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Antioxidant Properties and Chemical Profile of *Passiflora foetida* Extracts Determined by ATR-FTIR Metabolomics

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Passiflora foetida, which is also known in Malaysia as "Pokok Ulat Bulu", is widely found on the roadsides and coastal areas. The plant is traditionally used for hysteria, insomnia, diarrhea, fever and inflammation skin disease treatments. Considering the systematic way to prepare the *P. foetida* as herbal medicine has not been studied, this project was aimed to evaluate the potential of *P. foetida* as a natural source of antioxidant by using a rapid ATR-FTIR metabolomics method. The plant extracts were prepared and studied based on different postharvest treatments (fresh and dry samples), different extraction methods (maceration and sonication), different ethanol ratios (100%, 80%, 50%, and water), and different parts (leaf, stem, and fruit). All samples were evaluated for their antioxidant activities and chemical profiles by using ATR-FTIR metabolomics. The ATR-FTIR analysis detected metabolites such as phenolics and flavonoids, which were showed by the presence of functional groups at 3379 cm^{-1} (OH group), 1713 cm^{-1} (C=O group), 1651 cm^{-1} (C=C group) and 1034 cm^{-1} (C-O alkoxy group). Antioxidant properties results showed that 80% ethanol extract of leaf significantly exerted higher TPC (22.55±3.49 mg GAE/g sample) and TFC (69.11±13 mg QE/ g sample) as compared to 80% ethanol extracts from the stem and fruit. However, there was no significant difference in the DPPH scavenging activity of the different parts. Based on ATR-FTIR metabolomics, three different parts of the plant were clearly separated from each other, indicating that different metabolites were presented in the leaf, stem, and fruit. This study also indicated that the sonication method resulted in more uniform extracts as compared to maceration and freeze dry was the suitable postharvest treatment for maintaining the quality of herb.

Keywords: *Passiflora foetida*; antioxidant activity; chemical profile; ATR-FTIR metabolomics

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Passiflora foetida, or more commonly known as "Pokok Ulat Bulu" by the locals, is a very common plant in Malaysia. *P. foetida* is a Southern American native plant, and now it is being introduced to most tropical countries, including Malaysia. Also, researchers stated that *P. foetida* is a native plant of the West Indies. However, in Malaysia *P. foetida* is now categorised as a pantropical weed [1], which is considered as a weed that harms crops. This non-native plant has several habits like creeping and climbing that will compete and damage crops. As stated in the Malaysia Biodiversity Information System (MyBIS) [2], *P. foetida* in Malaysia usually have white flowers, orange or yellow fruits and lobe-shaped leaves.

P. foetida is used as a traditional medicine to treat skin diseases. Other than that, its leaves are used to cure sleeping and nervous disorders, but its unripe fruit was found to contain cyanide, which is poisonous to humans [3]. *P. foetida* is effective in traditional treatments which include to treat hysteria and insomnia [4], diarrhea, fever, and inflammation [5]. *P. foetida* has many medical values such as antibacterial and antioxidant bioactivities [6]. The leaf can be used for treating headaches and dizziness. Functions of the plant are also described in sexual dysfunction and cancer treatments [7].

Postharvest treatments which involve washing, sorting and drying are important stages in agriculture to prevent surface decontamination and maintain the quality of fresh fruits and vegetables [8]. Research studies showed that one-third of food produced was lost before consumption. Moreover, due to high humidity or high moisture content in plants and crops the production of mycotoxins has affected human health in serious cases [9]. The quality of extraction will be affected by the plant part used as starting material,

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solvent and the extraction procedure. In addition, the determination of a suitable solvent should include factors such as rate of extraction, toxicity and its preservation action towards the metabolites. The ratio of solvents used will affect the results of extraction. For example, water is a cheap and ready-to-use solvent, but it needs higher energy to concentrate the extract and may promote microorganism's growth. Ethanol is considered as a green solvent that is usually used to extract various classes of secondary metabolites [10]. Different ratios of ethanol and water are appropriate to extract compounds such as phenolic compounds and flavonoids from the plant [11].

It is a known fact that different parts of a plant may contain different chemical constituents which attribute to different biological activities. In previous studies, the preliminary phytochemical screening of *P. foetida* ethanol extraction showed the presence of phenols, carbohydrates, proteins, phytosterols, phenolic compounds, steroids, glycosides, gums, tannins, flavonoids (pachypodol, ermanin), cyanogenic compounds and alkaloids, such as Harman alkaloids and β-carbolines [12-15]. The raw fruits of *P. foetida* contain essential amino acids, unsaturated fatty acids, minerals, and phenolic compounds [16]. Phenolic compound is the main constituent that has antioxidant activity as it inhibits oxidative damage, maintains stability of cell membrane, as well as anti-inflammatory [17-18]. One of the common phenolic compounds is flavonoid. The major types of flavonoid reported in this plant are O-glycoside or C-glycoside [7].

Despite reports on its chemical constituents from previous studies, the systematic way to prepare *P. foetida* as an effective herbal medicine is still unavailable. In addition, there is a need to carry out proper laboratory investigations of *P. foetida* to explore its potential usage. Metabolomics provide a detailed characterisation of the phenotypes and aim to profile a larger number of metabolites. Based on polarity, functional group or structure of compound, the metabolomics approach takes the strategy of dividing the complex metabolome into subsets of metabolites [19]. Metabolomics is also very useful in natural products such as metabolite fingerprint, metabolic changes and toxicity, especially in drug discovery and development [20].

In this study, a comprehensive and cost-effective approach through ATR-FTIR metabolomics was applied to determine the chemical profile of *P. foetida* subjected to different postharvest treatments (fresh and dried) and extraction methods (maceration and sonication), different ethanol ratios of extraction (100%, 80%, and 50% ethanol, 100% water), and different parts (fruits, stems, and leaves). In addition, the anti-oxidant properties, including total phenolic content (TPC), total flavonoid content (TFC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity of the plant, were also evaluated.

EXPERIMENTAL

Plant Materials and Chemicals

P. foetida, including its aerial parts, fruits, stems and leaves were collected around the coastal areas of Universiti Malaysia Terengganu (UMT). The plant was identified by Dr. Shamsul Khamis (a Resident Botanist) and the voucher specimen (ID068/2023) was deposited at the Herbarium Universiti Kebangsaan Malaysia (UKMB). The chemicals used for determination of antioxidants were Folin-Ciocalteu reagent, sodium carbonate solution, standard gallic acid, DPPH, sodium nitrite, aluminium chloride and quercetin (Sigma-Aldrich, USA). Absolute ethanol (99.5%, Systerm, Malaysia) and de-ionised water were used in the extraction.

Sample Preparation

The collected *P. foetida* was rinsed by using tap water to remove heat and dust particles (include soils) on the plants. The plants were air-dried for 1h at room temperature to remove excess surface water. After that, the plant materials were divided into three groups, fresh samples (first group) were immediately proceeded to the next step of extraction, air-dried samples (second group) were stored at room temperature for 4d–5d until the samples were dried before extraction, and another samples (third group) were subjected to freeze drying. The samples for freeze-drying were separated into aerial parts, fruits, stems, and leaves. Then, the samples were ground with mortar and pestle to transform them into coursed particles and submitted for extraction. The powdered samples were kept in a refrigerator at 4 °C to avoid further degradation of their chemical composition.

Sample Extraction

In this study, sample extractions were done according to different extraction methods (maceration and sonication), different drying methods (fresh and dry), different ethanol ratios (100% ethanol, 80% ethanol, 50% ethanol and 100% water), and different parts (fruits, leaves, stems). For the maceration method, 2 g of each sample (fresh and dry of aerial parts) were soaked in 40 ml of 80% of ethanol solvent for 4 d in a sealed conical flask with occasional stirring. The sample was placed in a dark place to prevent light interference. Later, the sample solution was filtered by using a filter paper and the filtrate was concentrated by using a rotary-vacuum evaporator. All obtained crude extracts were further subjected to freeze drying to remove excess water.

For sonication, there were three types of sample extraction. Firstly, 2 g of each sample (fresh and dry of aerial parts) were added with 40 mL of 80% ethanol. Secondly, about 2 g of ground samples (aerial part) were placed in a conical flask and soaked with

40 mL of solvent at different ratios (100% ethanol, 80% ethanol, 50% ethanol and 100% water). Thirdly, about 2 g of *P. foetida* (fruits, leaves, stems) powder was mixed with 40 ml of 80% ethanol. Next, each sample was sonicated for 45 min at room temperature by using an ultra-sonicator. The mixture was filtered with a filter paper and concentrated by a rotary-vacuum evaporator. Similarly, through the maceration method, all crude extracts were subjected to freeze drying to ensure that water in the samples was removed. All extractions (maceration and sonication) were performed in six biological replicates.

Determination of Total Phenolic Content (TPC)

TPC was determined by using Folin-Ciocalteu assay. Firstly, about 20 µL of sample extract (concentration of 1 mg/mL) was placed in a 96-well microplate and 100 µL of Folin-Ciocalteu reagent was added and incubated for 5 min. Then, 80 µL of 7.5% of sodium carbonate was added. After 30 min of incubation, the absorbance was measured at 750 nm by using a micro-plate reader. The analysis was conducted for all samples with six biological replicates and three technical replicates. The standard curve was prepared by using gallic acid standard. The results were expressed as mg gallic acid equivalent (GAE)/g of sample [21].

Determination of Total Flavonoid Content (TFC)

Aluminium chloride colorimetric technique was used in the determination of TFC. About 20 µL of samples (concentration 1 mg/mL) were placed in a 96-well microplate and 120 µL of distilled water was added to each sample extract, followed by 10 µL of 5% sodium nitrite and incubated for 5 min. Then, 10 µL of 10% aluminium chloride was added to each sample extract. After 6 min of incubation, 40 µL of 1 M sodium hydroxide was added and incubated for another 15 min. The absorbance was then measured at 520 nm. The analysis was conducted for all samples with six biological replicates and three technical replicates. The standard curve was prepared by using quercetin standard. The results were expressed as mg quercetin equivalent (QE)/g extract.

2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Free Radical Scavenging Assay

In this bioassay, various concentrations of sample solutions (1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, and 15.625 µg/mL) were prepared. About 50 µL of samples were transferred to each the 96-well microplate and 50 µL of methanol was added. Following this, 100 µL of DPPH was added to each well. Then, the reaction mixture was left for 30 min at room temperature in a dark place. Microplate reader was used to record the absorbance at 517 nm. Six biological replicates and three technical replicates were performed. Quercetin was used as a positive control. To determine the concentration of sample required to scavenge 50% of DPPH free radicals, a graph of percentage inhibition was plotted against concentration (µg/mL). The results were reported as IC_{50} values (mg/mL).

ATR-FTIR Analysis

The Fourier transform infrared (FTIR) analysis was conducted in IRTracer-100 Shimadzu coupled with a MIRacle attenuated total reflection ATR accessory (Pike Technologies, Germany). To run ATR-FTIR spectroscopy, the ATR crystal was cleaned and the infrared background spectrum was first collected. Then, the sample (1 mg/mL, in acetone) was applied on the crystal and measurement was started. Six biological replicates and three technical replicates were performed for all analysed samples. All spectra obtained were converted to ACSII files prior to the multivariate data analysis.

Statistical Data Analysis

Multivariate data analysis (MVDA) was carried out by using SIMCA-P 14.1 (Umetrics AB, Umeå, Sweden) and pareto scaling was applied to all FTIR data [22]. MVDA models such as principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to determine the metabolite profile and classification of samples, respectively. Analysis of variance (ANOVA) and Tukey-HSD post hoc test were carried out to determine the significant difference between tested samples [23].

RESULTS AND DISCUSSION

Antioxidant Activity of *P. foetida*

In this study, total phenolic content (TPC), total flavonoid content (TFC) and 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assays were done to determine the antioxidant activities of *P. foetida* and results are shown in Table 1. The TPC values were predicted by using the gallic acid standard and interpreted as mg gallic acid equivalent/g extract. From Table 1, AP_SC_100 showed the highest TPC (28.40 ± 3.35 mg GAE/g extract), while F_SC_80 showed the lowest TPC (6.66 ± 2.13 mg GAE/g extract). Samples that used maceration as an extraction method contained a higher concentration of TPC as compared to sonication method. Besides, the freeze-dried samples had a higher concentration of TPC as compared to fresh samples. The aerial parts contained higher TPC as compared to the different parts of plant (fruits, stems and leaves), and amongst the parts, fruit sample contained the least TPC. The findings were in agreement with a previous study [24] which reported that the total phenolic of *P. foetida* leaf extract was higher than in fruit extract with value of 22.92 ± 0.18 mg GAE/g sample dry base and 6.53 ± 1.02 mg GAE/g sample dry base, respectively. Therefore, it was possible that *P. foetida* leaf had a high content of phenolic compounds. In comparison between different ethanol

ratios, 100 % ethanol extraction showed the highest concentration of TPC.

The TFC values were interpreted as mg of quercetin equivalent (QE)/g extract. Table 1 shows that the L_SC_80 had the highest TFC with a value of 69.11 ± 13.00 mg QE/g extract while the lowest concentration of TFC was AP_W with a value of 23.32 ± 3.84 mg QE/g extract. Based on this result, the leaf of *P. foetida* can be classified as the most active part with the highest value of flavonoid contents. A similar result reported that the concentration of flavonoid in leaf extract (7.01 ± 0.10 mg CE/g sample dry base) was higher than in fruit extract (1.56 ± 0.27 mg CE/g sample dry base) [24]. This indicated that the flavonoid content in the leaf was high, which was also supported by a previous study, whereby the leaves of *P. foetida* contained major O-glycoside or C-glycoside type flavonoids [7].

The results of DPPH were presented in the mean of percentage inhibition (IC_{50}) of samples and value expressed as mg/mL. Table 1 shows that the lower the IC_{50}, the higher the antioxidant activity of samples. AP_SC_50 had the lowest value of IC_{50} which was 0.50 ± 0.03 mg/ml, while the AP_SC_100 had the highest value of IC_{50} which was 3.28 ± 1.50 mg/mL. A previous study reported that 80% methanolic extract showed the highest scavenging potential [7], while in this study 50% ethanol extract (AP_SC_50) showed the highest scavenging potential. However, there were no significant differences between AP_SC_50, L_SC_80 and S_SC_80 samples.

ATR-FTIR Metabolomics Analysis

1. Chemical Profile of *P. foetida* Extracted in Different Postharvest Treatments

1.1. Air-dried Versus Freeze-dried Powder Samples of the Aerial Part of *P. foetida*

ATR-FTIR is one of the most common approaches in the metabolomic study. The chemical composition related to specific functional groups can be defined by using ATR-FTIR. In the preliminary study, the chemical profile of air-dried and freeze-dried powdered samples of *P. foetida* were compared by using ATR-FTIR metabolomics.

Table 1. The antioxidant properties (TPC, TFC and DPPH) of *P. foetida* extracts.

Sample	Extraction method	Postharvest treatment	Ethanol ratio (%)	Parts of plant	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	DPPH (IC_{50}, mg/mL)
AP_F_SK	Maceration	Fresh	80	Ariel parts	16.27 ± 2.11^{cd}	61.20 ± 11.88^{a}	1.27 ± 0.33^{def}
AP_SK	Maceration	Freeze dried	80	Ariel parts	18.75 ± 3.30^{bc}	52.46 ± 8.55^{ab}	1.91 ± 1.22^{ef}
AP_F_SC	Sonication	Fresh	80	Ariel parts	8.70 ± 5.27^{ef}	32.91 ± 15.86^{bc}	1.50 ± 0.13^{f}
AP_SC_80	Sonication	Freeze dried	80	Ariel parts	15.71 ± 1.55^{cd}	58.16 ± 9.97^{a}	0.82 ± 0.11^{cde}
AP_SC_50	Sonication	Freeze dried	50	Ariel parts	13.39 ± 2.95^{cde}	25.77 ± 7.36^{c}	0.50 ± 0.03^{a}
AP_SC_100	Sonication	Freeze dried	100	Ariel parts	28.40 ± 3.35^{a}	66.54 ± 16.06^{a}	3.28 ± 1.50^{f}
AP_W	Sonication	Freeze dried	0	Ariel parts	16.47 ± 2.63^{c}	23.32 ± 3.84^{c}	0.82 ± 0.17^{cd}
F_SC_80	Sonication	Freeze dried	80	Fruits	6.66 ± 2.13^{f}	25.63 ± 7.33^{c}	0.71 ± 0.14^{bc}
S_SC_80	Sonication	Freeze dried	80	Stems	10.55 ± 1.71^{def}	54.11 ± 9.10^{a}	0.60 ± 0.11^{abc}
L_SC_80	Sonication	Freeze dried	80	Leaves	22.55 ± 3.49^{b}	69.11 ± 13.00^{a}	0.56 ± 0.13^{ab}

The superscript letter (a, b, c) indicated the significant difference ($p \leq 0.05$). Same letter indicated that the samples were not significantly different, in which $p > 0.05$.
Sample code: AP, Aerial Part; F, Fresh; SK, maceration; SC, Sonication; 80, 80% ethanol ratio; 50, 50% ethanol ratio; 100, 100% ethanol ratio; W, water; F, Fruit; S, Stem; L, Leaf.

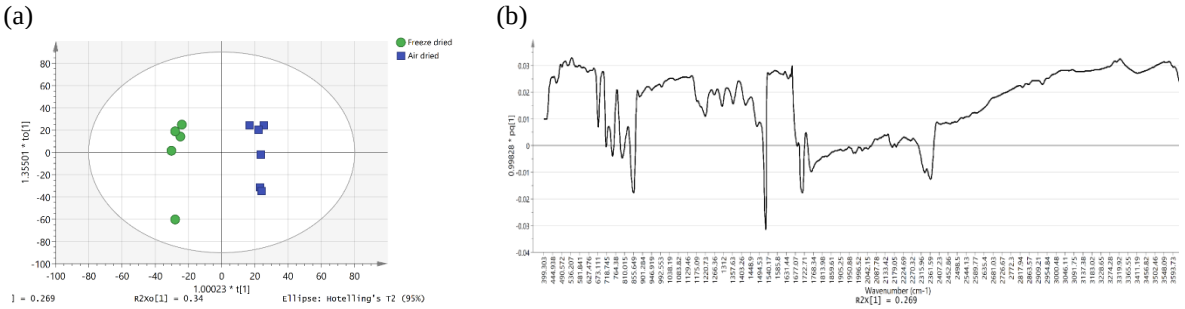

Figure 1. The OPLS-DA scores (a) and loadings line plots (b) of freeze-dried versus air-dried samples.

The ATR-FTIR spectra showed that peaks between air-dried and freeze-dried powdered samples were similar. A wavenumber at around 3283 cm^{-1} (OH stretching) was observed which was possible to indicate the presence of carbohydrates and phenolics [25]. In addition, a signal at 2923 cm^{-1} was observed, which was attributed to sp^3 CH stretching [26]. For peaks around 1658 cm^{-1} and 1433 cm^{-1}, there was a series of asymmetry and symmetry stretching of the COO^- group, and this might be the presence of unsaturation bonds of flavonoids and amino acids, respectively [25]. From their ATR-FTIR spectra it could be observed that the broad intensity represented by OH functional group of freeze-dried samples was a bit weaker than air-dried samples. This represented that the water content in the freeze-dried sample was much lower as compared to the air-dried sample.

MVDA was carried out to classify the samples as air-dried and freeze-dried. The OPLS-DA was used to discriminate the samples and identify important variables related to the separation of different samples. Figure 1 shows the OPLS-DA scores and loadings plots of freeze-dried against air-dried samples.

From the OPLS-DA scores plot, it was clearly shown that there were significant differences between air-dried and freeze-dried samples. Two clusters formed were designated for freeze-dried (green dots) and air-dried samples (blue boxes), as shown in Figure 1a. The OPLS-DA loadings plot shows the wavenumbers that were responsible for the separation of samples in the scores plot, whereby the upper part region belongs to air-dried samples while the bottom part region belongs to freeze-dried samples (Figure 1b). According to the loadings plot, air-dried samples had more moisture content (3283 cm^{-1}) as compared to the freeze-dried while the freeze-dried samples showed a prominent signal at 1723 cm^{-1}, indicating the presence of functional groups of C=O. It can be suggested that the freeze-dried sample was better because since the water content was low, the environment became unsuitable for the microorganism activity [27].

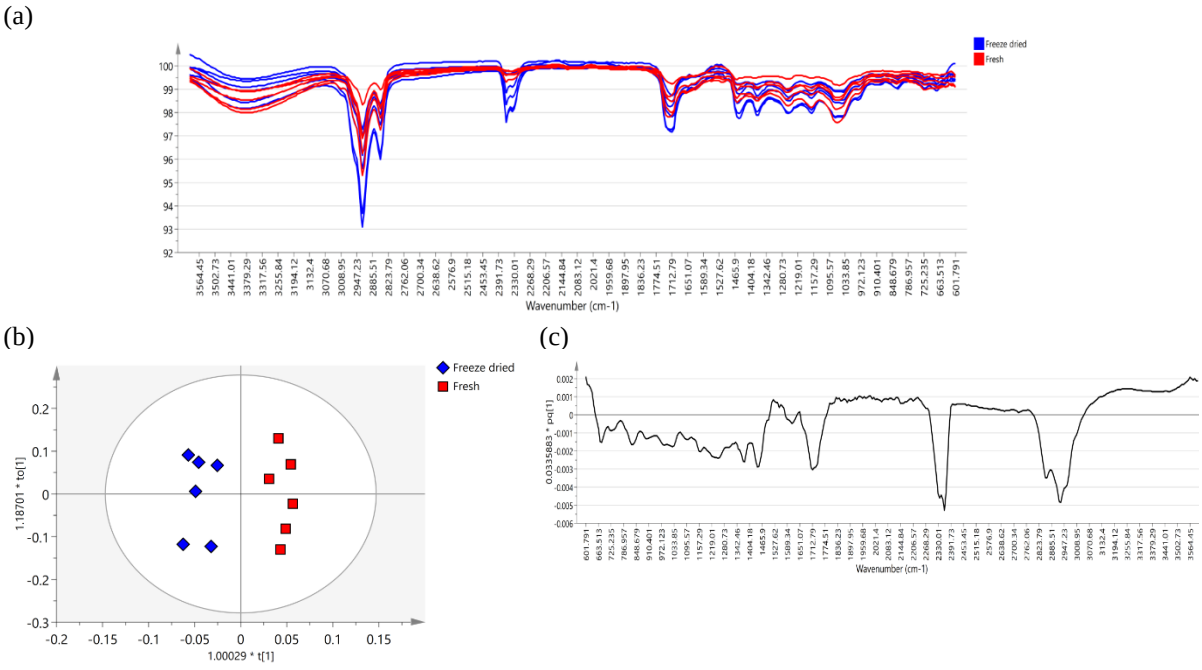

Figure 2. The ATR-FTIR spectra overlays of samples subjected to different postharvest treatments (a) and; the OPLS-DA scores (b) and (c) loading plots of the samples.

1.2. Freeze-dried Versus Fresh Extracts of *P. foetida*

In this study, the chemical profile of freeze-dried against fresh extracts of *P. foetida* was also compared. Same as the previous analysis, six biological replicates from each postharvest treatment proceeded to MVDA. Figure 2 shows the comparison between the ATR-FTIR overlay spectra of 12 samples of freeze-dried and fresh extracts. According to this, the fresh extracts showed a prominent signal at 3441 cm^{-1} (OH stretch), while the freeze-dried samples showed more intense signals at 2947 cm^{-1} (CH stretch) and 1712 cm^{-1} (C=O) [26]. Figures 2b and 2c showed the scores and loading plots of the OPLS-DA model of freeze-dried against fresh extracts. From the OPLS-DA scores plot could be observed that fresh and freeze-dried samples were well separated into two clusters, indicating there was a significant difference between the two different postharvest treatments (Figure 2a). The loading plot defined the IR spectra of the scores, which resulted from the linear combinations of data. The freeze-dried extract was recommended because the OH signal of its IR spectra that were attributed to water content was significantly lower as compared to fresh extract. Therefore, the freeze-dried extract was recommended and suitable for further analysis and storage purpose.

1.3. Comparison of Extraction Methods on the Chemical Profile of *P. foetida*

The freeze-dried powder samples of *P. foetida* were extracted using different extraction methods. For each extraction method (sonication and maceration), there were six biological replicates were subjected to MVDA analysis. Therefore, a total of 12 samples data were analysed and the results were as followed. Figure 3a shows the comparison of 12 samples by their spectra. From the OPLS-DA scores plot (Figure 3b), the samples seemed tight on the left side, except there were two samples situated on the right side of quadrant. Based on scores plot, it may be concluded that the extraction method of sonication is better than the maceration. Cluster of sonication remained highly regular on the left side, while cluster of maceration is irregularly scattered.

2. ATR-FTIR Metabolomics of *P. foetida* Extracted Using Different Ethanol Ratios.

Different ethanol ratio extracts were subjected to ATR-FTIR analysis for metabolite fingerprint. The samples were 100% water, 100% ethanol, 80% ethanol and 50% ethanol extracts. Figure 4 shows the ATR-FTIR spectra of the samples and Table 2 shows the wavenumber and functional groups presented in samples.

The FTIR spectra (Figure 4) showed the wavenumber at 3317 cm^{-1}-3564 cm^{-1} that corresponded to the presence of functional groups such as carbohydrates, phenolic acids and amino acids. Furthermore, the characteristic of carbohydrates was also shown by the presence of signal at 1034 cm^{-1}, suggesting that the C-O stretching that was available in carbohydrates [29]. The wavenumbers at 3318 cm^{-1}, 1713 cm^{-1}, and 1651 cm^{-1} attributed to OH, C=O, and C=C functional groups of phenolics and flavonoids [28].

Amino acids were also observed in the FTIR spectra according to the presence of signals at 3564 cm^{-1} and 1589 cm^{-1} due to N-H stretching and N-H bending, respectively [28]. Further analyses such as NMR and MS could be used to identify the exact chemical structures of compounds in the samples. To gain a clearer overview on the chemical profile of *P. foetida* extracts subjected to different ethanol ratios of extraction, OPLS-DA scores and loadings plots were conducted. The scores plot presented the clusters at different ethanol ratios extracts, in which the water extracts (blue) were significantly different with 50% ethanol extracts (yellow), 80% ethanol extracts (red) and 100% ethanol extracts (green).

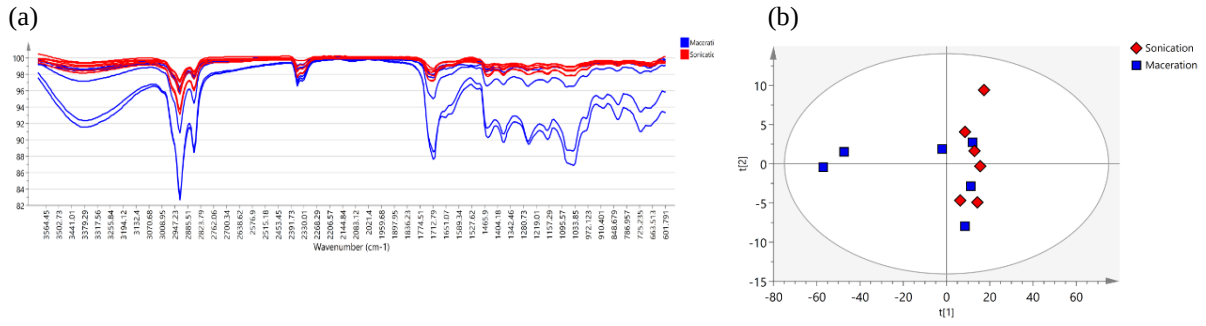

Figure 3. The ATR-FTIR spectra overlays of samples (a) and the PCA scores plot (b) of samples subjected to different extraction methods.

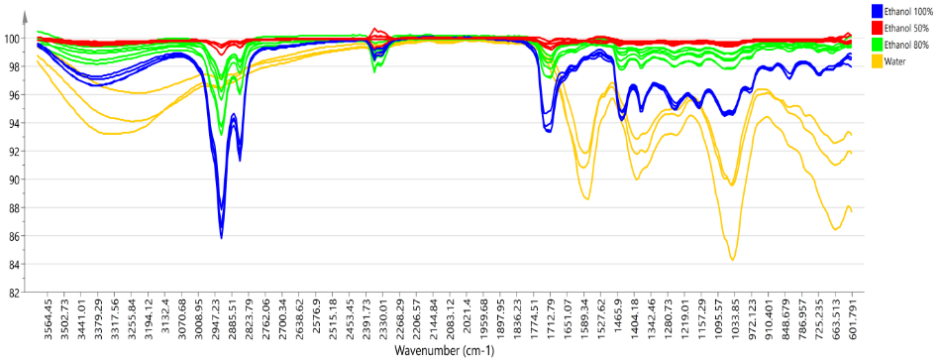

Figure 4. The ATR-FTIR spectra of samples subjected to different ethanol ratios of extraction.

Table 2. Wavenumber and the types of vibration in samples.

No	Wavenumber (cm^{-1})	Types of vibration	Intensity	Assignment	Availability
1	3317.56-3564.45	OH and N-H stretching	s-m	Alcohols, phenolic acids, amino acids, carbohydrates	Water, 100% ethanol, 80% ethanol
2	2823.79-2947.23	CH stretching (sp^3 hybridisation)	s	Alkanes, alkenes	100% ethanol, 80% ethanol
3	1712.79	C=O stretching	m	Phenolic acids	100% ethanol, 80% ethanol
4	1651.07	C=C bending	w	Phenolic acids	100% ethanol, 80% ethanol
5	1589.34	N-H bending	m	Amino acids	Water
6	1033.85	C-O stretching	s	Carbohydrates	Water

Note: w: weak; m: medium; s: strong

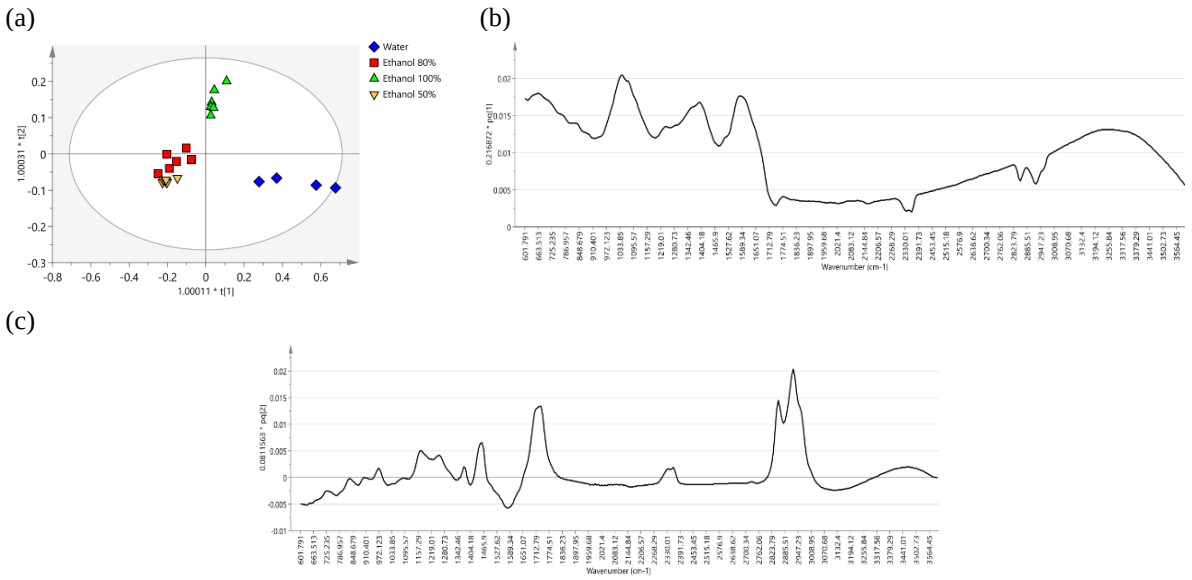

Figure 5. OPLS-DA scores plot (a), loadings line plot (PC1) (b), and loadings line plot (PC2) (c) of *P. foetida* extracted in different ethanol ratios.

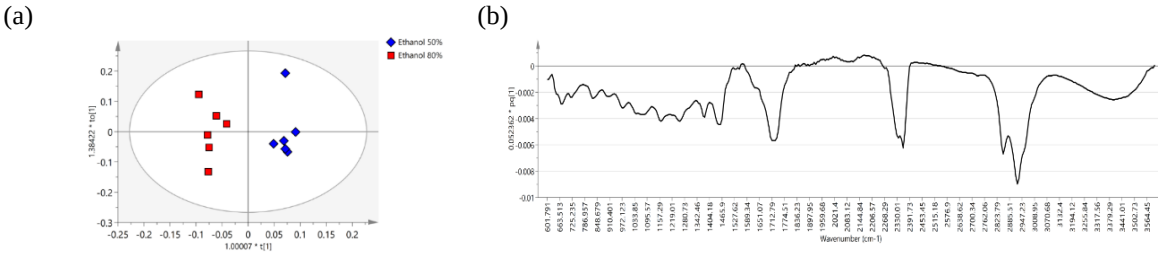

Figure 6. OPLS-DA scores plot (a) and loading line plot (b) of 50% ethanol extracts against 80% ethanol extracts

From the OPLS-DA scores plot, samples were separated into three clusters, whereas the water extracts were situated in lower right quadrant and 100% ethanol extracts in the upper right quadrant. The 50% ethanol and 80% ethanol extracts were situated in the left quadrant of OPLS-DA scores plot (Figure 5a). The OPLS-DA loadings plot (PC1) showed that wave-numbers that corresponded to the separation of water extracts (upper part of loadings line plot) from the rest of samples (Figure 5b). Furthermore, OPLS-DA loadings plot (PC2) identified wavenumbers that were related to the separation of 100% ethanol extracts (upper part of the loadings line plot) and the water extracts (lower part of the loadings line plot) (Figure 5c). From the OPLS-DA scores plot it could be observed that the clusters for 80% and 50% extracts were closed to each other. To differentiate the 50% ethanol extracts from 80% ethanol extracts, another OPLS-DA model was applied. Figure 6 shows the OPLS-DA scores and loading plots of 50% ethanol against 80% ethanol extracts.

The OPLS-DA scores plot shows a distinct two clusters of 50% ethanol (blue) and 80% ethanol (red) extracts (Figure 6a). The two clusters were separated by PC1 and indicated that there were significant differences amongst them. The loading plot shows the wavenumbers that correspond to the separation of samples, whereby upper part is 50% ethanol extracts and bottom part is 80% ethanol extracts. Based on the loading line plot, the 80% ethanol extracts showed more prominent wavenumbers as compared to 50% ethanol extracts (Figure 6b).

3. ATR-FTIR Metabolomics of Different Parts of *P. foetida* Extracts

The chemical profile of different parts of *P. foetida*, including leaf, stem and fruit extracts were studied. The FTIR overlay spectra of different parts of *P. foetida* extracts are shown in Figure 7. Almost all spectra exhibited comparable bands with varying peak intensities at around 3441 cm^{-1}, 2947 cm^{-1}, 2886 cm^{-1}, 2391 cm^{-1}–2330 cm^{-1}, 1713 cm^{-1}, 1564 cm^{-1}–1324 cm^{-1}, and 1280 cm^{-1}–1034 cm^{-1}. These wave-numbers represented various functional groups that belong to various types of phytochemical profiles.

According to Figure 7, the C-H with sp^3 hybridization signals at 2885 cm^{-1} –2947 cm^{-1} showed strong intensity in the order of fruit > leaf > stem. At around 1713 cm^{-1}, the fruit extracts showed a strong intensity signal as compared to leaf and stem extracts, which indicated that the fruit extracts contained high number of compounds with C=O functional group [30]. A weak signal occurred around 2391 cm^{-1}, which may represent the presence of carbon dioxide and moisture in the air [31]. From the result, different parts of *P. foetida* might contain various amounts of phenolics and flavonoids. These were due to the appearance of signals at around 3379 cm^{-1}, 1713 cm^{-1}, and 1651 cm^{-1} that attributed to the presence of OH, C=O, and C=C groups, respectively [30].

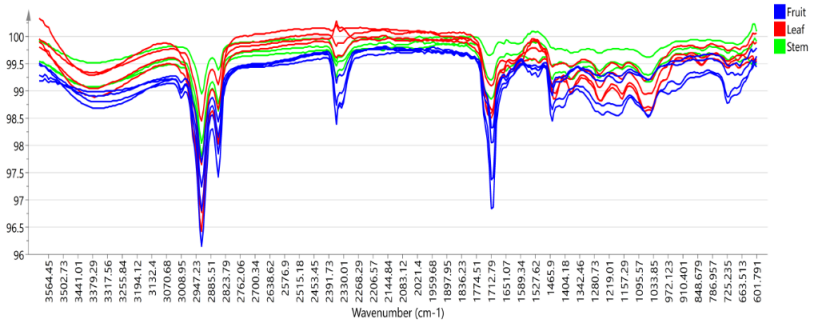

Figure 7. FTIR spectra of different parts of *P. foetida* extracts

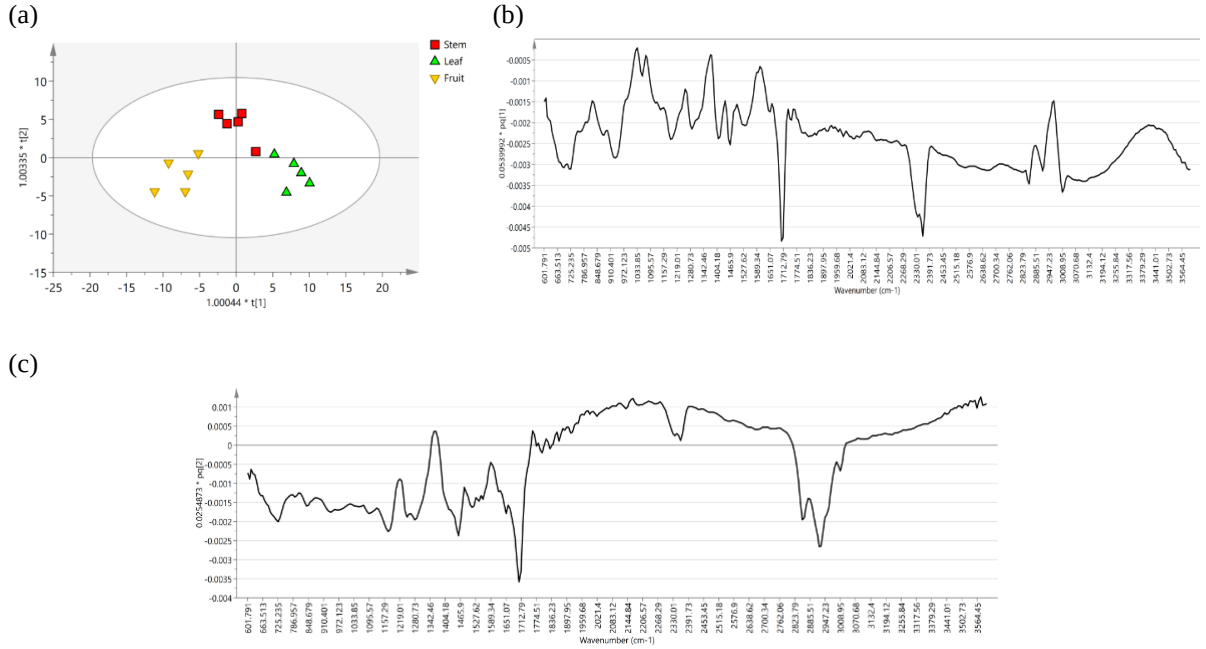

Figure 8. OPLS-DA scores (a) and loading line plots by PC 1 (b) and PC 2 (c) of different parts of *P. foetida.*

The chemical profile of different parts of *P. foetida* was further evaluated *via* MVDA. The scores plot of OPLS-DA is shown in Figure 8A. According to Figure 8A, a significant separation between samples of different parts of *P. foetida* could be observed, which resulted in the form of three clusters of stem, leaf and fruit. The fruit extracts were significantly different as compared to stem and leaf extracts according to PC1, while leaf and stem extracts showed differences according to PC2. This suggested that FTIR metabolomics was managed to distinguish the metabolite variations of different parts of *P. foetida.* The OPLS-DA loading plot showed the wavenumbers that corresponded to the separation of the different parts of *P. foetida* according to the scores plot (Figure 8b– Figure 8c).

CONCLUSION

In conclusion, the chemical profile of *P. foetida* may differ due to its postharvest treatments and extraction methods. The finding indicated that the sonication method was better than maceration, as the sonication produced more uniform extracts. Freeze-dried method was also the preferable postharvest treatment for better storage purpose and maintaining the quality of herb. For the different ethanol ratios of extract (100% water, 100% ethanol, 80% ethanol, 50% ethanol), the ATR-FTIR spectra showed that there were variations in the functional groups of phenolics and flavonoids present in the extracts. The clusters of three different parts (leaf, stem and fruit) were separated clearly according to the OPLS-DA scores plot and these results were supported by its loadings plot. The study of different parts of *P. foetida* showed that the leaves that were extracted by using an 80% ethanol ratio in the sonication method had the most potent antioxidant properties with TPC of 22.55 ± 3.49 mg GAE/g extract, TFC of 69.11 ± 13.00 mg QE/g extract, and IC_{50} value towards DPPH free radical scavenging activity of 0.56 ± 0.13 mg/ml. The outcome of study may provide potential impacts on many fields, especially in pharmaceutical research; the discovery of bioactive compounds in the plant will be useful in the development of drugs from natural resources. Additionally, it can contribute to a better understanding of the chemicals and antioxidant activity of *P. foetida*.

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