} = \frac{\text{WT}_{\text{dry extract}}}{\text{WT}_{\text{dry plant}}} \times 100 \quad (\text{Eq. 1})$$

where, $\text{WT}_{\text{dry extract}}$ = weight of each dry extract after solvent was removed, and $\text{WT}_{\text{dry plant}}$ = weight of the dry plant before extraction.

To obtain powder, each plant extract (1 mL) was incubated under 50°C until dry, then ground into powder, and used for FTIR analysis.

Preparation of natural dye powder

Dye powder was prepared by combining each dye extract of *S. javanica* flower with different carriers, namely white clay filler (used as an ingredient in cosmetics) and maltodextrin (used as a food additive). Briefly, each dye extract (25 mL) was combined with white clay filler powder (40 g), and incubated at 80°C for 1.30 h (95% ethanolic extract), and 16 h (aqueous extract) until dry. Each sample was then cooled down and ground to a powder, followed by refrigeration in a polyethylene tube at 4°C until used (Muangthai *et al.*, 2010). To prepare dye solution, each dye powder (20 g) was mixed with each solvent (95% ethanol and distilled water) at 20 mL. Each mixture was then left at 4°C for 1 h. The filtrate was kept in a polyethylene tube at 4°C, and used for FTIR analysis and determining biological activities.

For the maltodextrin method, the flower powder (10 g) was extracted with distilled water (250 mL) at 45°C for 48 h. Each extract was sieved through filter cloth. Each extract was combined with maltodextrin at ratios of 85:15, 80:20, 75:25, 70:30, and 65:35, and incubated at 60°C for 27 h. Each mixture was then cooled down, followed by grinding, and refrigeration in polyethylene tube at 4°C until used. For FTIR analysis and biological activity determination, the dye powder was dissolved in distilled water and adjusted to a final concentration of 1 g/mL.

Total phenolic contents

The total phenolic contents were determined by the Folin-Ciocalteu method (Thummajitsakul *et al.*, 2016). Each sample (300 µL) at a concentration of 5 and 20 mg/mL for extracts, and 1 g/mL for dye solution, was reacted with Folin-Ciocalteu reagent (1.5 mL) at room temperature for 5 min. Then, 7.5% (w/v) sodium carbonate (1.2 mL) was added and incubated at room temperature for 30 min, and absorbance was measured at 765 nm using a spectrophotometer (Model T60UV). Each sample was carried out in four replicates. The positive control was gallic acid at 0 - 0.5 mg/mL, and used to construct a calibration curve ($y = 6.4647x - 0.0356$; $R^2 = 0.99$), and expressed as mg gallic acid per g extract.

Total flavonoid contents

The total flavonoid contents were determined by the aluminium chloride colorimetric method (Chang *et al.*, 2002). Each sample (500 µL) at concentrations of 5 and 20 mg/mL for extracts, and 1 g/mL for dye solution, was added with 4.5 mL of a reaction mixture (1.5 mL of 95% ethanol, 0.1 mL of 10% aluminium chloride, 0.1 mL of 1 M potassium acetate, and 2.8 mL of distilled water). Each mixture was then incubated at room temperature for 30 min. Each sample was carried out in four replicates. The absorbance was detected at 415 nm by the spectrophotometer (Model T60UV). Rutin (0 - 500 µg/mL) was used as a positive control to construct a calibration curve ($y = 0.0018x - 0.002$; $R^2 = 1$), and expressed as mg rutin equivalent per g extract.

3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS)

The antioxidant activity was determined by ABTS assay (Thummajitsakul *et al.*, 2016). Briefly, 7 mM ABTS solution (10 µL) was converted to its cation radicals by reaction with 140 mM of potassium persulfate (179 µL) under dark conditions for 16 h. The ABTS⁺ solution was then diluted with distilled water until its absorbance was equal to 0.700 ± 0.050 at 734 nm. After that, the diluted ABTS⁺ solution (3.9 mL) was reacted with each sample (20 µL) at three different concentrations (5, 10, and 25 mg/mL for 95% ethanolic extracts; 20, 40, and 100 mg/mL for aqueous extracts; and 1, 0.5, and 0.25 g/mL for dye solution, respectively) in dark conditions for 6 min, then the absorbance was measured at 734 nm using a spectrophotometer (Model T60UV). Each sample was performed in six replicates. Ascorbic acid (0 - 1 mg/mL) was used as a positive control to construct a calibration curve ($y = 87.704x - 0.0786$; $R^2 = 1$). The antioxidant activity was expressed as mg ascorbic acid/g extract, and also performed as 50% effective concentration (EC₅₀) providing 50% free radical scavenging effect.

% antioxidant capacity =

$$\frac{OD_{ABTS} - OD_{Sample-ABTS}}{OD_{ABTS}} \times 100 \quad (\text{Eq. 2})$$

where, OD_{ABTS} = absorbance of the diluted ABTS⁺ solution, and OD_{Sample-ABTS} = absorbance of a reaction of the diluted ABTS⁺ solution and each sample.

α-glucosidase inhibitory activity

The *α*-glucosidase inhibitory activity was determined following the method described by Thummajitsakul *et al.* (2019). Each sample (100 μ L) at three different concentrations (5, 10, and 25 mg/mL for 95% ethanolic extracts; 20, 40, and 100 mg/mL for aqueous extracts; and 1, 0.5, and 0.25 g/mL for dye solution) was reacted with a mixture of 3 mM glutathione (25 μ L), 67 mM potassium phosphate buffer pH 6.8 (250 μ L), and 0.3 unit/mL *α*-glucosidase (25 μ L) at 37°C for 10 min. Then, 10 mM of PNP (25 μ L) was added and incubated at 37°C for 10 min, followed by mixing 0.1 M sodium carbonate (400 μ L). Each sample was carried out in six replicates. Its absorbance was then determined at 400 nm using a spectrophotometer (Model T60UV). Acarbose (0 - 25 mg/mL) was used as a positive control, and a calibration curve was constructed ($y = 3.178x + 15.919$; $R^2 = 0.82$). The percentage of *α*-glucosidase inhibition was calculated using Eq. 3:

$$\% \text{ glucosidase inhibition} = \frac{[(\text{OD}_{\text{water}} - \text{OD}_{\text{waterblank}}) - (\text{OD}_{\text{sample}} - \text{OD}_{\text{sampleblank}})]}{(\text{OD}_{\text{water}} - \text{OD}_{\text{waterblank}})} \times 100$$

(Eq. 3)

where, OD_{water} and $\text{OD}_{\text{sample}}$ = absorbance of distilled water and sample with *α*-glucosidase, and $\text{OD}_{\text{waterblank}}$ and $\text{OD}_{\text{sampleblank}}$ = absorbance of distilled water and sample without *α*-glucosidase. The *α*-glucosidase inhibitory activity was expressed as mg rutin equivalent/g extract, and also performed as 50% effective concentration (EC_{50}) that produced 50% *α*-glucosidase inhibition effect.

Fourier-transform infrared spectroscopy (FTIR)

Each solvent extract and dye solution were placed in FTIR spectroscope (Spectrum Two™, Perkin Elmer, USA), and analysed in a range of 550 to 4000 cm^{-1} with a resolution of 4 cm^{-1} . Each sample was analysed in four replicates. Each FTIR spectra was then determined by using PerkinElmer spectrum IR version 10.6.0, and compared with data of Coates (2006), Caunii *et al.* (2012), Cao *et al.* (2017), and Topalãa *et al.* (2017).

Data analysis

Statistical analysis was performed using paleontological statistic program version 3.16 (Hammer *et al.*, 2001). Replication results were averaged and expressed as mean \pm SD. The FTIR

spectra was scored as binary data (presence (1) or absence (0) of a peak), which was used for principal component analysis (PCA), partial least squares structural equation (PLS), cluster analysis, and Pearson's correlation. The PCA analysis was performed to reduce the number of variables. Moreover, similarity among samples was performed by unweighted pair group method (UPGMA), based on FTIR data, different sample preparations, total phenolic contents, total flavonoid contents, antioxidant activity ($1/\text{EC}_{50}$ values), and *α*-glucosidase inhibitory activity ($1/\text{EC}_{50}$ values). Pearson's correlation coefficients (r) between five different assays of determining total phenolic contents, total flavonoid contents, antioxidant activity ($1/\text{EC}_{50}$ values), anti-glucosidase activity ($1/\text{EC}_{50}$ values), and FTIR data were determined. Additionally, the direct relationships between independent and dependent variables, namely between bioactive compound content and chemical fingerprint, between bioactive compound content and biological activity, and between chemical fingerprint and biological activity, were performed by the partial least squares structural equation from SmartPLS version 3 (Ringle *et al.*, 2015).

Results and discussion

In the present work, the objectives were to determine total phenolic contents, total flavonoid contents, antioxidant activity, and *α*-glucosidase inhibitory activity, including FTIR spectra analysis of ethanolic and aqueous extracts, and dye solution of *S. javanica* flower. To this end, flower and dye extracts were extracted by using either 95% ethanol (samples ESJ1 and ESJ2 were flower ethanolic extracts; PESJ1 and PESJ2 were dye ethanolic extracts) or distilled water (samples WSJ1 and WSJ2 were flower aqueous extracts; samples PWSJ1 and PWSJ2 were dye aqueous extracts). Moreover, dye solution 1 (samples PESJ1P, PESJ2P, PWSJ1P, and PWSJ2P) was prepared by mixing white clay filler with either ethanolic dye extract (samples PESJ1 and PESJ2) or aqueous dye extract (samples PWSJ1 and PWSJ2). Dye solutions 2 (samples WSJM1_1 and WSJM1_2) and 3 (samples WSJM2_1 and WSJM2_2) were prepared by mixing 20 and 25% maltodextrin with aqueous extract, respectively.

Results showed that differentiation of total phenolic contents, total flavonoid contents, antioxidant activity, and *α*-glucosidase inhibitory

activity was significantly found among sample groups namely flower extracts, dye extracts, dye solution 1, dye solution 2, and dye solution 3 (Table 1). The orders of total phenolic contents, total flavonoid contents, antioxidant, and α -glucosidase inhibitory activities were: flower extract > dye extract > dye solution 2 > dye solution 3 > dye solution 1; flower extract > dye extract > dye solution 2 > dye solution 1 > dye solution 3; dye solution 1 > flower extract > dye extract > dye solution 2 > dye solution 3; and dye solution 1 > dye solution 2 > dye solution 3 > flower extract > dye extract, respectively.

Results indicated that the ethanolic flower extracts (ESJ1 and ESJ2) and dye extracts (PESJ1 and PESJ2) showed higher total phenolic contents and total flavonoid contents, antioxidant activity, and α -glucosidase inhibition than the aqueous extracts. However, dye solution 1 (PWSJ1P and PWSJ2P), obtained from mixing white clay filler with aqueous dye extract, showed higher total phenolic contents and total flavonoid contents, antioxidant activity, and α -glucosidase inhibition than ethanolic extracts (Table 1).

When transmission FTIR spectrums of ethanolic flower and dye extracts, aqueous flower and dye extracts, and dye solution were compared (Figure 1), difference was observed in peak positions in a range of 4000 - 550 cm^{-1} . The presence of functional groups of the plant samples and their products were indicated by peaks at twenty-one wavenumber range in FTIR spectrum (Table 2). As a result, the existence of phenolic compounds was shown by transmittance peaks at wavenumber ranges of 3005.45 - 3339.64, 2927.5 - 2951.11, and 1315.96 - 1410.89 cm^{-1} , respectively. The presence of isoprenoids, carbohydrates, lipids, proteins, amino acids, aromatic secondary amines, acids, esters, DNA, and RNA were shown by transmittance peaks at each wavenumber range shown in Table 2.

Two specific peaks at a wavenumber range of 2885.37 - 2895.66 cm^{-1} , corresponding to C-H stretch (polysaccharides, lipids, and carbohydrates), were found for ethanolic flower and dye extracts in powdered form, while one specific peak at the wavenumber range was found in ethanolic flower and dye extracts in liquid form. One specific peak at a wavenumber range of 1734.5 - 1740.54 cm^{-1} , corresponding to C=O of lipids, was found in ethanolic flower and dye extracts in powdered form, and dye solution 1, which was prepared from mixing white clay filler with the ethanolic dye extract.

However, one specific peak at 2546.32 - 2604.95 cm^{-1} , which could not be designated to functional group, was found for aqueous flower and dye extracts in liquid form and dye solutions 2 and 3, which were prepared from mixing 20 and 25% maltodextrin with aqueous flower extract, respectively (Figure 1).

Interestingly, the difference between flower extracts and methods of dye preparation were observed. Hence, solvent types and the dye preparation methods might have affected FTIR spectrum and biological activities of the samples. As a result, the positions of the peaks of the samples, extracted with the same solvent, were similar in location, but different in percentage of transmittance. Therefore, peak position could be used to identify similarity of the samples. This result confirmed that FTIR was an effective technique for identification of processed plants from different methods. It has been reported that FTIR fingerprint has been used to detect contamination and composition of plants, and monitor quality of plants and their products (Joshi, 2012). Moreover, several forms of samples can be analysed by FTIR technique. Several previous studies have reported that powder dried plants, semi-liquid plants, and fresh plants can be placed directly on attenuated total reflectance (ATR)-crystal of FTIR spectroscopy (Liu *et al.*, 2016; Skolik *et al.*, 2019).

Additionally, strong positive correlations were significantly found between total phenolic contents and total flavonoid contents ($r = 0.88$, p value < 0.05), between total phenolic contents and FTIR data ($r = 0.82$, p value < 0.05), and between total flavonoid contents and FTIR data ($r = 0.78$, p value < 0.05) (Table 3). As the total flavonoid contents increased, total phenolic contents also increased; flavonoid is a major group of phenolic compounds generally found in plants (Cheynier *et al.*, 2013). Moreover, very strong positive correlation between antioxidant activity and α -glucosidase inhibitory activity was significantly found ($r = 0.92$, p value < 0.005). Interestingly, negative strong correlations were significantly found between antioxidant activity and FTIR data ($r = -0.792$, p value < 0.005), and between α -glucosidase inhibitory activity and FTIR data ($r = -0.689$, p value < 0.005) (Table 3). However, negative correlations were insignificantly found between bioactive compound contents and biological activities (Table 3).

Additionally, the partial least squares structural equation (PLS) was used to confirm a causal effect

Table 1. Total phenolic contents, total flavonoid contents, antioxidant activity, and α -glucosidase inhibition of 95% ethanolic extract, aqueous extract, dye extracts, and dye powders of *S. javanica* flower.

Sample	Solvent used for flower extraction	Total phenolic content		Total flavonoid content		Antioxidant activity		α -glucosidase inhibition	
		(mg gallic acid equivalent/g extract)*	(mg gallic acid equivalent/g extract)*	(mg rutin equivalent/g extract)*	(mg rutin equivalent/g extract)*	EC ₅₀	1/EC ₅₀	EC ₅₀	1/EC ₅₀
Flower extract	95% ethanol	43.63 ± 10.46***	100.90 ± 10.21***	40.87 ± 6.54***	0.0245	12.77 ± 1.62***	0.0783		
	Distilled water	28.92 ± 0.73***	29.62 ± 5.41***	154.35 ± 7.44***	0.0065	47.95 ± 3.99***	0.0208		
Dye extract	95% ethanol	29.30 ± 0.18	84.69 ± 3.99***	53.50 ± 7.36***	0.0187	19.59 ± 2.54***	0.0510		
	Distilled water	27.91 ± 6.88	29.20 ± 4.05***	81.65 ± 4.23***	0.0122	49.55 ± 0.41***	0.0202		
Dye solution 1 ^a	95% ethanol	0.06 ± 0.01***	0.20 ± 0.021***	4.82 ± 1.73***	0.2073	2.05 ± 0.19***	0.4876		
	Distilled water	0.35 ± 0.12***	0.57 ± 0.077***	2.03 ± 0.12***	0.4935	0.34 ± 0.07***	2.9671		
Dye solution 2 ^b	Distilled water	0.88 ± 0.00	0.43 ± 0.01	161.86 ± 39.67	0.0062	2.61 ± 0.33	0.3825		
Dye solution 3 ^c	Distilled water	0.82 ± 0.00	0.35 ± 0.02	315.45 ± 37.34	0.0032	3.38 ± 0.20	0.2963		
	<i>p</i> -value**	0.000	0.015	-	0.001	-	0.062		

^aDye solution 1 was filtrate obtained from mixing white clay filler and dye extract from *S. javanica* flowers. ^bDye solution 2 was solution obtained from mixing 20% maltodextrin and aqueous extract from *S. javanica* flowers. ^cDye solution 3 was solution obtained from mixing 25% maltodextrin and aqueous extract from *S. javanica* flowers. *Total phenolic contents, total flavonoid contents, antioxidant activities and α -glucosidase inhibition of dye solutions 1, 2, and 3 were respectively expressed as mg gallic acid equivalent/g powder sample, mg rutin equivalent/g powder sample, mg ascorbic acid/g powder sample, and mg acarbose equivalent/g powder sample. ***p*-value from One way ANOVA analysis ($p < 0.05$) is statistically significant, and indicated differentiation of each assay among sample groups. ****p*-value from independent *t*-test ($p < 0.05$) is statistically significant, and indicated differentiation of each assay between ethanolic and aqueous extracts of flower extract, dye extract, and dye solution 1.

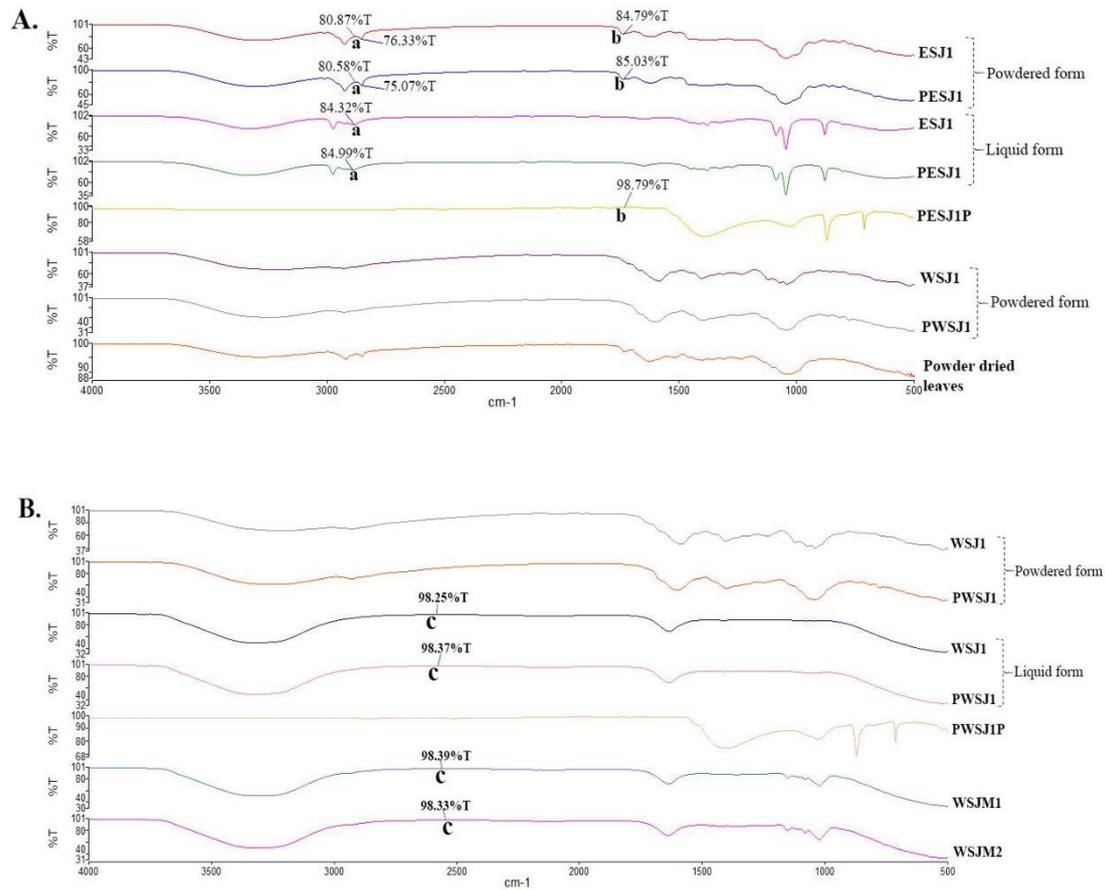


Figure 1. Examples of FTIR spectra in a range of 4000 - 550 cm⁻¹. **(A)** compares spectrum of flower and dye extracts in liquid and powdered forms, powder dried leaves, and dye solution 1. **(B)** compares spectrum of aqueous flower and dye extracts in liquid and powdered forms, and dye solution. Specific peaks of FTIR spectra were identified at wavenumber ranges namely 2885.37 - 2895.66 (a), 1734.5 - 1740.54 (b), and 2546.32 - 2604.95 (c). ESJ1 and ESJ2 were ethanolic extracts from *S. javanica* flower. WSJ1 and WSJ2 were aqueous extracts from *S. javanica* flower. PESJ1 and PESJ2 were ethanolic dye extracts from *S. javanica* flower. PWSJ1 and PWSJ2 were aqueous dye extracts from *S. javanica* flower. PESJ1P and PESJ2P were dye solutions obtained from mixing white clay filler and ethanolic dye extract from *S. javanica* flower. PWSJ1P and PWSJ2P were dye solutions obtained from mixing white clay filler and aqueous dye extract from *S. javanica* flower. WSJM1_1 and WSJM1_2 were solutions obtained from mixing 20% maltodextrin and aqueous extract from *S. javanica* flower. WSJM2_1 and WSJM2_2 were solutions obtained from mixing 25% maltodextrin and aqueous extract from *S. javanica* flower.

Table 2. Wavenumbers of FTIR peaks and functional groups of each extract of *S. javanica* flower.

Wavenumber range (detected in this study, cm ⁻¹)	Wavenumber range (reference, cm ⁻¹)	Assignment	Functional group	Reference
3005.45 - 3339.64	3000 - 3600	O-H and N-H stretch	Water, alcohols, phenols	Caunii et al. (2012)
3005.45 - 3339.64	3500 - 3000		Carbohydrates, peroxides	Cao et al. (2017)
2885.37 - 2895.66 ^a	2800 - 2900, 2850	C-H stretch	Polysaccharides, lipids, and carbohydrates	Cao et al. (2017), Topalaa et al. (2017)
2546.32 - 2604.95 ^c	unknown	-	-	-
2927.5 - 2951.11	2925.4 - 2960.0	Intramolecular bonded alcohol O-H stretching	Alcohols	Kumar et al. (2015)
2851.55 - 2997.8	3000 - 2800	CH ₂ and CH ₃ stretching vibrations	Lipid acyl chains	Lahlali et al. (2014)
1973.77 - 2033.83	unknown	-	-	-
2157.67 - 2159.54	unknown	-	-	-
1601.93 - 1740.54	1600 - 1760	N-H bending vibrations, C=O bending vibrations	Amino acids, fatty acids, esters	Topalaa et al. (2017)
1734.5 - 1740.54 ^b	1700 - 1799	δ C=O of lipids	Lipids	Hands et al. (2016)
1601.93 - 1697.74	1600 - 1706	Amide I of proteins (α-helix structures, β-pleated sheet structures, turns, random coils), ν C=O (76%), ν C-N (14%), CNN (10%)	Proteins	Hands et al. (2016)

1541.51 - 1595.12	1500 - 1600	Aromatic and N-H bending vibrations	Amino acids	Caunii et al. (2012), Topalãa et al. (2017)
1380.51 - 1456.19	1380 - 1465	CH ₃ lipids/proteins and COO- of amino acids	Lipids/proteins/amino acids	Hands et al. (2016)
1482.25 - 1585.51	1460 - 1590	Amide II of proteins (α -helix structures, β -pleated sheet structures, turns, random coils), δ N-H (60%), ν C-N (40%)	Proteins	Hands et al. (2016)
1315.96 - 1410.89	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)
1315.96 - 1377.24	1300 - 1380	CH ₃ bending	Lipids	Baker et al. (2014).
1281.48 - 1294.91	1350 - 1280	C-N	Aromatic secondary amine, CN stretch	Coates (2006)
1151.9 - 1235	1150 - 1270	C-O stretching vibrations	Acids or esters	Caunii et al. (2012), Topalãa et al. (2017)
1022.36 - 1187.41	1008 - 1230	C-O stretch, deoxyribose/ribose, DNA, RNA (PO ₂ -), C-C stretch, C-H bend	Deoxyribose/ribose, DNA, RNA	Hands et al. (2016)
1022.36 - 1119.06	997 - 1130, 997 - 1140	C-O stretching vibrations	Mono-, oligo-carbohydrates, oligosaccharides, glycoproteins	Caunii et al. (2012), Topalãa et al. (2017)
503.15 - 994.13	< 1000	C-H bending vibrations	Isoprenoids	Caunii et al. (2012), Topalãa et al. (2017)

Table 3. Pearson's correlation coefficients (r) between five different assays to determine total phenolic contents, total flavonoid contents, antioxidant activity, α -glucosidase inhibitory activity, and FTIR data.

	Total flavonoid content	Antioxidant activity	α -glucosidase inhibition	FTIR
Total phenolic content	0.88*	-0.47	-0.48	0.82*
Total flavonoid content	-	-0.38	-0.40	0.78*
Antioxidant activity	-	-	0.92*	-0.79*
α -glucosidase inhibition				-0.69*

* $p < 0.05$ is statistically significant, and indicated correlation between each two different methods.

between independent and dependent variables, namely between bioactive compound contents and chemical fingerprints, between bioactive compound contents and biological activities, and between chemical fingerprints and biological activities, respectively.

The relationship between variables can be indicated by path coefficient, known as standardised beta (β). If it showed a positive value, this indicated that one variable and the other variable increased or decreased in the same direction. In the present work, independent variables were bioactive compound

contents and chemical fingerprints, while dependent variable was biological activities. Results showed that a strongly positive relationship was significantly demonstrated between chemical fingerprints and bioactive compound contents ($\beta = 0.825$, $t = 17.037$, p value = 0.000), followed by the relationship between bioactive compound contents and biological activities ($\beta = 0.563$, $t = 3.000$, p value = 0.003). However, a strongly negative relationship was significantly found between chemical fingerprints and biological activities ($\beta = -1.204$, $t = 6.065$, p value = 0.000) (Figure 2).

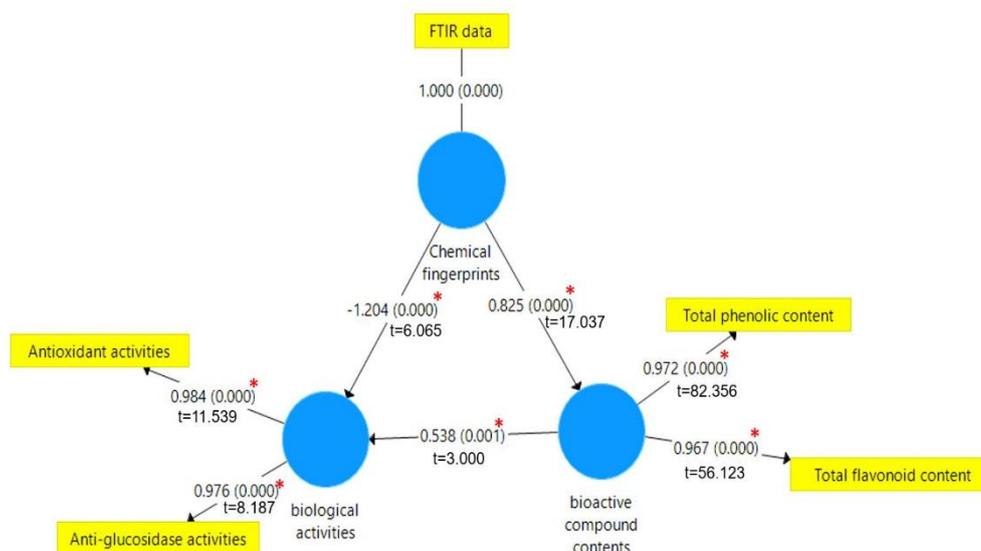


Figure 2. The path diagram and path coefficients generated by the partial least squares structural equation. Arrows indicate a causal effect between chemical fingerprints and bioactive compound contents, chemical fingerprints and biological activities, and between bioactive compound contents and biological activities. * p -value less than 0.05 ($p < 0.05$) in parentheses indicate statistically significant data obtained from bootstrapping.

Positive correlation between antioxidant activities and α -glucosidase inhibition in herbs has been detected and reported (Lee *et al.*, 2014). Besides, several bioactive agents (*i.e.* kaempferol, rutin, hesperetin 5-O-glucoside, kaempferol-coumaroyl-glucoside, and luteolin 3-glucoside) in

plant extracts have been reported as both antioxidant compounds and α -glucosidase inhibitors (William *et al.*, 2019). Interestingly, antioxidant activities and α -glucosidase inhibition of dye solution 1, obtained from mixing white clay filler and dye extract, showed the highest quantity. The antioxidant and α -

glucosidase inhibitory capacities of the dye solution may be the result of a synergistic effect, which is commonly present in natural products (Adamska-Patruno *et al.*, 2018).

However, relationships between total phenolic contents and total flavonoid contents on antioxidant and α -glucosidase inhibitory capacities were not detected. It may involve other factors. Non-phenolic or non-flavonoid compounds (*i.e.* fatty acid, phytol, and plant sterols) can also function as antioxidant compounds and α -glucosidase inhibitors. For example, it has been reported that fatty acids (*i.e.* palmitic acid and heptadecanoic acid), plant sterol (*i.e.* stigmasterol), and phytol (*i.e.* isoprenoids) have α -glucosidase inhibitory capacity (Murugesu *et al.*, 2018). Moreover, plants have both enzymatic system (*i.e.* catalase and glutathione peroxidase) and non-enzymatic system (*i.e.* ascorbic acid, glutathione, and carotenoids) to scavenge free radicals (Kasote *et al.*, 2015). Interestingly, it has been reported that *S.*

javanica flower has high total carotenoid contents, namely β -carotene, lutein, and β -cryptoxanthin that is used as a pigment in egg yolk (Kijparkorn *et al.*, 2010).

The FTIR result was further analysed by a PCA scatter plot. PCA analysis has been applied in analysing FTIR spectra data, and providing data about grouping and FTIR peaks of samples in PC scatter plots (Durak and Depciuch, 2020). As mentioned earlier, this PCA result was also observed in PCA analysis of FTIR peaks from flower and dye extracts in liquid form, powder dried leaves, and dye solution (Table 2). PC1 was 42.62% of total variance, while PC2 was 36.77% of total variance. Results showed low separation among powder dried leaves, dye extract, and flower extract (Figure 3A). However, the PCA analysis also showed that flower extract, powder dried leaves, and dye extract were similar, while dye solutions 2 and 3 were similar.

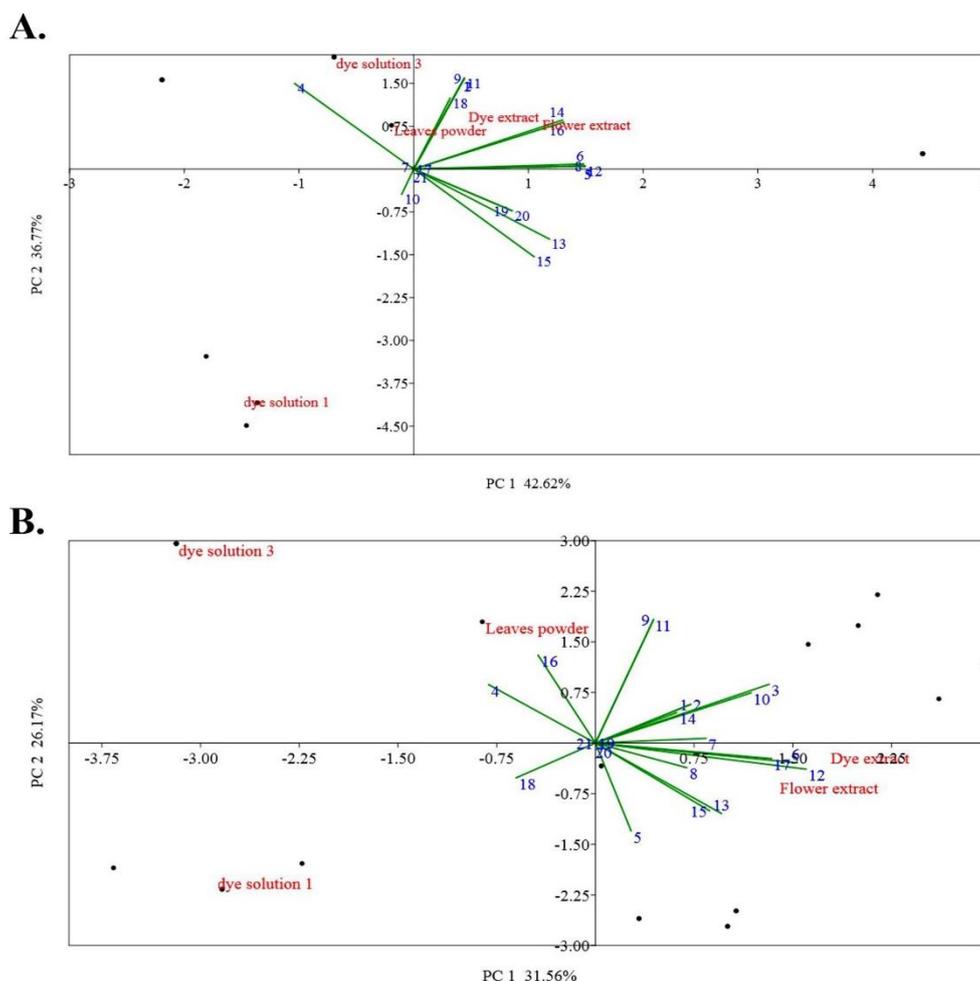


Figure 3. Principal component analysis (PCA). (A) PCA based on transmittance values of the FTIR spectra of flower and dye extracts in liquid form, powder dried leaves, and dye solution. (B) PCA based on transmittance values of the FTIR spectra of flower and dye extracts in powdered form, powder dried leaves, and dye solution.

The PCA result from FTIR data of flower and dye extracts in powdered form, powder dried leaves, and dye solution are shown in Table 2. The PC1 was 31.56% of total variance, while PC2 was 26.17% of total variance. Results showed good separation among powder dried leaves, dye extract, flower extract, dye solution 1, dye solution 2, and dye solution 3 (Figure 3B). This indicated that PCA analysis from sample extracts in powdered form provided greater separation than those in liquid form.

The similarity among samples based on total phenolic contents, total flavonoid contents, antioxidant activity, and α -glucosidase inhibition was also determined by an unweighted pair group method (UPGMA). The cluster analysis showed that the samples were classified into two groups. The first group consisted of ethanolic flower and dye extracts. The second group consisted of two subgroups namely aqueous flower and dye extracts, and dye solutions 1, 2, and 3 (Figure 4A). The cluster analysis showed a similarity between ethanolic flower extract and dye extract, between aqueous flower extract and dye extract, and among dye solutions 1, 2, and 3.

The cluster analysis from FTIR data of flower and dye extracts in liquid form, powder dried leaves, and dye solution was also performed. The samples were also grouped into two groups. The first group consisted of ethanolic flower and dye extracts in liquid form. The second group consisted of two subgroups: (1) dye solution 1, and (2) powder dried leaves, aqueous flower and dye extracts in liquid form, and dye solutions 2 and 3 (Figure 4B). The cluster analysis showed a similarity between ethanolic flower and dye extracts in liquid form, and among powder dried leaves, aqueous flower and dye extracts in liquid form, and dye solutions 2 and 3.

The cluster analysis from FTIR data of flower and dye extracts in powdered form, powder dried leaves, and dye solution was also carried out. The similarity among the FTIR spectra of samples was classified into two groups. The first group consisted of dye solution 1. The second group consisted of two subgroups: (1) dye solutions 2 and 3, and (2) ethanolic flower and dye extracts, aqueous flower and dye extracts, and powder dried leaves (Figure 4C). The cluster analysis also showed a similarity between flower extract and dye extract in powdered form, and between dye solutions 2 and 3.

Dye solution 1 was obtained from mixing white clay filler and dye extract from *S. javanica*

flowers. White clay filler was compared with data in spectral libraries implemented in PerkinElmer Spectrum IR program. The result showed that white clay filler was calcium carbonate (correlation score = 0.87039). Calcium carbonate is a porous inorganic material with biocompatible and biodegradable properties, and can act as drug carrier for anticancer, antitumor, and gene delivery (Guragain *et al.*, 2018; Khan *et al.*, 2019).

In the present work, white clay filler carrier in aqueous condition led to improve antioxidant activity and α -glucosidase inhibitory activity. This carrier may absorb biological compounds, and may help to increase dissolve phytochemicals in aqueous medium. Previous studies reported that calcium carbonate has a suitable structure for drug absorption, leading to increased synergistic antibacterial effect of drugs (*i.e.* gentamicin) (Pan *et al.*, 2018). Moreover, it has been reported that porous calcium carbonate can also be used for food as carrier compounds to increase solubility of flavouring agents in aqueous solution (Johnson *et al.*, 2017).

In addition, maltodextrin was also compared with data in the spectral libraries. Results showed that maltodextrin used in the present work was similar to maltodextrin from the spectral libraries (correlation score = 0.97543). Maltodextrin has been widely applied in microencapsulation of bioactive agents as a carrier and drying agent in foods, cosmetics, and pharmaceuticals (Plainfossé *et al.*, 2018; Corrêa-Filho *et al.*, 2019). For example, maltodextrin increased yields and total sugar content, but decreased the antioxidant activity of dried gelatine and gelatine hydrolysate powder (Chuaychan and Benjakul, 2016). Additionally, maltodextrin has been used as a solid cosmetic support by mixing with plant (*Quercus pubescens*) extract, which did not influence antihyaluronidase, antioxidant, and anti-inflammatory properties of the extract but led to decreased anti-elastase activity (Plainfossé *et al.*, 2018). Maltodextrin has also been used as a carrier of bioactive compounds in plant (*Satureja montana*) dry powder, yielding high total phenolic content, total flavonoid content, and antioxidant activity (Vidović *et al.*, 2014). Maltodextrin is also useful in powder production of fruit juices and plant extracts (Phisut, 2012).

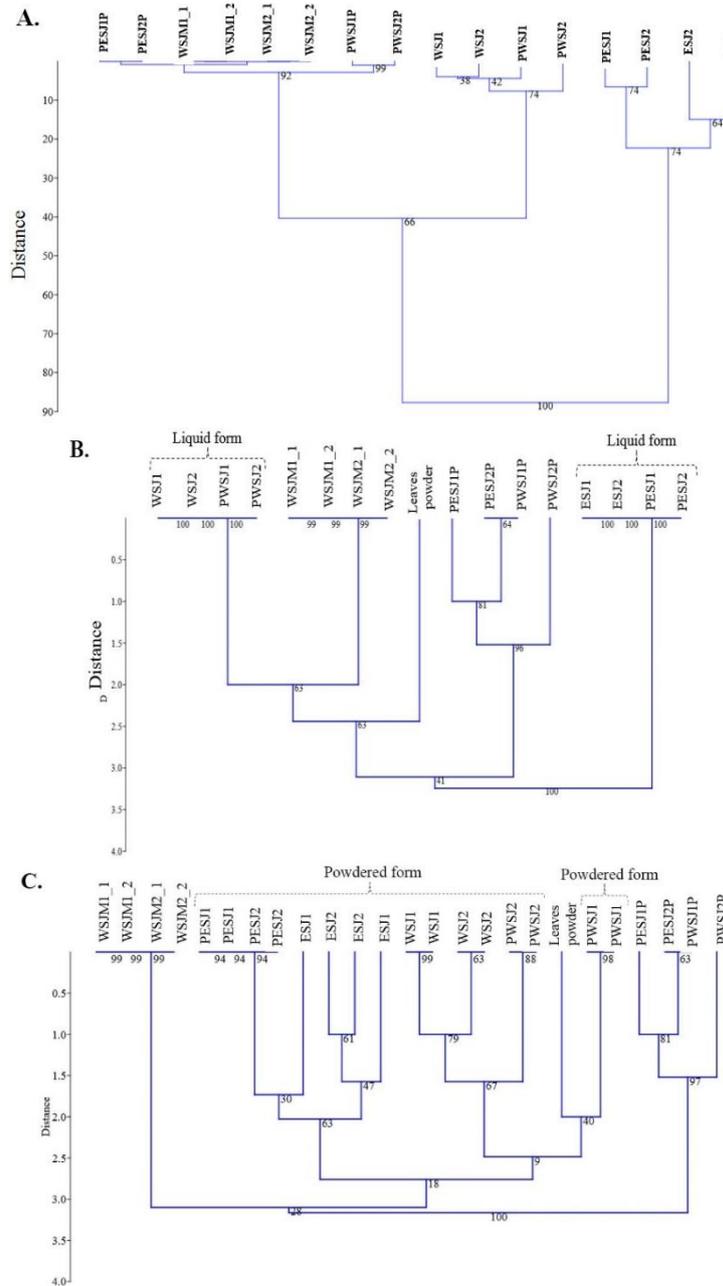


Figure 4. Cluster analysis of all samples performed by an unweighted pair group method (UPGMA). (A) was UPGMA tree of similarity among flower and dye extracts in liquid form, dye solution 1, dye solution 2, and dye solution 3 based on total phenolic contents, total flavonoid contents, and biological activity; (B) was UPGMA tree of similarity among flower and dye extracts in liquid form, powder dried leaves, and dye solution based on FTIR spectra; and (C) was UPGMA tree of similarity among flower and dye extracts in powdered form, powder dried leaves, and dye solution based on FTIR spectra. ESJ1 and ESJ2 were ethanolic extracts from *S. javanica* flower. WSJ1 and WSJ2 were aqueous extracts from *S. javanica* flower. PESJ1 and PESJ2 were ethanolic dye extracts from *S. javanica* flower. PWSJ1 and PWSJ2 were aqueous dye extracts from *S. javanica* flower. PESJ1P and PESJ2P were dye solutions obtained from mixing white clay filler and ethanolic dye extract from *S. javanica* flower. PWSJ1P and PWSJ2P were dye solutions obtained from mixing white clay filler and aqueous dye extract from *S. javanica* flower. WSJM1_1 and WSJM1_2 were solutions obtained from mixing 20% maltodextrin and aqueous extract from *S. javanica* flower. WSJM2_1 and WSJM2_2 were solutions obtained from mixing 25% maltodextrin and aqueous extract from *S. javanica* flower.

Conclusion

In conclusion, flower and dye extract of *S. javanica* were a source of biological agents, antioxidant activity, and α -glucosidase inhibitory activity. The ethanolic flower and dye extracts of *S. javanica* showed higher total phenolic and flavonoid contents, antioxidant activity, and α -glucosidase inhibition than those of aqueous extracts. However, dye solution, obtained from mixing white clay filler and dye aqueous extract, showed higher total phenolic and flavonoid contents, antioxidant activity, and α -glucosidase inhibition than those of dye solution, obtained from mixing white clay filler and dye ethanolic extracts. The FTIR fingerprint revealed the presence of phenolics, flavonoids, isoprenoids, carbohydrates, lipids, proteins, amino acids, aromatic secondary amines, acids, esters, DNA, and RNA in *S. javanica* flower and its dye products. Furthermore, FTIR could be used to detect similarity among samples from different methods. The PCA and cluster analysis, obtained from FTIR data and biological activities, led to well grouping in PC plots and phylogenetic trees based on solvent types and dye preparation methods. The present work demonstrated that a combined FTIR spectrum, PCA, and cluster analysis of plant extracts in powdered form provided better separation in PC plots and phylogenetic trees than in liquid form. These could be valuable data for detecting an important ingredient in health foods and pharmaceutical cosmetics.

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