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PROXIMATE COMPOSITION OF DRIED POWDER OF *Passiflora Foetida* LEAVES AND FRUITS AND ITS PHYTOCHEMICAL CONTENT OF CRUDE AQUEOUS AND ETHANOL EXTRACT

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Abstract: *Passiflora foetida* is an edible plant that is underappreciated by most local communities even though it grows widely in many areas. The aim of this study is to measure the proximate composition of *P. foetida's* leaves and fruits as well as to determine the most efficient solvent to extract phytochemicals from the plant parts. The proximate values determined in this study include the moisture, ash, protein, lipid, fiber and carbohydrate. Meanwhile for extraction efficiency, aqueous and ethanol were selected to quantitatively evaluate the composition of total phenolic (TPC), flavonoid and tannin. The result shows that the leaves and fruits of *P. foetida* contain 4.80±0.17% and 3.52±0.17% of ash, 18.41±0.47% and 10.00±0.45% of crude protein, and 15.28±0.49% and 27.49±0.52% of fiber, respectively. In terms of the yield of extraction, it shows that aqueous capable of extracting higher yield than ethanol. However, comparison between aqueous and ethanol for extracting phytochemicals, revealed that ethanol is capable of extracting significantly higher amount of TPC in both leaves and fruits, as well as flavonoid and tannin from the fruit material. Aqueous extract was identified to be the best extract for tannin in the leaves. Ethanol extracts lower yield, but the efficiency to extract most phytochemicals in the plant materials was considered to be better than aqueous.

Keywords: *Passiflora foetida*, proximate composition, phytochemical content, solvent extraction, plant materials.

Introduction

Passiflora foetida L. is a plant locally known as 'pokok ulat bulu' or 'letup-letup'. The *Passifloraceae* family comes from Angiosperms group and covers about 20 genera and 600 species (Antonio *et al.,* 2018). *Passiflora* is the largest genus in the *Passifloraceae* family which comprises about 500 species. The plant of *P. foetida L.* was originally from the South American region, but currently spreads to other tropical regions. In Malaysia, this plant species can be found growing in riverbeds, dry forest floors, roadside and wasteland. The fruits are normally yellowish-orange when ripe, 2-3 cm in size and contain a bluish-white pulp which is mildly sweet and delicately flavoured. Meanwhile, the leaves are heart shaped and three lobed arising from the common point and covered with fine hairs.

Although this *P. foetida* can be found in many areas of the region, not many of us know about this plant. Moreover, few of the younger generation know that this plant is edible. This plant is rich with many beneficial nutrients and medicinal values but are less exposed to the public resulting in it being marginalized and not fully utilized by the local community. Although there are previous reports on the *P. foetida* nutrients and phytochemicals composition, most of them rely on species found in their locality. Since the nutrient and phytochemical composition could be influenced by the climate, soil and other environmental factors, it is suggested that a specific study is carried out on the plant species that grows locally

P. foetida is a plant that has potential to be used not only as a food ingredient but also for health benefits. The leaves of ethanol extract from *P. foetida* indicated immunomodulation

activity for the dose of 200, 400 and 600 mg/BW (Andi *et al.,* 2017). *P. foetida* has been used as analgesic agents as well as for the treatment of insomnia, epilepsy and hysteria (Saravanan *et al.,* 2014). In Sri Lanka, the leaves are commonly used to treat type II diabetes (Siriwardhene *et al.,* 2013). The *P. foetida* fruit was identified to exhibit an intense and characteristic aroma due to the presence of esters, terpenes and some sulphur compounds (Martínez *et al.,* 2014). Analytical result by Song *et al*. (2018), reported that the fruits of *P. foetida* were rich in amino acids, minerals and unsaturated fatty acids.

Polyphenols are chemical compounds, often a product of secondary plant metabolisms**,** which are characterized by the presence of multiple phenolic groups. Among the different groups of phenolic compounds, the primary dietary phenolic compounds are phenolic acids, flavonoids and tannins (King & Young, 1999). The antioxidant activity of phenolic compounds is mainly associated with the presence of polyphenols aromatic ring structures and the hydroxyl groups (Minatel *et al.,* 2016). Phytochemicals such as phenolic compounds, flavonoids and tannins are normally attributed to health benefits due to their capability of reactions including absorption, distribution and metabolism in the body system (Neilson *et al.,* 2017).

In order to extract or isolate the intended compounds from plant materials, many methods have been tried including solvent extraction. According to Zhang *et al*. (2018), solvent extraction gives stronger separation effect than other separation methods, and possesses higher degree of selectivity and faster mass transfer compared to the ion exchange process. In fact, solvent extraction has advantages in comparison with distillation, such as low energy consumption, high production volume, fast action, easy continuous operation and ease of automation. Solvent extraction method basically based on the law of "like dissolve like" in which the solvent with a polarity value close to the solute polarity are likely to perform better and vice versa (Zhang *et al.,* 2018).

This study intends to provide information on the nutritional composition in the leaves and fruits of *P. foetida* and to compare the efficiency between aqueous and ethanol solvents in order to extract most phytochemicals. The information from this research may be useful to those who are interested to have knowledge about the proximate composition and selection of the best solvent extraction especially for the most antioxidant active compounds in the leaves and fruits of *P. foetida*. Figures 1 and 2 show the *P. foetida* leaves and fruits.

Figure 1: *P. foetida* leaves

Figure 2: *P. foetida* fruits

Methodology

Preparation of Samples

The samples were prepared according to Antonio *et al*. (2018) with slight modification. The unripen leaves and fruits of *P, foetida* were collected from bushes in the area of Gong Badak, Terengganu and dried in the oven dryer at 60°C for 19 h (leaves) and 24 h (fruits). Both samples were tested for moisture content (low than 11%). It was ground using a blender until the powder form was obtained. The samples were weighed and kept in an airtight container for future use.

The samples were extracted according to the method reported by previous researchers with a slight modification (Zarina & Tan, 2013). 50 g of samples were separately extracted sequentially with 250 ml of solvents (aqueous and ethanol). The conical flask of 250 ml was filled with both types of solvent and samples. The conical flask was transferred into water bath shaker at temperature 60°C for 24 hours. The sample then was cooled at room temperature. The whole solution was filtered through the Whatman filter paper No. 1. The filtrate was then transferred into a rotary flask evaporator. After the extraction was completed, the sample was collected and stored in a vial for further studies.

Determination of Proximate Composition

The proximate analysis of the samples was determined based on the dry weight basis for moisture content, ash content, crude protein, crude fat and fiber, and were carried out in triplicate using a method by AOAC (2000). Moisture content was determined based on the dry weight basis by heating the sample at 105°C until a constant weight. Ash content was determined by weighing the residue obtained after incineration at 550°C for 4 hours until constant weight. Crude protein content (N × 4.38) of the sample was determined using the Kjeldahl method. Crude fat content was determined by Soxhlet extraction with petroleum ether as the solvent. Meanwhile, the carbohydrate content was calculated using the following formula:

Percentage of carbohydrates

= 100 – (percentage [protein + moisture + crude fat + ash + crude fibre])

Determination of Total Phenolic Content (TPC)

Total polyphenol content was determined by the Folin-Ciocalteu method (Liang *et al*., 2012) with slight modification. 1 ml standard solution was mixed with 1.5 ml of 7.5% sodium carbonate and 1 ml of 5% Folin-Ciocalteu reagent. The solution was incubated at room temperature for 30 min in the dark. After that, the absorbance of the reaction mixture was measured at 765 nm against reagent blank. The standard curve was plotted by a graph absorbance (nm) against the concentration (mg/L) by using the concentration of 25, 50, 75, 100 and 125 mg/L respectively. The analysis was conducted as in the standard of gallic acid solution. The total phenolic content was expressed as mg GAE/L of gallic acid extract.

Determination of Flavonoids

The flavonoid content was determined using the aluminium chloride colorimetric method as described by Makris *et al.* (2007) with slight modification. 1 ml sample solution was mixed with 4 ml distilled water in a tube, with 0.3 ml 5% $NaNO_3$ added and allowed to react for five minutes. 0.3 ml of 10% $AlCl_3$ was added and the mixture was allowed to stand for a further five minutes. Finally, 2 ml of 1 M NaOH and 2.4 ml distilled water were added to the reaction mixture. The absorbance was measured at 510 nm against a blank. The standard curve was plotted by a graph absorbance (nm) against the concentration (mg/L) by using the concentration of 100, 200, 300, 400 and 500 mg/L respectively. The analysis was conducted as in the standard of quercetin solution.

Determination of Tannin

The tannins were determined by Folin-Ciocalteu method according to the Vijay and Rajendra (2014) with slight modification. Approximately 0.1 ml of the standard solution was added to a

test tube. After that, 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu phenol reagent and 1 ml of 35 % Na_2CO_3 solution were added. The mixture was shaken well and kept at room temperature for 30 minutes. A set of reference standard solutions of gallic acid (20, 40, 60, 80 and 100 mg/L) was prepared. Absorbance of the test and standard solutions were measured against the blank at 725 nm using UV/Visible spectrophotometer. The analysis was conducted as in the standard of gallic acid solution.

Data Analysis

The amounts of phytochemical components present in the fruits and leaves of *P. foetida* between ethanol and aqueous extracts were analyzed using independent t-test. P value less than 0.05 considered as significantly different between samples. All the analysis were conducted in triplicates.

Results and Discussion

The percentage of yield for different solvent extractions and plant parts are presented in Table 1. The result shows that higher yield was observed in the aqueous extracts than in the ethanol extract for both of the *P. foetida* leaves and fruit.

Table 1: Percentage yields of extraction for different types of samples

Samples	Weight of Samples (g)	Weight of Sample Extract (g)	Yield of Percentage (%)
Leaves aqueous	100.04	37.76	37.74
Leaves (Leaf) ethanol	199.81	37.26	18.65
Fruit aqueous	100.06	36.44	36.42
Fruit ethanol	200.20	62.24	31.09

By comparison, the different of extraction yield between solvents for the leaves was 19.09% while for the fruit was 5.33%. This explained that the extraction yield increased with the increasing polarity of the solvent used in the extraction. This is due to aqueous with higher polarity gave larger amount of extracted yield than the ethanol extract in both samples of the leaves and fruits. Thus, it can be concluded that more extractable polar compound was measured in the plant of *P. foetida.* The result is in agreement with the study by Ramaiya *et al*. (2014) who reported that maximum extraction yields of antioxidant components from *Passiflora* species were observed in the methanol extract which is considered as the most polar solvent rather than petroleum ether or acetone.

Proximate Composition

The summary of the proximate composition of *P. foetida* leaves and fruits are reported in Table 2. Both parts have shown that there was no significant difference in moisture content ($p>0.05$). The moisture content for fruits and leaves of the plant were 17.78±0.24% and 14.78±1.04%, respectively. Higher moisture content in the samples could be due to this experiment being based on the fresh weight instead of dry weight content basis. According to a study carried out by Odewo *et al*. (2014), moisture content in leaves of *P. foetida's* (% dry weight) was 1.88%, which was quite low compared to the current study. However, it should be noted that for dry matter, moisture content would depend on the degree of drying.

Table 2: The proximate composition of dried powder of the leaves and fruits *P. foetida*

	Mean ± Standard Deviation	
	Leaves (%)	**Fruits (%)**
Moisture	14.78±1.04[a]	17.78±0.24[a]
Ash	4.89±0.04[a]	3.60±0.12[b]
Crude fat	6.39±0.57[a]	6.47±0.50[a]
Crude protein	18.68±0.13[a]	10.22±0.36[b]
Fiber	15.63±0.49[b]	27.49±0.52[a]
Carbohydrate	39.64±0.08[a]	34.77±0.75[a]

The ash and crude protein content in the leaves of *P. foetida* showed significantly higher amount than in the fruits. This result differs from the findings of Odewo *et al*. (2014) which indicated high in ash content (28.70%) in the plant leaves. The amount of ash presence in the samples gives us an idea of the quantity of minerals present in the sample (Michael & David, 2002). The amount of crude protein which was 18.68±0.13 in the leaves and 10.22±0.36 in the fruits could be said that this plant has a moderate amount of protein.

This study showed that the fruit of *P. foetida* contain higher amount of fiber compared to leaves. High fiber in the fruit sample could be due to the fiber content in the seed of *Passiflora* sp. which was reported rich in insoluble dietary fiber (Ramaiya *et al.,* 2018). Normally for commercial fruits, fiber content is around 2.50%. Fiber content in this study might be useful in the production of juices or other product since the amount of fiber content was higher than in commercial.

In terms of fat and carbohydrate content in the leaf and fruit parts, it showed no significant difference between the samples. Interestingly, this study shows that *P. foetida* is low in fat but high in carbohydrate. In fact, carbohydrate was considered as the highest composition in both the leaf and the fruit parts. The amount of carbohydrate content is in agreement with that of Odewo *et al*. (2014), which was also reported as the highest components in the plant species. In general, *P. foetida is* considered as having good nutritional value of plant source since it contains of all proximate composition as required by our body.

Total Phenolic Content (TPC)

The quantity of TPC in *P. foetida's* fruit ethanol and aqueous were determined using a method known as Folin-Ciocalteu assay. The standard of gallic acid was used as representative of total phenolic content present in the ethanol and aqueous crude extract. The graph of the standard curve showed the equation as $y = 0.0016x + 0.4094$ and $R^2 = 0.9486$. Figures 3 and 4 represent the comparison of TPC in leaves and fruits between different mediums of extraction: aqueous and ethanol.

Figures 3 (a) and 3 (b) indicated that significant difference ($p<0.05$) of TPC was measured in the fruit and leaves of *P. foetida* when using different types of solvent. This study revealed that ethanol was more efficient in extracting higher amount of TPC than aqueous. Therefore, it can be considered that in the leaves and fruit of *P. foetida,* more ethanol soluble phenolic exists than the water soluble. It also shows that the phenolic properties have considerably lower polarity due to them being soluble in the medium polarity of the ethanol solvent of extraction.

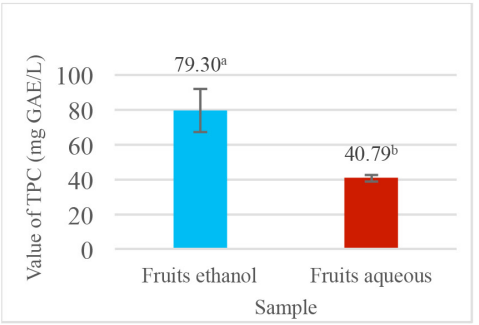

Figure 3 (a): The value of total phenolic content in *P. foetida's* fruit ethanol and fruit aqueous

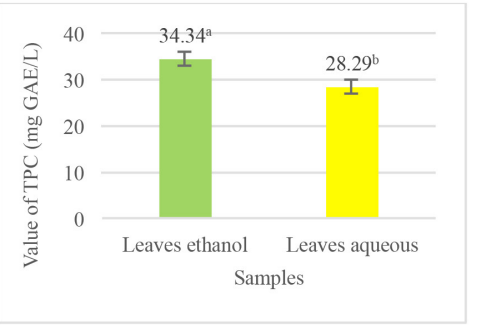

Figure 3 (b): The value of total phenolic content in *P. foetida's* leaves ethanol and leaves aqueous

Note: Mean values represent triplicates of different samples analyzed. Values with different (a,b) are significantly differences ($p<0.05$) between samples

The result also indicated that fruit ethanol extracted significantly higher total phenolic content than the leaf ethanol. The presence of various antioxidant compounds with different chemical characteristics and polarities may or may not be soluble in a particular solvent (Turkmen *et al.,* 2006). Similarly, the fruits aqueous indicated higher total phenolic content than in the leaves aqueous. Thus, it can be concluded that the fruit of *P. foetida* contains higher TPC compared to its leaves. This is due to different parts of plants which consist different amount of phenolic compound in its composition. Phenolic compounds are widely distributed in plants and have garnered attention due to their anti-mutagenic, antitumor and antioxidant properties, which contribute to human health (Li *et al.,* 2006).

Flavonoid Content

Flavonoid content was determined using the aluminum chloride colorimetric method and quercetin was used as a standard solution. The equation of standard curve for flavonoid content was $y= 0.0005x + 0.117$ and $R^2 = 0.9763$.

There were no significant differences in both fruit ethanol and leaf ethanol ($p>0.05$) as shown in Figure 4 (a) and 4 (b). This indicated that the fruit ethanol of *P. foetida* contained equal amount of flavonoid content compared to the fruits aqueous. This study is supported by Paulraj *et al*. (2014) who indicated similar concentration of flavonoid between the ethanol and the aqueous fruit extracts (Correct usage). They also reported that the flavonoid content was not affected by different ripeness stages of the fruits. In this study, equal amount of flavonoid also indicated in the leaves of ethanol and aqueous extracts. However, comparison between fruit ethanol (346.67±36.3mg/mL) and leaves ethanol (268±23.07mg/mL) showed significant difference ($p<0.05$) of flavonoids. This shows that higher flavonoid content was observed in the ethanol extract of fruits than in the leaves of *P. foetida.* It was recognized that solubility of flavonoids was strongly affected by the nature of solvent used and the structure of the flavonoids itself. For example**,** the presence of a sugar group in flavonoid structure was one of the reasons for low solubility in a particular extraction solvent (Chebil *et al.,* 2007). A previous study by Ulubelen *et al*. (1982) indicated that the leaves of *P. foetida* contain major C-glycosyl flavanoids such as vitexin and isovitexin.

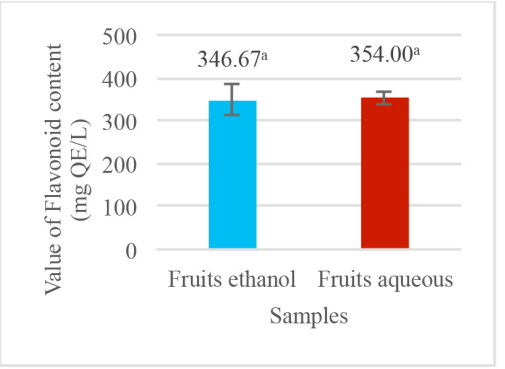

Figure 4 (a): The value of flavonoid content in *P. foetida's* fruit ethanol and fruits aqueous

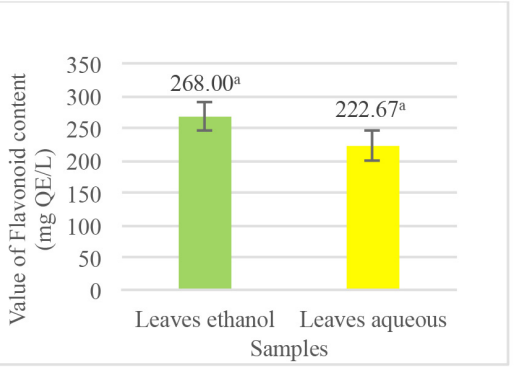

Figure 4 (b): The value of flavonoids content in *P. foetida's* leaves ethanol and leaves

Note: Mean values represent triplicates of different samples analyzed. Values with different (a, b) are significantly different ($p<0.05$) between samples

Tannin Content

Tannin content was determined using the Folin-Ciocalteu assay system and gallic acid was used as a standard solution. From the graph of standard curve, the equation of graph is $y = 0.0027x + 0.0058$ and $R^2 = 0.9608$. The tannin was expressed as mg GAE/L of Gallic acid of extract.

Figures 5 (a) and 5 (b) show the amount of tannin content in *P. foetida's* in both fruit and leaves extracts using ethanol and aqueous as medium of extractions. As indicated in Figure 7, there was a significant difference of tannin content between fruit ethanol and fruit aqueous ($p< 0.05$). Higher tannin is observed in the fruit ethanol rather than in the fruit aqueous extract. This means that this particular fruit of *P. foetida* contains more ethanol soluble tannin than the water-soluble tannin. Thus, it shows that the tannin properties in the fruits were considerably lower polarity due to being soluble in the medium polarity of the ethanol solvent. However, result from the leaves extract of ethanol and aqueous showed the other way around.

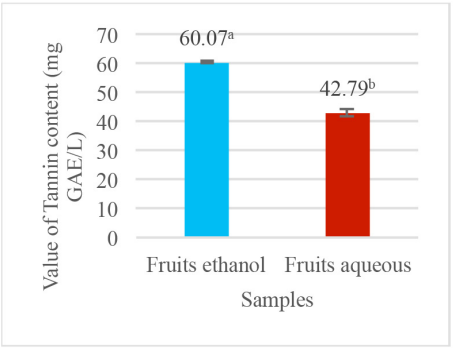

Figure 5 (a): The value of tannin content in *P. foetida's* fruits ethanol and fruits aqueous

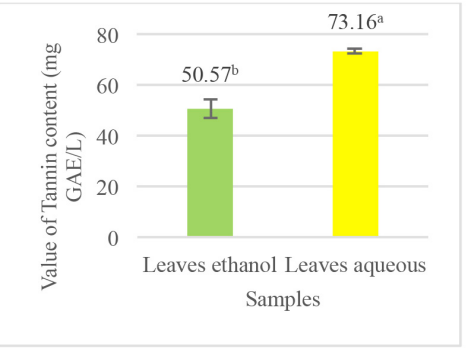

Figure 5 (b): The value of tannin content in *P. foetida's leaf ethanol and leaves aqueous*

Note: Mean values represent triplicates of different samples analyzed. Values with different (a, b) are significantly differences ($p<0.05$) between samples

This result is consistent with the previous study which indicated that tannin content is lower than flavonoid content in the ethanolic leaves extract of *P. foetida* (Siriwardhene *et al.,* 2013). Meanwhile, comparison of tannin content in the leaves extract between different solvents, showed that this study has a similar trend with Paulraj *et al*. (2014) who indicated that aqueous as higher concentration than ethanol. The aqueous extract of leaves showed significantly higher ($p<0.05$) as compared to the ethanol extract. It showed that the tannin properties had considerably higher polarity due to being it soluble in high polarity of the aqueous solvent. This means that the properties of tannin present in the leaves and fruit were quite different in their properties. Although tannins are water-soluble compounds, the solubility depends mainly on its hydrophobic interactions and hydrogen bonding (Tanaka *et al.,* 2018). This study shows that leaves contain most of the aqueous soluble tannin compared to the fruits. It is accepted that tannins are recognized to be distributed in various locations of plant tissues including leaf, root, seed, stem and bud (Upadhyaya & Ashok, 2012). Generally, this study show that tannin content has appeared higher in fruit ethanol as compared to leaves ethanol.

Conclusion

P. foetida of leaves and fruits contained various proximate compositions such as ash, fat, protein, fiber and carbohydrate that could give benefits to humans. The current study shows that the amount of ash and protein was significantly higher in leaves compared to fruits. However, fruits have a higher amount of fiber than the leaves. Aqueous solvent appeared to be the most efficient to extract phytochemical from leaves and fruits of *P. foetida* compared to ethanol since the percentage yield of extraction for both

parts were higher in aqueous except for total phenolic content. Phytochemical content that has been quantified in *P. foetida* shows that the fruits contain higher content than the leaves for most of the total phenolic content, flavonoid and tannin. Information from this study will give an idea for the selection of appropriate solvent for extracting bioactive compounds from the fruit and leaves of P. *foetida* for nutraceutical and pharmaceutical.

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