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Research Article

Comparison of FTIR fingerprint, phenolic content, antioxidant and anti-glucosidase activities among *Phaseolus vulgaris* L., *Arachis hypogaea* L. and *Plukenetia volubilis* L.

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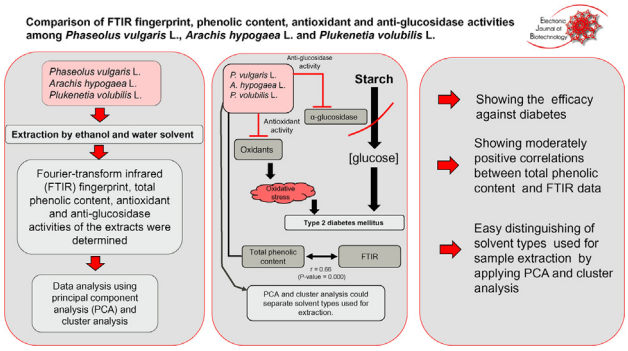
GRAPHICAL ABSTRACT



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ABSTRACT

Background: Inhibition of starch-hydrolysing enzymes is one of the major methods to reduce the risk of type−2 diabetes mellitus. Nowadays, there are no reports involving oil-rich and oil-low seeds of different botanical origins. The current study intended to extract *Phaseolus vulgaris* L. and *Arachis hypogaea* L. including *Plukenetia volubilis* L. using ethanol and water solvents, and to analyse Fourier-transform infrared (FTIR) fingerprint, total phenolic content, antioxidant and anti-glucosidase activities of the extracts by principal component analysis (PCA) and cluster analysis.

Results: The result showed that the ethanol extracts of *P. vulgaris* L., *A. hypogaea* L., and *P. volubilis* L. showed total phenolic content higher than those of the aqueous extracts. The result also demonstrated that the aqueous and ethanol extracts from *P. volubilis* L. seed showed the highest antioxidant and anti-glucosidase activities, respectively. In comparison with the efficacy of the aqueous extracts of beans, *A. hypogaea* L. showed the highest antioxidant activity and anti-glucosidase activity. For the ethanol extract of beans, *P. vulgaris* L. (red kidney bean) showed the highest antioxidant activity, while *P. vulgaris* L. (white kidney beans) showed the highest anti-glucosidase activity. Moreover, significantly positive correlations between total phenolic content and anti-glucosidase activity (r = 0.41, P-value = 0.018), and between total phenolic content and FTIR data (r = 0.66, P-value = 0.000) were found.

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Conclusions: FTIR of the extracts showed functional groups corresponding with phenolic compounds. Moreover, the PCA and cluster analysis from FTIR data, phenolic content and biological activity could separate solvent types used for extraction.

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1. Introduction

The prevalence of chronic non-communicable diseases (i.e. diabetes mellitus, hypertension, heart diseases, cerebrovascular disease and cancers) has now become increasingly more severe in people worldwide, which is influenced by lifestyle changes. Among the lifestyle diseases, type−2 diabetes is one of the major chronic non-communicable diseases, and Thailand is facing adverse effects of the diseases. In Thailand, the prevalence of diabetes mellitus in the Thai population (age 20–79 years) has become an increasing trend between 2021 and 2045 from 9.7% in 2021 to 11.0% in 2045 [1]. Moreover, the trend of type−2 diabetes among Thai people is continuously rising. People with type−2 diabetes have increasing trend at 1.59 million in 2025 [2].

Therefore, Thailand is attempting to reduce risk factors, complications, and mortality, which occur from type−2 diabetes. However, inhibition of enzyme function involving carbohydrate digestion is one of the major methods to reduce the problems. For example, glucosidase is an exoenzyme corresponding to hydrolyse glycosidic bonds in carbohydrates into glucose, which is absorbed via the small intestine epithelium into blood [3]. Thus, inhibiting this enzyme can help to decrease the level of blood sugar after a carbohydrate meal. Plant is one of the healthy choices which can promote human health and disease prevention, due to which it contains several bioactive agents with pharmaceutical effects. For example, phenolic agents can be displayed as an antioxidant by scavenging free radicals based on the mechanism of transferring hydrogen atom, a single electron, sequential proton loss electron, and chelating transition metals [4]. Moreover, several reports demonstrated that some plant extracts comprise agents which show glucosidase inhibitory activities [3,5]. Recently, many reports put effort to identify natural glucosidase inhibitors from plants, due to which natural agents provide serious adverse effects lower than those of synthetic drugs (i.e. biguanide and sulfonylureas) [6,7]. In current research, *Phaseolus vulgaris* L., *Arachis hypogaea* L. and *Plukenetia volubilis* L. were focused, due to which the plants were popularly used as the major ingredient of many healthy foods and were generally cultivated in Thailand.

P. vulgaris L. is a common bean in Fabaceae family which is well known and commonly consumed worldwide. *P. vulgaris* L. consists of a large number of cultivars that show variations in morphological characteristics (i.e. seed size and color) [8]. The seed coat color (i.e. white, white with speckle, yellow, cream, brown, red, pink, grey and black) can affect the amount of phytochemicals and bioactivities [9]. Recently, *P. vulgaris* L. is becoming increasingly popular as a functional food because it comprises high nutrients (i.e. protein, complex carbohydrates, vitamins and minerals) and phytochemicals with nutritional and functional values [10,11]. *P. vulgaris* L. seeds have been described for pharmacotherapeutic effects such as bifidogenic effect [12], antioxidant activity, and antiproliferative effects [13].

Arachis hypogaea L., known as peanut, is an oil-yielding legume in Fabaceae family and is one of the important commercial plants in Thailand, and is commonly consumed as high nutrient food [14,15]. *A. hypogaea* L. has been described in health benefits, due to which it is rich in unsaturated fatty acids, proteins, fibre, micronutrients, and phytochemicals with great positive biological effects (i.e. reducing cardiovascular disease risk, decreasing LDL oxidation and lowering type−2 diabetes risks) [16].

P. volubilis L., called sacha inchi or inca peanut, is a climbing plant in Euphorbiaceae family, which is commonly grown in tropical rain forest in Peru, Brazil and South East Asia namely Thailand, Vietnam and Malaysia [17,18]. Its seed has a lenticular shape that contains high levels of unsaturated fatty acids (i.e. α-linoleic acid and linolic acid), vitamins, minerals, proteins and phytochemicals, providing therapeutic and nutritional uses for patients with coronary heart disease, arthritis, diabetes, ADHD, and inflammatory skin diseases [19]. Moreover, the seed coat of *P. volubilis* L. is popular to consume as functional food, so it was used in this study. It has been reported that ethanol extract from the non-cooked shell of *P. volubilis* L. consists of flavonoids and triterpenoids, and provides high phenolic content, antioxidant activity, and anti-tyrosinase activity [20]. Although *P. volubilis* L. is popular to economically cultivate in Thailand as foods and omega supplements, it is high cost.

However, a comparative knowledge of antioxidant activity, glucosidase inhibition and chemical fingerprint among oil-rich and oil-low seeds of different botanical origins were unknown. Interestingly, the application of Fourier-transform infrared (FTIR) spectra for phytochemical screening was rapid and low cost. Therefore, our objectives focused on a comparison of total phenolic content, antioxidant activity, anti-glucosidase activity among seed extracts of *P. vulgaris* L., *A. hypogaea* L. and *P. volubilis* L. including seed coat of *P. vulgaris* L. Moreover, Fourier-transform infrared (FTIR) spectroscopy was used to detect functional groups of the chemical components in the extracts. The FTIR data and biological activities from this study are useful for screening some popular plant foodstuffs.

2. Methods and materials

2.1. Chemicals

Alpha-glucosidase from *Saccharomyces cerevisiae*, acarbose, 4-nitrophenyl-α-D-glucopyranoside and 3-ethylbenzothiazoline-6-sulfonic acid diammonium salt (ABTS) were received from Sigma. Gallic acid, L-glutathione reduced and absolute ethanol were received from Sigma-Aldrich. Potassium persulfate and sodium carbonate were received from Ajax Finechem. Folin–Ciocalteu's phenol reagent was received from Merck. Potassium phosphate was received from Bio Basic Canada Inc.

2.2. Sample preparation and extraction

Seeds of *P. vulgaris* L. (white and red kidney beans), *A. hypogaea* L., including seed and seed coat of *P. volubilis* L., were purchased from a market. Seed size, shape and color were identified by comparing the report of Kodahl [21], Farber et al. [22], Kläsener et al. [23]. Each sample (500 g) was cleaned with water, followed by dry-

ing at 60°C for 48 h. The dried seeds and seed coat were finely crushed by a homogeniser. Each powder sample (10 g) was extracted with a 250 ml solvent namely ethanol and water at 45°C for 24 h. After that, each extract was sieved through a filter cloth, and then concentrated by using a rotary evaporator (IKAa RV10) at 45°C for 20 min for ethanol extract, and at 45°C for 30 min for water extract [24]. Finally, each extract was adjusted to final concentration (0.1 g/ml). Each sample was extracted with each solvent in duplicate (n = 10) and was kept at 4°C until used.

2.3. Anti-glucosidase activity

Anti-glucosidase activity was carried out according to the report of Thummajitsakul et al. [25]. Each extract at 100, 50 and 20 mg/ml concentration was used for 100 µl to react with 3 mM glutathione (25 µl) and 0.3 unit/ml glucosidase enzyme (25 µl) in 0.067 M potassium phosphate buffer pH 6.8 (250 µl) at 37°C for 10 min. Then, 10 mM 4-Nitrophenyl-α-D-glucopyranoside (25 µl) was gently mixed and incubated at 37°C for 10 min, followed by mixing 0.1 M sodium carbonate (400 µl) [25]. Two assays were run per each extract, and acarbose (25 mg/ml) was used as positive control. The absorbance of each reaction was determined at 400 nm using a spectrophotometer (Model T60UV). The percentage of inhibition was calculated by following **Equation (1)**:

$$\%\ glucosidase\ inhibition = \frac{[(A_{water} - B_{water}) - (A_{sample} - B_{sample})]}{(A_{water} - B_{water})} \times 100\% \quad \text{Equation1}$$

where A_{water} and A_{sample} represented the absorbance of negative control (distilled water) and sample with alpha-glucosidase; B_{water} and B_{sample} represented the absorbance of distilled water and sample without alpha-glucosidase.

The percentage of glucosidase inhibition of each extract at 100, 50 and 20 mg/ml concentration was used to generate a simple linear regression (R^2 = 0.5–0.9) for calculating 50% effective concentration (EC_{50}), which was concentration of the extract used to inhibit 50% of glucosidase activity.

2.4. Antioxidant activity

Antioxidant activity was estimated by 2,2-Azino-bis-3-ethylben zothiazoline-6-sulfonic acid (ABTS) method [24,25]. Firstly, $ABTS^{\bullet+}$ free radicals were prepared by mixing between 7 mM ABTS solution (10 µl) and 140 mM potassium persulfate (179 µl) and then left at room temperature in the dark condition overnight. Before using, the free radical cation solution was diluted until an absorbance of 0.700 ± 0.050 was obtained. For each reaction, the diluted $ABTS^{\bullet+}$ solution (3.9 ml) was mixed with each sample at 100, 50 and 20 mg/ml concentration (20 µl) and was incubated for 6 min in a dark condition; then, the absorbance was detected at 734 nm. Two assays were run per each extract. The percentage of antioxidant capacity was performed by following **Equation (2)**:

$$\%\ Antioxidant\ capacity = \frac{A_{ABTS} - A_{Sample-ABTS}}{A_{ABTS}} \times 100\% \quad \text{Equation2}$$

where A_{ABTS} represented the absorbance of the diluted $ABTS^{\bullet+}$ solution. $A_{Sample\text{-}ABTS}$ represented the absorbance of the tested sample with the diluted $ABTS^{\bullet+}$ solution.

A simple linear regression (R^2 = 0.6–1.0) was generated from the percentage of antioxidant capacity of each extract at 100, 50 and 20 mg/ml concentration and used to calculate EC_{50}, which was concentration of the extract needed to scavenge 50% of $ABTS^{\bullet+}$ free radicals.

2.5. Total phenolic content

Total phenolic content was estimated by the Folin-Ciocalteu method [24,25]. Each extract (300 µl) was mixed with Folin-Ciocalteu reagent (1.5 ml) for 5 min under room temperature, then reacted with 7.5% w/v sodium carbonate (1.2 ml) for 30 min under room temperature. Each extract was performed in duplicate. An absorbance was determined at 765 nm. Total phenolic content was calculated by comparing with a standard graph (R^2 = 0.9) of gallic acid at concentrations 0.0625, 0.125, 0.25, 0.50 and 1.00 mg/ml.

2.6. Fourier-transform infrared (FTIR) spectroscopy

Each extract was loaded onto a crystal plate of a Fourier-transform infrared (FTIR) spectroscopy (PerkinElmer spectrum IR version 10.6.0, USA), and scanning absorption were recorded at wavenumber ranges in the range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} [24]. Each FTIR spectrum of each sample was detected in duplicate. For identifying functional groups, the FTIR spectra in wavenumber ranges were compared with the reports of Lingegowda et al. [26], Caunii et al. [27], Lahlali et al. [28], Kumar et al. [29], Hands et al. [30], Cao et al. [31], Topală et al. [32], Abbas et al. [33], and Noh et al. [34]. For FTIR data analysis, binary data (1/0) was carried out by scoring the presence of peak in each wavenumber range as 1 and the absence as 0. The binary data were used for determining Pearson's correlation, principal component analysis (PCA), and cluster analysis.

2.7. Data analysis

Descriptive statistics (i.e. mean, SD and percentage) were used to express total phenolic content, antioxidant activity and anti-glucosidase activity. One-way ANOVA was used to test the difference of total phenolic content, antioxidant activity and anti-glucosidase activity among sample groups. Pearson's correlation was used to express the relationship among total phenolic content, antioxidant activity, anti-glucosidase activity, and FTIR data. The descriptive and inference statistics were performed by the PSPP program version 0.10.5 [35]. Moreover, principal component analysis (PCA) and cluster analysis in PAST 3.25 software were used to demonstrate similarity of the sample extracts [36].

3. Result and discussion

The total phenolic content of seed extracts from *P.vulgaris* L. (white and red kidney bean), *P. volubilis* L., and *A. hypogaea* L., and seed coat extract of *P. volubilis* L. were found in ranging from 445.98 ± 128.17 to 1389.11 ± 272.17 mg gallic/g extract. The result showed that ethanol extracts had total phenolic content (1084.43 ± 63.67 to 1389.11 ± 272.17 mg gallic/g extract) higher than that of aqueous extracts (445.98 ± 128.17 to 836.61 ± 223.84 mg gallic/g extract). The maximum amount of total phenolic content was found in ethanol extract of *P. volubilis* L. seed coat (1389.11 ± 272.17 mg gallic/g extract), followed by seed ethanol extract of *P. vulgaris* L. (white kidney beans) (1328.30 ± 156.63 mg gallic/g extract) and seed ethanol extract of *P. volubilis* L. (1251.31 ± 13.22 mg gallic/g extract) (Table 1).

The antioxidant activity was determined by ABTS method. The $1/EC_{50}$ values obtained from ABTS method were in the range of 0.0014 ± 0.0003 to 0.0158 ± 0.0006. The result showed that aqueous extracts ($1/EC_{50}$ = 0.0026 ± 0.0009 to 0.0158 ± 0.0006) showed higher antioxidant activity than that of ethanol extracts ($1/EC_{50}$ = 0.0014 ± 0.0003 to 0.0150 ± 0.0017). The highest $1/EC_{50}$ value was found in seed aqueous extract of *P. volubilis* L. ($1/EC_{50}$ = 0.0158 ± 0.

Table 1
Total phenolic content, antioxidant activity and anti-glucosidase activity of aqueous and ethanol extracts of *P. vulgaris* L. (white and red kidney bean), *P. volubilis* L., and *A. hypogaea* L.

Samples	Solvent types	Total phenolic content (mg gallic/g extract)	Antioxidant activity (mean ± SD) $1/EC_{50}$ (ml/mg)	Anti-glucosidase activity (mean ± SD) $1/EC_{50}$ (ml/mg)
P. vulgaris L. (red kidney bean)	Seed aqueous	445.98 ± 128.17	0.0046 ± 0.0001	0.0125 ± 0.0026
	Seed ethanol	1174.32 ± 103.11	0.0061 ± 0.0008	0.0214 ± 0.0032
P. vulgaris L. (white kidney bean)	Seed aqueous	720.47 ± 161.15	0.0026 ± 0.0009	0.0123 ± 0.0010
	Seed ethanol	1328.30 ± 156.63	0.0035 ± 0.0005	0.0388 ± 0.0287
A. hypogaea L.)	Seed aqueous	712.16 ± 218.59	0.0070 ± 0.0048	0.0200 ± 0.0037
	Seed ethanol	1084.43 ± 63.67	0.0041 ± 0.0013	0.0163 ± 0.0038
P. volubilis L.	Seed aqueous	836.61 ± 223.84	0.0158 ± 0.0006	0.0151 ± 0.0050
	Seed ethanol	1251.31 ± 13.22	0.0014 ± 0.0003	0.0577 ± 0.0621
	Seed coat aqueous	647.20 ± 358.99	0.0112 ± 0.0018	0.0052 ± 0.0021
	Seed coat ethanol	1389.11 ± 272.17	0.0150 ± 0.0017	0.0120 ± 0.0001
P-value		0.000*	0.232	0.030*

Note: Asterisk symbol (*) indicated differentiation of total phenolic content and anti-glucosidase activity between ethanol and aqueous extracts by using an independent t-test statistically significant at P-value < 0.05.

0006), followed by ethanol extract of *P. volubilis* L. seed coat ($1/EC_{50}$ = 0.0150 ± 0.0017), seed aqueous extract of *P. volubilis* L. seed coat ($1/EC_{50}$ = 0.0112 ± 0.0018) and seed aqueous extract of *A. hypogaea* L. ($1/EC_{50}$ = 0.0070 ± 0.0048) (Table 1).

The $1/EC_{50}$ values for the anti-glucosidase activity were found in range of 0.0120 ± 0.0001 to 0.0577 ± 0.0621. The result showed that ethanol extracts ($1/EC_{50}$ = 0.0120 ± 0.0001 to 0.0577 ± 0.0621) showed anti-glucosidase activity higher than that of aqueous extracts ($1/EC_{50}$ = 0.0052 ± 0.0021 to 0.0200 ± 0.0037). The highest $1/EC_{50}$ value was found in seed ethanol extract of *P. volubilis* L. ($1/EC_{50}$ = 0.0577 ± 0.0621), followed by seed ethanol extract of *P. vulgaris* L. (white kidney beans) ($1/EC_{50}$ = 0.0388 ± 0.0287), seed ethanol extract of *P. vulgaris* L. (red kidney bean) ($1/EC_{50}$ = 0.0214 ± 0.0032) (Table 1). From the result, differentiation of total phenolic content and anti-glucosidase activity between ethanol and aqueous extracts was significantly found at P-value < 0.05.

According to the result, a comparison of the efficacy of the aqueous extracts of beans, *A. hypogaea* L. showed the highest antioxidant activity and anti-glucosidase activity. For the ethanol extract of beans, *P. vulgaris* L. (red kidney bean) showed the highest antioxidant activity, while *P. vulgaris* L. (white kidney beans) showed the highest anti-glucosidase activity. In current study, water and ethanol solvents were used to extract the samples. Water solvent was safe for food and health applications and used to enhance the solubility of polar compounds, while ethanol was used as a nonpolar solvent to improve antioxidant and anti-glucosidase activities and solubility of phenolic compounds [24,25].

Moreover, the FTIR result showed 15 weverange peaks. Of these, two specific peaks of ethanol extract were 2853.87–2925.91 and 1743.72–1744.4 cm^{-1}, corresponding to CH_2 and CH_3 stretching vibrations, and C═O of lipids, respectively. Additionally, FTIR peaks showed different peak patterns and high weveranges of 571.09–1744.4 cm^{-1} among types of samples and solvents (Fig. 1A and 1B). Functional groups, found in the samples after extraction, were shown in Table 2. The result indicated that there were many functional groups associated with phenolic compounds, which were in weverange of 3008.64–3356.84 cm^{-1}, 1632.61–1744.4 cm^{-1}, 1743.72–1744.4 cm^{-1}, 1632.61–1638.05 cm^{-1}, 1377.67–1415.32 cm^{-1}, 1377.67–1379.42 cm^{-1}, 1155.41–1269.5 cm^{-1}, 1085.68–1118.83 cm^{-1} and 1046.37–1078.95 cm^{-1}. Previously, it has been reported that phenolic acids were identified by weveranges of 700−1640 cm^{-1}, 950−1470 cm^{-1} and 1630–1755 cm^{-1} involving aromatic six-membered rings, methoxy group and carboxylic acids, respectively [33].

In order to determine the correlation between chemical content and biological activities of the extracts, moderately positive correlations between total phenolic content and FTIR data (r = 0.66, P-value = 0.000) and low positive correlations between total phenolic content and anti-glucosidase activity (r = 0.41, P-value = 0.018) were significantly found by Pearson's correlation analysis (Table 3). This indicated that some phenolics in the extract showed anti-glucosidase activity. In case of FTIR, positive correlation was significantly found with total phenolic content (Table 3). This indicated that some functional groups shown in Table 2 were phenolic compounds. However, Pearson's correlation between antioxidant activity and total phenolic content, and between anti-glucosidase activity and FTIR data were not found.

Previously, it has been reported that several α-glucosidase inhibitors (i.e. flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, phenolics) are isolated from plants [5]. Moreover, several α-glucosidase inhibitors from plants, such as taxumariene F, akebonoic acid, morusin, rhaponticin, procyanidin A2, alaternin, mulberrofuran K and psoralidin, have ability for the treatment of type-2 diabetes [37]. Nonetheless, α-glucosidase inhibitors of *P. vulgaris* L. (white and red kidney beans) have been reported, but beans (*Phaseolus* spp.) have high phenolic compounds (i.e. quercetin 3-glucoside and genistein), antioxidant activity, and antinutritional components [38]. Moreover, seed extract from *P. volubilis* L. has been showed high flavonoids and phenolic compound contents with antioxidant activities [39]. However, fresh *P. volubilis* L. seeds and leaves have saponins, alkaloids, and lectins, which are phytotoxins and can be unstable by heating [40]. *A. hypogaea* L. have high phytonutrient, such as resveratrol, isoflavonoids, phenolic acids, and phytosterols, which relate to health and wellness [41].

However, it has been reported that some non-phenolic agents show anti-glucosidase activity. Several previous reports show that many non-phenolic compounds have anti-glucosidase activity, such as bioactive peptide, non-starch carbohydrates and lipids [42]. It has been reported that peptides obtained from gastrointestinal digestion of germinated soybean proteins show anti-glucosidase activity [43]. Additionally, it has been reported that *P. vulgaris* L. has higher polyphenols and antioxidant activity in dark beans than in lighter color beans, due to which the seed coats consist of greater polyphenols and reveal higher antioxidant activity than cotyledons [44].

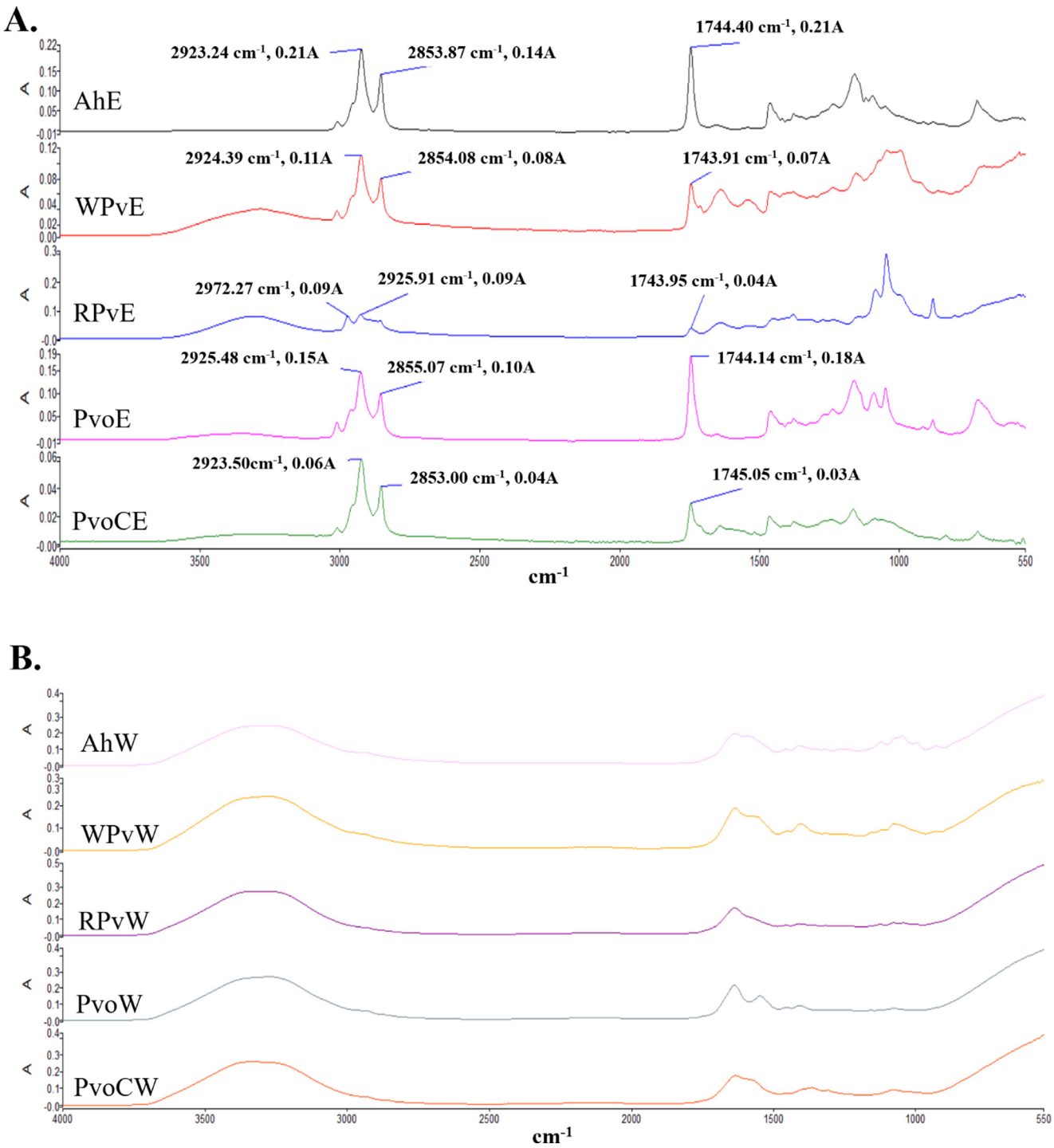

Fig. 1. FTIR spectra after extraction of *P. vulgaris* L. (white and red kidney beans), *P. volubilis* L. and *A. hypogaea* L. WPvE: seed ethanol extract of *P. vulgaris* L. (white kidney bean); RPvoE: seed ethanol extract of *P. vulgaris* L. (red kidney bean); AhE: seed ethanol extract of *A. hypogaea* L.; PvoE: seed ethanol extract of *P. volubilis* L.; PvoCE: seed coat ethanol extract of *P. volubilis* L.; WPvW: seed aqueous extract of *P. vulgaris* L. (white kidney bean); RPvoW: seed aqueous extract of *P. vulgaris* L. (red kidney bean); AhW: seed aqueous extract of *A. hypogaea* L.; PvoW: seed aqueous extract of *P. volubilis* L.; PvoCW: seed coat aqueous extract of *P. volubilis* L.

Generally, pigments in seed coats are better dissolved in organic solvent (i.e. ethanol and methanol) than in water solvent. In the previous study, anthocyanin pigments in seed coats of black soybean (*Glycine* max (L.) Merr.) are extracted by 1% HCl in methanol solvent, in which major anthocyanins (i.e. delphinidin-3-glucoside, cyanidin-3-glucoside, and petunidin-3-glucoside) are found in the extract [45]. Moreover, it has been reported that cyanidin and catechin pigments of adzuki bean (*Vigna angularis*) are extracted by 1% HCl in methanol solvent [46].

However, *P. vulgaris* L. (white kidney bean) has the ability to reduce blood glucose levels and can be used as a commercial functional food for the treatment of diabetes [47,48]. The *P. vulgaris* L.

Table 2
Functional groups and its FTIR wavenumber ranges after extraction of *P. vulgaris* L. (white and red kidney bean), *P. volubilis* L. and *A. hypogaea* L.

Peak number	Wavenumber ranges (cm^{-1})	Function groups	References
1	3008.64–3356.84	O—H and N—H stretch	[27,31]
2	2925.48–2925.91	Intramolecular bonded alcohol O—H stretching	[29]
3	2853.87–2925.91	CH_2 and CH_3 stretching vibrations	[28]
4	1632.61–1744.4	N—H bending vibrations, C=O bending vibrations	[32,33]
5	1743.72–1744.4	C=O	[30,33]
6	1632.61–1638.05	Unsaturation bonds of flavonoids	[33,34]
7	1547.12–1599.67	Aromatic and N—H bending vibrations	[27,32]
8	1403.98–1464.77	CH_3 lipids/proteins and COO^- of amino acids	[30]
9	1464.36–1548.15	Amide II of proteins (α-helix structures, β-pleated sheet structures, turns, random coils)	[30]
10	1377.67–1415.32	Primary or secondary O—H bending (in-plane), and phenol or tertiary alcohol (O—H bend)	[27,33]
11	1377.67–1379.42	CH_3 bending, C—O—H deformation	[33]
12	1155.41–1269.5	C—O stretching vibrations	[27,32,33]
13	1085.68–1118.83	Secondary alcohol, C—O stretching vibrations	[26,33]
14	1046.37–1078.95	Primary alcohol, C—O stretching vibrations	[26,33]
15	571.09–996.99	C—H bending vibrations	[27,32]

Table 3
Pearson correlation coefficients between biological activity and chemical component measured in extracts of *P. vulgaris* L. (white and red kidney beans), *A. hypogaea* L. and *P. volubilis* L.

	Total phenolic content	Antioxidant activity	Anti-glucosidase activity
Antioxidant activity	r = 0.09 (P-value = 0.591)		
Anti-glucosidase activity	r = 0.41 (P-value = 0.018)*	r = −0.32 (P-value = 0.074)	
FTIR data	r = 0.66 (P-value = 0.000)*	r = 0.10 (P-value = 0.547)	r = 0.30 (P-value = 0.098)

Note: Asterisk symbol (*) showed statistically significant at P-values < 0.5.

(white kidney beans) contain rich nutrients such as resistant starch, oligosaccharides, and bioactive peptide nutrients [49]. Bioactive peptides can act as antioxidant and can be used to produce health-promoting foods. Recently, it has been reported that pepsin hydrolysate and papain hydrolysate from *P. vulgaris* L. (white kidney beans) show 85% and 89% antioxidant activity [50,51].

For *A. hypogaea* L., its pericarp consists of resveratrol compound in ethanol extract, which helps to inhibit α-amylase, α-glucosidase and β-galactosidase enzymes [52], whereas its kernel and seed coat show high phenolic content and antioxidant property [53,54]. Interestingly, ethanol extracts of *A. hypogaea* L. skin show high α-glucosidase inhibitory activity and contain 26 bioactive compounds namely catechin, epicatechin, and 24 proanthocyanidins [55].

Moreover, *P. volubilis* L. seed contains phytotoxins namely saponins, alkaloids, and lectin [40]. However, it consists of several bioactive compounds and high nutrients, such as phenolics, tocopherol, phytosterol, high essential fatty acid levels (i.e. ω-3 and ω-6), amino acids and vitamin E [56,57,58].

For PCA result from FTIR data, PC1 was 42.66% of total variance, whereas PC2 was 22.63%. According to the result, ethanol and aqueous extracts were separated on opposite side in the PCA biplot. Peak numbers 6 and 14 were mostly found in aqueous extracts, which were in wavenumber ranges of 1632.61–1638.05 and 1046.37–1078.95 cm^{-1}, respectively, whereas peak numbers 2, 3, 5, 9, 10, 11, 12, 13 and 15 were mostly found in ethanol extracts, which were in wavenumber ranges of 2925.48–2925.91 cm^{-1}, 2853.87–2925.91 cm^{-1}, 1743.72–1744.4 cm^{-1}, 1464.36–1548.15 cm^{-1}, 1377.67–1415.32 cm^{-1}, 1377.67–1379.42 cm^{-1}, 1155.41–1269.5 cm^{-1}, 1085.68–1118.83 cm^{-1} and 571.09–996.99 cm^{-1}, respectively (Fig. 2A). For PCA result based on chemical content and biological activity, PC1 was 59.06% of total variance, whereas PC2 was 33.00% of total variance. The result confirmed that the aqueous extracts had higher antioxidant activity than ethanol extracts, whereas ethanol extracts had stronger anti-glycosidase activity and total phenolic content than aqueous extracts. Moreover, it confirmed positive correlation between antioxidant activity and total phenolic content.

The dendrograms were generated by paired group (UPGMA) method based on FTIR data, total phenolic content, antioxidant activity, and anti-glycosidase activity of samples. For FTIR data, the dendrogram was separated into 2 groups namely ethanol extracts and aqueous extracts. Similarly, the dendrogram based on chemical content and biological activity was classified into 2 groups namely ethanol and aqueous extracts (Fig. 3). Thus, differentiation between ethanol extracts and aqueous extracts was identified by the PCA and cluster analysis based on FTIR data, total phenolic content, antioxidant activity, and anti-glycosidase activity. The analytical discriminability of the ethanol extracts from the aqueous extracts can be influenced by the polarity of extracting solvents. It has been reported that the polarity of solvents can affect extraction yield, phytochemical content and antioxidant activity, in which polar solvents provide higher extract yield but lower phenolic and flavonoid content than those of non-polar solvents [59]. Therefore, *A. hypogaea* L. and *P. vulgaris* L. are healthy food choices which have efficacy against diabetes, which can be consumed as substitute for inca peanut, which has high cost.

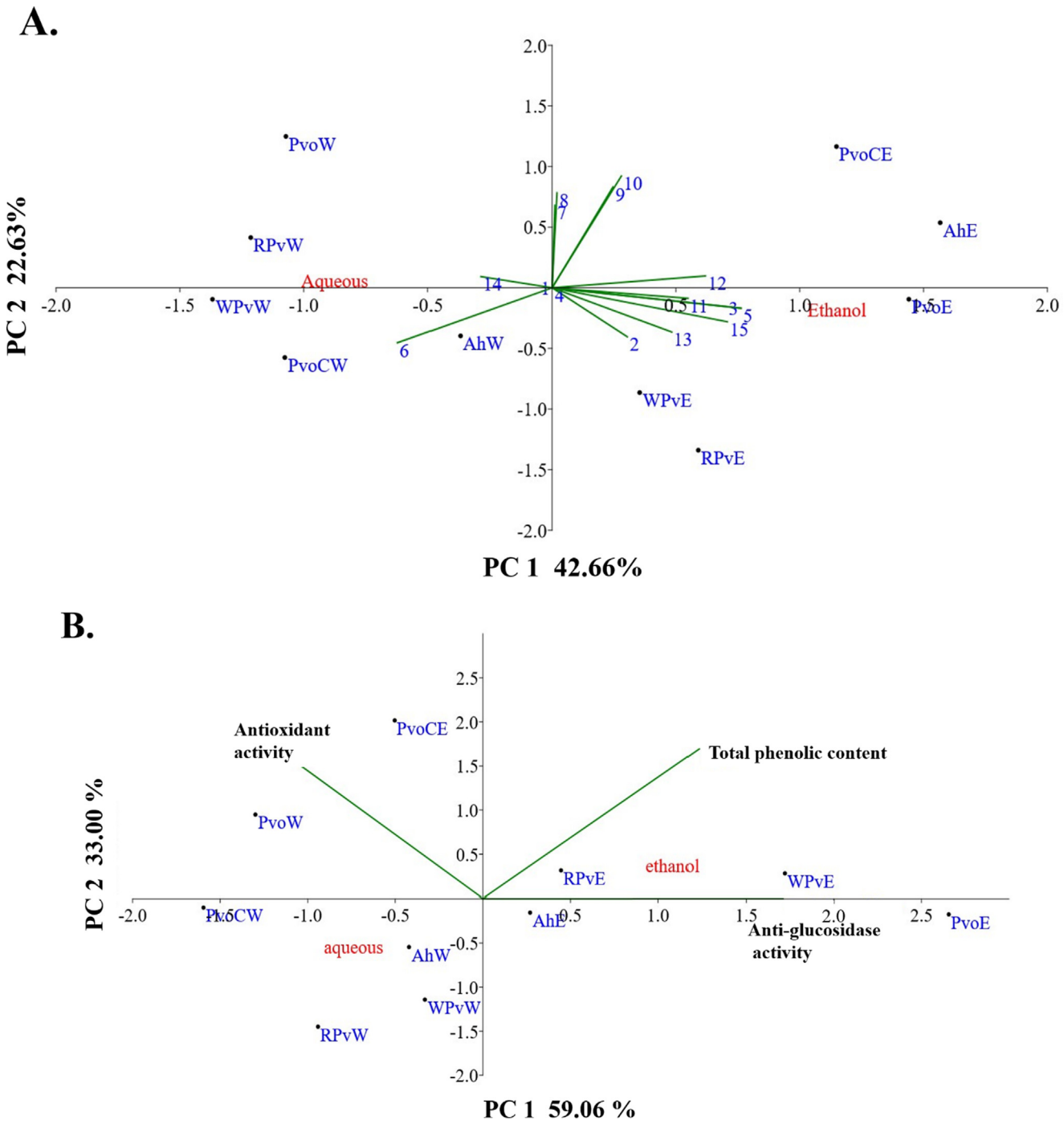

Fig. 2. Principal component analysis (PCA) of FTIR spectra (A), total phenolic content, free radical scavenging activity and anti-glycosidase activity of samples. WPvE: seed ethanol extract of *P. vulgaris* L. (white kidney bean); RPvoE: seed ethanol extract of *P. vulgaris* L. (red kidney bean); AhE: seed ethanol extract of *A. hypogaea* L.; PvoE: seed ethanol extract of *P. volubilis* L.; PvoCE: seed coat ethanol extract of *P. volubilis* L.; WPvW: seed aqueous extract of *P. vulgaris* L. (white kidney bean); RPvoW: seed aqueous extract of *P. vulgaris* L. (red kidney bean); AhW: seed aqueous extract of *A. hypogaea* L.; PvoW: seed aqueous extract of *P. volubilis* L.; PvoCW: seed coat aqueous extract of *P. volubilis* L.

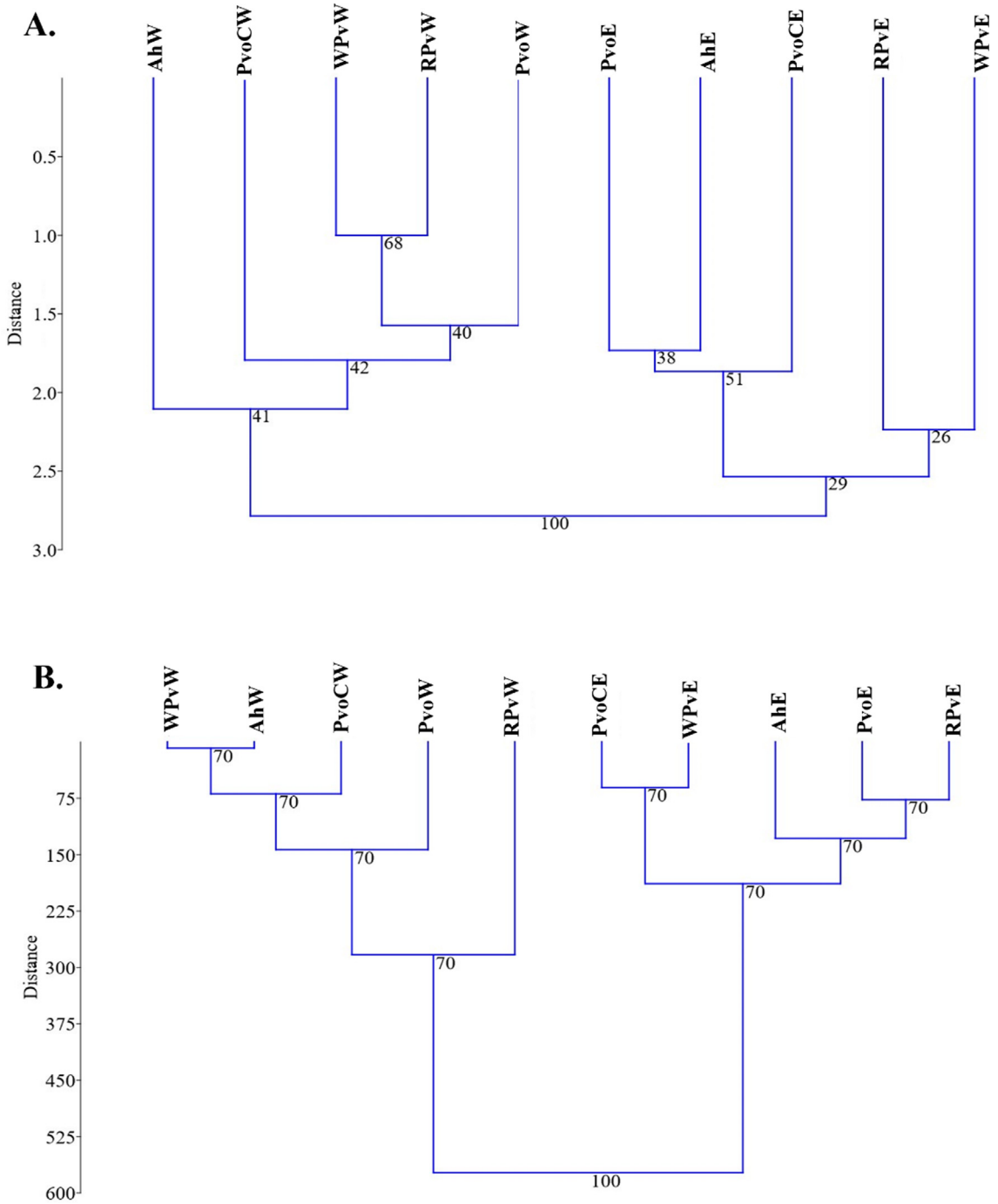

Fig. 3. Dendrogram generated by paired group (UPGMA) method based on FTIR data (A), total phenolic content, free radical scavenging activity and anti-glycosidase activity (B) of samples. WPvE: seed ethanol extract of *P. vulgaris* L. (white kidney bean); RPvoE: seed ethanol extract of *P. vulgaris* L. (red kidney bean); AhE: seed ethanol extract of *A. hypogaea* L.; PvoE: seed ethanol extract of *P. volubilis* L.; PvoCE: seed coat ethanol extract of *P. volubilis* L.; WPvW: seed aqueous extract of *P. vulgaris* L. (white kidney bean); RPvoW: seed aqueous extract of *P. vulgaris* L. (red kidney bean); AhW: seed aqueous extract of *A. hypogaea* L.; PvoW: seed aqueous extract of *P. volubilis* L.; PvoCW: seed coat aqueous extract of *P. volubilis* L.

4. Conclusions

This current study showed that the ethanol extracts of *P. vulgaris* L. (white and red kidney bean), *A. hypogaea* L., and *P. volubilis* L. showed total phenolic content stronger than those of the aqueous extracts. The result also demonstrated that the aqueous extracts from *P. volubilis* L. seed showed the highest antioxidant activity, while the ethanol extract of *P. volubilis* L. seed showed the highest anti-glucosidase activity. In comparison with the efficacy of the aqueous extracts of beans, *A. hypogaea* L. showed the highest antioxidant activity and anti-glucosidase activity. For the ethanol extract of beans, *P. vulgaris* L. (red kidney bean) showed the highest antioxidant activity, while *P. vulgaris* L. (white kidney beans) showed the highest anti-glucosidase activity. Interestingly, the Fourier-transform infrared (FTIR) result confirmed the presence of functional groups corresponding with phenolic compounds. Moreover, significantly positive correlations were found between total phenolic content and anti-glucosidase activity, and

between total phenolic content and FTIR data. The PCA analysis from FTIR data demonstrated that the number of FTIR peaks found in the ethanol extracts was higher than that found in aqueous extracts. Additionally, the PCA analysis based on chemical content and biological activity confirmed that aqueous extract had higher antioxidant activity than ethanol extract, while anti-glycosidase activity and total phenolic content were higher in ethanol extracts than aqueous extracts. Similarly, the cluster analysis based on FTIR data, chemical content and biological activity showed two groups of the samples, which were extracted with different solvents. Thus, differentiation between ethanol extracts and aqueous extracts could be identified by PCA and cluster analysis based on FTIR data, total phenolic content, antioxidant activity, and anti-glycosidase activity. The FTIR data and biological activities from this study are further applied for screening bioactive compounds by using other effective techniques such as gas chromatography–mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC).

Author contributions

- Study conception and design: S Thummajitsakul; P Piyaphan; S Khamthong; M Unkam; K Silprasit
- Data collection: S Thummajitsakul; P Piyaphan; S Khamthong; M Unkam; K Silprasit
- Analysis and interpretation of results: S Thummajitsakul; K Silprasit
- Draft manuscript preparation: S Thummajitsakul
- Revision of the results and approved the final version of the manuscript: S Thummajitsakul.

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Conflict of interest

The authors declare that there are no conflicts of interest in this research.

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