PHYTOCHEMICAL COMPOUNDS AND ANTIOXIDANT CAPACITY OF *Paederia foetida*

Nor Habibah Mohd Rosli[1*], Nurdiyana Amira Mohamad Shahrul[1], Siti Nur Liyana Darimi[1], Melur Asmida Mohd Hassan[1], Afiqah Nasuha Samsudin[1], Siti Nurulhajar Razihan[1]

[1]Faculty of Applied Science
Universiti Teknologi MARA, Cawangan Pahang, 26400 Bandar Jengka, Pahang

[*]Corresponding author: norha505@uitm.edu.my

Abstract

Paederia foetida is locally known as *'akar sekentut'* or *'daun sekentut'* because of the foul smell produced when the leaves are touched. It was used traditionally to reduce bloating and used by women after giving birth. This local species is overlooked because it is rarely found and often chopped off. Performing research on this species could help to enhance its medicinal value. Thus, this study attempts to analyse antioxidant capacity of fresh and dried leaves and twigs of *P. foetida*. The phytochemical constituents were initially screened, and the antioxidant capacity was evaluated by determining Total Phenolic Content (TPC), Total Flavonoid Content (TFC) and IC_{50} values using Follin-Ciocalteu method, $AlCl_3$ method and DPPH assay, respectively. *P. foetida* showed the presence of phenolics, glycosides, alkaloids, saponins, tannins and terpenoids. The highest TPC and TFC were observed for methanolic extract of fresh twigs with 0.0536 GAE/g and methanolic extract of fresh leaves with 0.1545 QE/g respectively. The IC_{50} values ranged from 1.50 – 4.88 for all the ethanolic and methanolic extracts except for ethanolic extract of dried twigs of which the value was 16.88. The results obtained from this study revealed the potential of this local species as a promising natural antioxidant.

Keyword: Antioxidant, *Paederia foetida*

Introduction

Interest in the exploitation of medicinal plants has always been prevalent as it offers many benefits to humankind. The medical values of these plants commonly come from their antioxidant properties and because of this reason, scholars continue conducting research on uncommon medicinal plants species to study their potential in pharmaceuticals, cosmeceuticals, agrochemicals, and other fields. The supremacy of natural antioxidants is undeniable as they have strong ability to destruct free radicals (Lim et al., 2006). Additionally, the use of synthetic antioxidants such as butylatedhydroxytoluene (BHT) and butylatedhydroxyanisole (BHA) has been heavily censured by many health critics and researchers because they are suspected to be the promoters to carcinogenesis (Islam et al., 2018). Thus, finding newer natural antioxidants is in great demand. Secondary metabolites, such as phenolic compounds which include their derivatives such as flavonoids and carotenoids, are biologically active compounds which accountable for the antioxidant properties of plants and herbs. They function as antioxidants because they are significantly involved in the protection against free radicals due to the presence of phenolic rings and hydroxyl groups (Minatel et al., 2017).

Paederia foetida or locally known as '*akar sekentut'* is one of the overlooked medicinal plants in Malaysia. The leaves of *P. foetida* contain sulphur compounds including dimethyl disulphide (Uddin et al., 2013). Due to the sulphur compounds, it produces a fetid smell which diminishes when cooked. The local community serve this plant as salad or '*nasi ulam'*

rather than using it as a traditional remedy (Rosli et al., 2013). Old Malay folks use this plant in treating women during confinement and reducing bloating. In India, young leaves and shoots of *P. foetida* are used to prepare curry. They believe it is good for women after giving birth, excellent as a treatment for kidney problem (Kumar et al., 2014), diarrhea (Chanda et al., 2015), piles (Devi & Sarkar, 2017), and jaundice (Bhattacharya et al., 2015), as well as important during drought as it has nutritional values (Srianta et al., 2012). In addition to that, Chinese used this plant to make a nutritious soup for the sick (Wong & Tan, 1994) and Indonesians use it to cure bloating (Hossan et al., 2010).

A small number of studies on this species in other countries have been done, but study on the potential of *P. foetida* from local species is scarce. This is due to the plant being regularly chopped off, mainly found in undisturbed areas, or rarely planted in home garden unlike 'ulam raja' (*Cosmos caudatus*) and 'pegaga' (*Centella asiatica*). Previous studies have shown that *P. foetida* contains bioactive compounds such as amino acids, saponin, tannin, phenolics, flavonoid, terpenoid, glycoside, alkaloid, carbohydrates, iridoid, sitosterol, stigmasterol, volatile oil, protein (Chanda et al., 2014; Mazumder et al., 2018). The biological properties of *P. foetida* have been tested in a few studies such as gastroprotective for gastric ulcer (Chanda et al., 2015), antidiabetic (Vikas et al. 2009), hepatoprotective (Peng et al., 2015), and antidiarrheal activity (Mazumder et al., 2018). A recent study revealed that the extract from *P. foetida* twigs had antidiabetic potential (Tan et al., 2019). Majority of these studies mainly used *P. foetida* dried leaves. Herein, we report the phytochemical compounds and the antioxidant capacity of both fresh and dried local *P. foetida* leaves and twigs.

Materials and Methods

Materials

The leaves and twigs of *Paederia foetida* (*P. foetida*) were collected at Pasir Mas, Kelantan, Malaysia. The fresh samples were rinsed with tap water followed by distilled water and air dried before use. The dried samples were subsequently oven dried at 60℃ for 72 hours and then stored in an airtight container for further use.

Extraction

The fresh and dried leaves and twigs of *P. foetida* were macerated separately with n-hexane, methanol and ethanol at ambient temperature for 3 days following method as described by Dev & Kumar, (2011) and Handrianto & Surahmaida, (2018). The extracts were then evaporated using rotary evaporator at 40℃.

Screening for phytochemical constituents

The screening tests were conducted using the methods as described by Rao et al., (2016). All samples were screened for phenols, glycosides, saponins, alkaloids, terpenoids, flavonoids and tannins.

Total Phenolic Content

The total phenolic content (TPC) was determined using Follin-Ciocalteu method. Firstly, stock solution was prepared by dissolving 0.025 gram of Na_2CO_3 in ethanol and further diluted to 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. 1 mL of extract was transferred into a 10 mL volumetric flask with 5 mL of distilled water and 1 mL of Follin reagent. After 5 minutes, Na_2CO_3 solution was added followed by distilled water. The absorbances of the standard and samples were measured at 760 nm with UV-1800 spectrophotometer (Shimadzu Japan). The total phenolic content was expressed as mg gallic acid equivalent per gram of the sample

(GAE/g). The measurements were made in triplicate. The total phenolic content was calculated using equation (1):

$$TPC = \frac{c \times V}{m} \quad (1)$$

where C is sample concentration from the calibration curve (mg/mL), V is volume (mL) of the solvent used in the extraction and m is weight (g) of sample.

Total Flavonoid Content

The Total Flavonoid Content (TFC) in *P. foetida* was identified by using quercetin as the standard. 3.2 mg of quercetin was dissolved in ethanol and diluted to 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL. Then, 50 µL of extract, 0.1 mL of 1M potassium acetate, 0.1 mL of 10% $AlCl_3$, distilled water and ethanol were transferred into a 10 mL volumetric flask and mixed well. The absorbances of the standard and samples were measured at 415 nm with a UV-1800 spectrophotometer (Shimadzu Japan). TFC was determined by using equation (2):

$$TFC = \frac{R \times V \times D.F \times 100}{W} \quad (2)$$

where R is the obtained result from the standard curve, V is the volume (mL) of stock solution, D.F is the dilution factor, 100 for 100 g plants and W is the weight (g) of plants used in the experiment.

DPPH Assay

The radical scavenging activity of extracts against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was performed following the method described by Yeap et al., (2017) with slight modifications. 0.004 g of DPPH crystalline solid was dissolved in 100 mL of methanol. Samples were prepared by adding 2 mL of DPPH solution after dissolving 0.2 mL of extract in 2 mL of methanol. The absorbances of the standard (0.1, 0.2, 0.4, 0.6, 1.0 mg/mL) and samples were recorded at 517 nm using a UV-1800 spectrophotometer (Shimadzu Japan) after 30-minute incubation in the dark. The antioxidant capacity of DPPH was determined by using equation (3):

$$\%DPPH\ antioxidant\ capacity = \frac{Acontrol - Asample}{Acontrol} \times 100\% \quad (3)$$

where $A_{control}$ is the mixture of DPPH solution and methanol while A_{sample} is the mixture of DPPH solution and sample extract.

Results and Discussion

Screening for phytochemical constituents

Phytochemical screening tests of fresh and dried twigs as well as leaves of *P. foetida* have revealed the presence of phenolics, glycosides, alkaloids, saponins, tannins and terpenoids. The results are tabulated in **Table 1**. These findings are almost similar with previous studies (Chanda et al., 2014; Handrianto & Surahmaida, 2018; Ojha et al., 2018). It shows that extraction efficiency of phytochemical compounds favors the highly polar solvent.

Table 1 Phytochemical screening test of *P. foetida*

	Phenolics	Saponins	Glycosides	Flavonoids	Tannins	Alkaloids	Terpenoids
Ethanol extracts							
Fresh leaves	+	-	+	-	+	-	-
Dried leaves	+	-	+	-	+	-	+
Fresh twigs	+	-	+	-	+	-	-
Dried twigs	+	-	+	-	+	-	+
Methanol extracts							
Fresh leaves	+	+	+	-	+	+	+
Dried leaves	+	+	-	-	+	+	+
Fresh twigs	+	-	-	-	+	-	+
Dried twigs	+	-	+	-	+	-	+
Hexane extracts							
Fresh leaves	-	-	+	-	-	+	-
Dried leaves	-	-	+	-	+	-	+
Fresh twigs	-	-	+	-	-	+	-
Dried twigs	-	-	-	-	-	+	-

+ = present, - = absent

Antioxidant activity

The phenolic compounds which act as free radical terminators (Omoregie et al., 2014) contain polyphenols and aromatic benzene ring and are also able to absorb free radical (Iqbal et al., 2015). This makes them as vital antioxidants. In this study, TPC was measured using Folin – Ciocalteu's reagent method. This method allows the estimation of all anthocyanins, flavonoids, and non-flavonoid phenolic compounds of all the phenolics available in the samples (Rao et al., 2016). A good correlation (R^2=0.999) was obtained from the calibration of the standard (y = 4.5011x + 0.2977). TPC of every sample was measured as gallic acid equivalent (GAE) and the results are shown in **Figure 1.** The higher the phenolic content, the higher the antioxidant activity (Mwonjoria, 2019). The obtained TPC for methanol, ethanol and n-hexane extract of fresh twigs were 0.0536 GAE/g, 0.0219 GAE/g and 0.0111 GAE/g respectively. The obtained TPC of dried twigs in methanol, ethanol and n-hexane extract were 0.0399 GAE/g, 0.0388 GAE/g and 0.01026 GAE/g respectively. The TPC values for methanol, ethanol and n-hexane extract of fresh leaves were 0.0407 GAE/g, 0.0357 GAE/g and 0.00752 GAE/g respectively. The TPC values for methanol, ethanol and n-hexane extract of dried leaves were 0.0267 GAE/g, 0.0137 GAE/g and 0.00767 GAE/g respectively. The results show that methanolic extract showed the highest TPC value compared to ethanolic and hexane extracts. This is due to the fact that a wide range of phenolic compounds may dissolve in this solvent due to its ability to inhibit the reaction of polyphenols oxidase that causes the oxidation of phenolic and its ease of evaporation compared to water (Ngo et al., 2017).

Flavonoids are important biological compounds because they are responsible in managing biological activities such as anti-microbial, anti-allergic and anti-carcinogen and cannot be synthesized by human (Chanda et al., 2015). The TFC of various parts of *P. foetida* was quantified using Aluminium chloride method. The principle of this method is based on the oxidation of flavonoid which forms colored flavonoid-aluminium complex that can be monitored spectrophotometrically at 415 nm (Rao et al., 2016). In this method, quercetin was used as the standard and the linear regression line of standard equation is y = 4.0516x –

0.0369 with $R^2 = 0.999$. The TFC of fresh leaves, dried leaves, fresh twigs, and dried twigs of *P. foetida* are presented in **Figure 2.** The results showed that TFC for methanol, ethanol, and n-hexane extracts of fresh twigs of *P. foetida* were 0.0738 QE/g, 0.0151 QE/g and 0.0172 QE/g respectively. The TFC for methanol, ethanol, and n-hexane extracts of dried twigs were 0.0175 QE/g, 0.0137 QE/g and 0.0134 QE/g respectively. The measured TFC for methanol, ethanol and n-hexane extracts of fresh leaves were 0.1545 QE/g, 0.1509 QE/g and 0.00107 QE/g respectively. The measured TFC for methanol, ethanol and n-hexane extracts of dried leaves were 0.125 QE/g, 0.0632 QE/g and 0.0667 QE/g respectively. Similar findings were also observed in TFC as methanol extracts in fresh and dried twigs and leaves of *P. foetida* indicated the highest TFC. These results are in accordance with Peng et al. (2015), who used the same assay and confirmed the high TFC in methanolic extracts. A study done by Kakati and Sikdar (2017), also revealed the presence of high content of flavonoid in methanol compared to the other solvents.

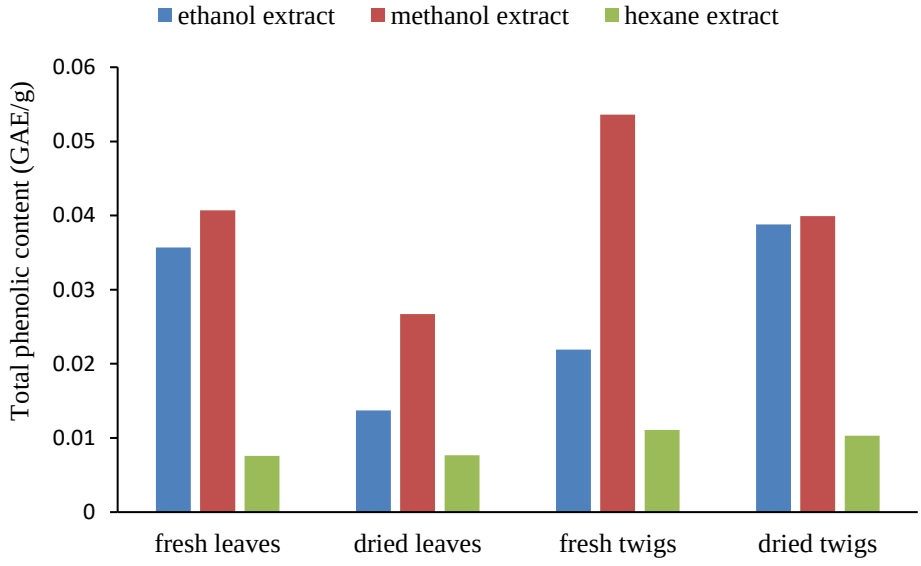

Figure 1 Total phenolic content of fresh leaves, dried leaves, fresh twigs, and dried twigs of *P. foetida*

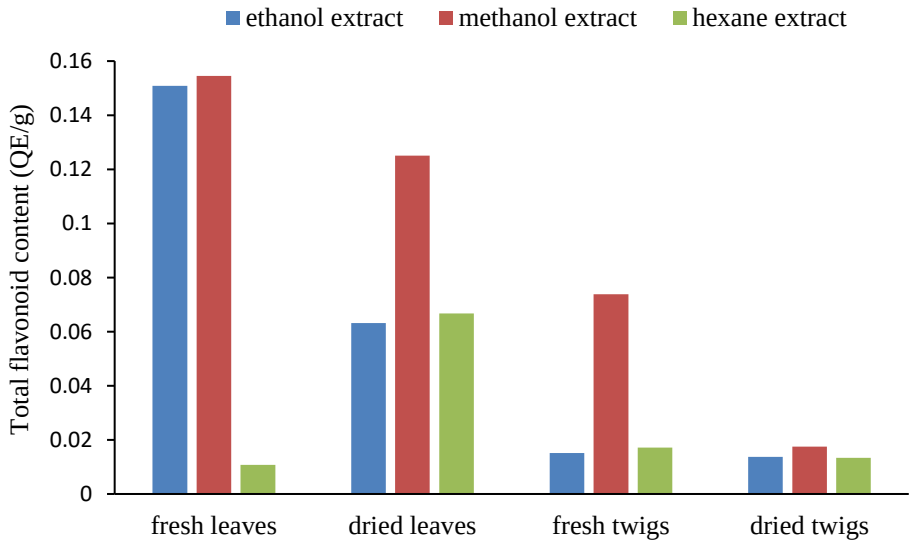

Figure 2 Total flavonoid content of fresh leaves, dried leaves, fresh twigs, and dried twigs of *P. foetida*

The present study shows that the fresh samples contain averagely higher TPC and TFC content than in the dried samples. The findings by Osman et al. (2009), also indicated similar results. The results obtained show that TPC in the twigs of *P. foetida* is slightly higher than the leaves whereas the TFC shows an opposite result. These results are supported by the IC_{50} values of all samples as presented in **Table 2.** The presence of phenolic compounds including flavonoids in most natural plant extracts show antioxidant activity and this proved that they are responsible for the scavenging of DPPH radicals (Mwonjoria, 2019).

The IC_{50} of fresh leaves, dried leaves, fresh twigs and dried twigs of *P. foetida* were calculated as the effective concentration of the sample required to scavenge 50% radical. The percentage of inhibition was measured using DPPH assay with ascorbic as the standard ($y=83.55x + 8.461$, $R^2= 0.998$). Hence, higher IC_{50} value corresponds to lower antioxidant capacity (Omoregie et al., 2014). The IC_{50} values of fresh samples were lower than that of the dried samples which indicate that the fresh twigs and leaves of *P. foetida* have higher antioxidant activities. The results obtained also show that the leaves possess more antioxidant capacity than the twigs. This is because as stated by Ghaffar et al. (2015), the antioxidant content is not only available in one part of the plant, but other parts as well. Moreover, the properties of phenolic compounds and their derivatives in concerned plant might be different than others (Khoddami et al., 2013) and differences in geographical condition could significantly affect the contents of phenolic compounds including flavonoids, thus, varying the antioxidant activity (Jovancevic et al., 2011).

Table 2 IC_{50} of fresh leaves, dried leaves, fresh twigs, and dried twigs of *P. foetida*

	fresh leaves	**dried leaves**	**fresh twigs**	**dried twigs**
ethanol extract	1.7	4.88	4.37	16.88
methanol extract	1.6	3.19	1.5	2.8
hexane extract	29.5	12.35	<100	<100

Conclusion

The biologically active constituents present in the local species of *P. foetida* are phenolics, glycosides, alkaloids, saponins, tannins and terpenoids. The total phenolic content, total flavonoid content and IC_{50} values show that local *P. foetida* has a good antioxidant capacity. Thus, the findings from this study reveal the high potential of local *P. foetida* as natural antioxidant, and a detailed study of its biological functions will substantiate its medicinal values.

Acknowledgement

The authors would like to thank all chemistry laboratory staff of UiTM Pahang for their cooperation.

Conflict of interests

Author hereby declares that there is no conflict of interests.

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