Total Phenolic Content of Five Selected Malaysian Herbs

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Abstract
Malaysian rainforests support a vast diversity of plant which approximately 15,000 plants species. These plants are diverse in chemical complexities that undoubtedly important source of bioactive compounds such as phenolic compounds. The objectives of this research are to extract and compared the total phenolic content (TPC) from *Solanum lasiocarpum*, *Euodia redlevi, Colubrina asiatica, Ipomoea aquatica s. and Amaranthus viridis* in hexane, dichloromethane (DCM) and methanol extracts respectively by using Soxhlet extractor. TPC are later determined using Folin-Ciocalteu reagent using the UV-Vis Spectrometer at 765 nm with Gallic acid as the standard. First plants were stripped, washed, and dried in an oven at 40 ^{o}C for 96 h and ground to powder and kept in a tight container until further use. Dried powders were extracted in Soxhlet extraction units with three different solvents until the solvent became colourless. Phenolic compound was measured according to the Folin-Ciocalteu method at absorbance of 765 nm using the UV-Vis Spectrometer. The statistically significant difference ($p<0.05$) between phenolic content among samples was analyzed by one-way ANOVA by using Microsoft excel. Results showed that *Amaranthus Viridis extracted with DCM* had the lowest phenolic content, which was 2.73±0.16 mg GAE/g sample. The highest phenolic content found at *Ipomoea Aquatica S.* which was extracted by Methanol was 50.6 mg GAE/g sample. Phenolic content for most samples shows significant differences between types of sample and types of solvent extraction. There are no significant differences between *Colubrina Asiatica* extract with hexane and methanol, *Ipomoea Aquatica S.* extract with hexane and DCM, *Amaranthus Viridis* extract with hexane and DCM. For hexane extraction, there are no significant differences between *Solanum Lasiocarpum* and *Euodia Redlevi; Colubrina Asiatica and Ipomoea Aquatica S.* For DCM extraction, there are no significant different between *Solanum Lasiocarpum and Colubrina Asiatica; Colubrina Asiatica and Amaranthus Viridis; Euodia Redlevi and Ipomoea Aquatica S.* For methanolic extract of all plants are significantly different ($p<0.05$) with each other.

Keywords: Total phenolic content, Folin-Ciocalteu method, Malaysian herbs

1.0 Introduction

In Malaysia, plants are widely used as traditional medicines to cure some diseases such as fever, cough, headache and so on (Omar et al. 2022). Malaysians also like to eat local plants as salad, or sometimes they cooked. Most of these local plants are rich in antioxidant (Awang et al. 2020). Antioxidants are compounds that can delay, inhibit, or prevent processes oxidation from free radicals. Free radicals are very reactive because they have one or more unpaired electrons, so they are unstable. The stability of free radicals can occur by way of antioxidants donating their hydrogen atoms to

free radicals. Attacks from free radicals can cause several diseases such as cancer, heart coronary, even accelerate aging (Winarsi, 2007).

Although plants in Malaysia are rich in antioxidants, the actual antioxidant value has not been discovered yet. Knowing the antioxidant content and their importance, may encourage the next generation in Malaysia to take these plants as their daily food. Besides, crude extract rich in phenolics compounds in plants can be used as further application in the food industry to inhibit or delay the oxidation of lipids hence to improve the nutritional value of food (Admassu & Kebede, 2019).

Selected plants were yellow *Solanum lasiocarpum* (Terung Asam), *Euodia redlevi* (Tenggek Burung), *Colubrina asiatica* (Peria Pantai), *Ipomoea aquatica s.* (Sudu Itik) *and Amaranthus viridis* (Bayam Hijau). These plants were collected from Kampung Pandan and wet market in Kuantan, Pahang. Samples were washed, cleaned and the damaged parts were removed. The undamaged parts were then dried in the oven at 40^{o}C for 96 h, before ground to powder form. The powdered form samples were then kept in a tight container and stored at cool environment until further use. The solvent used for extraction were hexane, DCM and methanol. The samples were extracted until the solvent became colorless. Phenolic compound was determined according to the Folin-Ciocalteu method at absorbance of 765 nm using the UV-Vis Spectrometer. The statistically significant difference ($p<0.05$) between phenolic content among samples was analyzed by one-way ANOVA by using Microsoft excel. The research aim to identify and compare the total phenolic content of five selected plants namely *Solanum lasiocarpum*, *Euodia redlevi, Colubrina asiatica, Ipomoea aquatica s. and Amaranthus viridis* in hexane, dichloromethane (DCM) and methanol extracts.

2.0 Literature Review

Polyphenols from plants have at least one aromatic ring containing hydroxyl substituent and obtained from the secondary metabolism of flora and most of which are found in many kinds of plant. These natural antioxidants can be found in fruits, seeds, cereals, berries and vegetables. Antioxidant agent may base on the structural and compositional properties of the individual phenolic compound. These natural antioxidants are potential to prevent toxicity and/or carcinogenic (Maqsood et al. 2014).

Solanum lasiocarpum is also called 'terung asam' as a local name in Malaysia which is popular among Sarawak community as a wild vegetable in fruit form. Visitors to Sarawak also like the unique flavor of *Solanum lasiocarpum.* Now it has been planted all over Sarawak, due to high demand and high market price. Estimation nutrient content for 100g of fresh fruit is 1.1g protein, 0.9g oil, 5.8g carbohydrate, 1.7 g fiber, 27mg phosphate, 188 mg Kalium and 8mg vitamin C. *Solanum lasiocarpum* has been given the status of "Protected Geographical Indication" under Intellectual Property Corporation of Malaysia (MyIPO) in 2011 as Sarawak Sour Eggplant (GI No. 2010-00002) to ensure its authenticity are protected. (Umikalsum et al. 2019)

Euodia Redlevi grow naturally and are easily found in the bush. The local name is 'Tenggek burung'. This tree can reach a height of 4-5 m if allowed to grow wild. It has large -sized tree trunks and three-branched leaves.

The sprouts are picked and made into a side dish; it tastes a little gelatinous. This tree is planted well in moist soil. Based on previous community practices, the sprouts are believed to have various properties such as treating high blood pressure, promoting blood circulation, removing fatigue from the body, shrinking the uterus after childbirth and refreshing the body. Studies also show it has a high antioxidant activity (Umikalsum et al. 2019).

Colubrina asiatica, called 'Peria Pantai' as its local name, is a type of shrub that is commonly found in coastal areas. The tree grows up to 20 feet tall, but because the branches are soft, they bend down and make this tree very low at only 6-10 feet. The shoots are commonly used as salad and side dishes by the Malay community. Previous studies showed that this plant contains compounds of saponins and alkaloids. The minerals content in 100g fresh plants are 0.59g calcium, 0.31g magnesium, 2.54g potassium and 0.031g sodium. The ancient community believed that leaves can relieve inflammation by rubbing the juice of the leaves on the parts of infection. In addition, *Colubrina asiatica* is also used to heal mouth ulcers, stabilize blood pressure, and help to control diabetes (Umikalsum et al. 2019).

Ipomoea Aquatica S. also known as 'Sudu itik' is a plant whose stem is soft. It can be planted with seeds. This herb grows in the bush and has skinny leaves. Local communities consume this vegetable either cooked or raw. This herb is believed to increase appetite and digestive system. *Ipomoea Aquatica S.* is believed to treat ear pain, asthma and so on. To treat ear pain, repeatedly put the juice of leaves into the ear until it recovers (Norhayantie, 2013).

Amaranthus Viridis comes from the *Amaranthaceae* family and often grows wild in Malaysia and is usually used as a vegetable. The stems are soft, but they become hardened as they begin to flower. Local name is called 'Bayam Hijau'. *Amaranthus Viridis* has wide and oval-shaped leaves. A traditional practice, *Amaranthus Viridis* is believed to kill worms in the intestines. It is also believed to relieve swelling, relieve urination. The compounds found in *Amaranthus Viridis*There are iron, vitamin B, C, sugar, tannin, and saponin. *Previous studies found it* acts as a good antioxidant. (Umikalsum et al. 2019)

3.0 Research Methodology

Five type of plants *Solanum lasiocarpum* (Terung Asam), *Euodia redlevi* (Tenggek Burung), *Colubrina asiatica* (Peria Pantai), *Ipomoea aquatica s.* (Sudu Itik) *and Amaranthus viridis* (Bayam Hijau) were collected from plants farm at Kampung Pandan and wet market in Kuantan, Pahang. All samples were washed, cleaned and the damaged parts were removed. All the edible part and undamaged part of plants were stripped from its stem and cleaned. The plants were then dried in the oven at 40^{o}C for 96 h, ground and kept in a tight container and cool environment until further use.

Powdered samples from the five plants were extracted in Soxhlet extractors using hexane, dichloromethane (DCM) and methanol, respectively. About 5g of powdered samples were placed in thimble in the Soxhlet extractor. Some 200ml solvent was filled in a round bottom flask and plants samples were extracted until the solvent became colourless. The solvent was removed by using a water bath until the solvent was dried. The extract yield is calculated as using equation 1.

Eq 1: yield of extraction (%) = weight of crude extract/ weight of sample in thimble x100%

About 0.01g of gallic acid were dissolved in 10ml of ethanol and topped up with distilled water until 100ml. From this stock solution, gallic acid standard with the concentration of 2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm were prepared. The absorbance of standards was read at 765 nm using UV-Spectrophotometer (Shimadzu UV-1650 PC Spectrophotometer). A standard curve was drawn, and an equation was produced based on the spectrophotometer standard readings. All procedures were carried out in triplicate and for concordant values.

TPC were measured by the modified Folin-Ciocalteu method (Hudz et al. 2019). About 0.01 g extracted samples were diluted to 10ml with their solvent. Then, 1ml sample was added to 5ml of Folin-Ciocalteu reagent. The mixture was mixed by using a vortex for 1 min. The mixture then was added with 4ml of 5% Na_2CO_3 and left in the dark place for 1 hour. This allowed the sample to be oxidized by reagent and neutralized by Na_2CO_3. The absorbance of samples was read using a UV-Spectrophotometer. The phenolic compounds (mg GAE/g extract) of samples were calculated based on the equation shown below:

Eq 2: $y=mx + c$

Where, y = absorbance ; x=total phenolic content.

All procedures were carried out in triplicate and for concordant values. The statistically significant difference ($p<0.05$) between phenolic content among samples was analyzed by one-way ANOVA by using Microsoft excel.

4.0 Result and Discussion

Table 1 shows the yield of extraction for five herbs. *Solanum lasiocarpum* extracted with methanol showed the highest yield which was 37.55%, followed by *Colubrina asiatica* extracted by methanol too showed 31.67%. The third higher extraction was *Ipomoea Aquatica S.* extracted by methanol showed 24.30%. The lowest yield extraction was *Solanum lasiocarpum* which was extracted by hexane showed 1.87%.

From this result, different solvent showed different yield of extraction. Methanol showed the highest yield of extraction for all herbs. Compounds of herbs can be polar or nonpolar in nature. Phenolic compounds presence of hydroxyl substituents are more soluble in methanol which is polar solvent (Aryal et al. 2019). These herbs present more polar compounds in their nature.

Table 1: Yield Extraction of Herbs

Samples	% Crude extract		
	Hexane	DCM	Methanol
Solanum lasiocarpum	1.87	3.03	37.55
Euodia Redlevi	10.02	4.77	19.00
Colubrina asiatica	11.29	3.60	31.67
Ipomoea Aquatica S.	6.78	2.67	24.30
Amaranthus Viridis	6.44	4.71	15.25

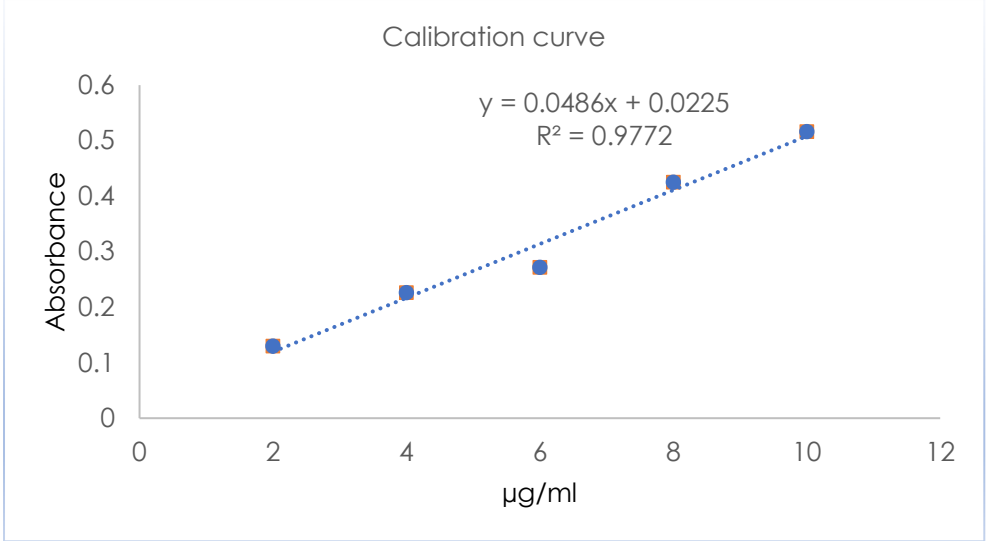

Figure 1: Absorbance versus standard of gallic acid

Gallic acid was used to represent the polyphenol compound of herbs. This calibration curve was used to estimate the phenolic compound for five selected herbs. The equation of curve was as below:

Eq3: $y=0.486x + 0.0225$ with $R^2 = 0.9772$.

Table 2 shows total phenolic content of herbs in mg GAE / g extract. Results found that *Amaranthus Viridis extracted with DCM* had the lowest phenolic content, which was 2.73 ± 0.16 mg GAE/g sample. The highest phenolic content found at *Ipomoea Aquatica S.* which was extracted by Methanol was 50.6 mg GAE/g sample. Phenolic content for most samples shows significant differences between types of sample and types of extraction. There are no significant differences between *Colubrina Asiatica* extract with hexane and methanol, *Ipomoea Aquatica S.* extract with hexane and DCM, *Amaranthus Viridis* extract with hexane and DCM. For hexane extraction, there are no significant different between *Solanum Lasiocarpum and Amaranthus Viridis; Colubrina Asiatica and Ipomoea Aquatica S.* For DCM extraction, there are no significant differences between *Solanum Lasiocarpum and Colubrina Asiatica; Colubrina Asiatica and Amaranthus Viridis; Euodia Redlevi and Ipomoea Aquatica S.*

Table 2: Total Phenolic Content of Herbs

Solvent / Herbs	mg GAE/ g extract		
	Hexane	DCM	Methanol
Solanum Lasiocarpum	2.78±0.02 a, a	4.80±0.165 b, a	43.52±0.06 c, a
Euodia Redlevi	12.15±0.25 a, c	6.10±0.06 b, c	31.91±0.13 c, b
Colubrina Asiatica	6.66±0.07 a, b	4.00±0.10 b, ab	7.82±0.01 a, c
Ipomoea Aquatica S.	7.60±0.23 a, b	6.91±0.10 a, c	50.63±0.27 b, d
Amaranthus Viridis	3.72±0.15 a, a	2.73±0.16 a, b	11.02±0.06 b, e

Compare results with previous study, research of Zaidan et al. (2019), they extracted parts seed, peel, and pulp for yellow and purple of 'terung asam'. Samples were dried in the oven at 50°C overnight, ground and extracted with 100% water, 100% methanol, 50% methanol at pH 5 and 50% methanol at pH 9. Results showed the highest TPC in peels extracted with 100% methanol were 2.26 ± 1.13 and 2.35 ± 0.21 mg GAE per g sample for yellow and purple 'terung asam'. Ab Rahman et al. (2019) studied TPC of 'terung asam' by drying it in an oven at 55˚C for 72 hours. Ground samples extracted with distilled water from 60˚C to 90˚C and from 20 mins to 180 mins. Sample was extracted using a hot plate with stirrer speed at 90 rpm. Study found that the highest TPC was at 60°C for 180 min (5.95 mg GAE/dry extract). Hwong et al. (2020) studied freeze-dried ground 'terung asam' extracted with 80% (v/v) methanol, samples extracted from 30 to 60°C and duration from 0.5 h to 16 h with centrifuge speed (0-250 rpm). They found that the highest TPC was 16.16 mg GAE/g which extracted at 60°C for 16 hours and speed at 250 rpm.

Water and 80 % ethanolic extracts of 'Tenggek burung' for 2 h at 50°C were studied and found that around 1.62 g GAE/ 100g fresh herbs for ethanolic extract and around 0.9 g GAE / 100g fresh herbs for water extract (Othman et al. 2014). 'Tenggek burung' and 'peria pantai' were dried in oven at 40°C for 14 days, then ground dried samples were extracted in water at 60°C for 1 day. The TPC of 'tenggek burung (44.56 ± 0.35) was higher than 'peria pantai' (20.97 ± 0.53) mg GAE/ g extract (Ravi et al. 2016). Young leaves of 'tenggek burung' were air dried, ground and dissolved using hexane, ethyl acetate, methanol and water at 37 °C for 6 h. Result showed 'tenggek burung' extracted with methanol contain the highest phenolic compound which were 61.31 ± 1.39 mg GAE/g dried extract (Kabir et al. 2017). Leaves of 'tenggek burung' were dried at room temperature in dark place, ground and extracted in 95% ethanol at room temperature for 3 days in the dark. Result found that there are 65.33 ± 1.91 mg GAE/g sample (Musyirna, et al. 2019). Young leaves and old leaves of 'tenggek burung' were extracted by 96% ethanol and place at ultrasonic bath for 30 minutes at 50°C with frequency 50 kHz. Result showed TPC of samples were at 0.594 to 0.797 µg/mg extract. The highest content was young leaves of 'tenggek burung' at 0.797±0.0021 µg GAE/mg extract (Nasution & Ardhiyati, 2019).

'Peria pantai' were collected for two groups where rainy season (Sept) as group 1 and dry season (Feb) as group 2. Fresh herbs were dried at oven below 40 °C and blend to fine grains. Fine grains were extracted using methanol which extracted with 0.1% HC1 for 1 h. Result showed TPC of 'peria pantai' were 105 ± 5.6 mg GAE /kg fresh weigh for batch 1 and 62 ± 2.5 mg/kg GAE fresh weigh for batch 2. It found there are significant different between two batches ($p<0.05$). (Shukri et. al, 2011) 'Tenggek burung' and 'Sudu itik' extracted by using water at three different durations for 4, 8 and 12 hours at 100°C. Result showed 'sudu itik' the highest content of polyphenol compounds (15.01 mg GAE / g sample) at 8h extraction (Rahman, 2013).

'Bayam hijau' was grown with five treatments and fresh sample will be cut and chopped became fine. After that, ethanol was added to the chopped sample and sonicated around 15 minutes. After that incubated at -20 °C for 24 h. TPC showed from 0.652 ± 0,001 to 0.940 ± 0,004 mg GAE/ g to the

samples (Naspera et al. 2013). Direct comparison among studies has not been possible due to differences in methods of extraction. The readings of polyphenol content in this research are different compared to those in the previous study. This may be due to the different geographical environment, different growing condition, and the experimental conditions, which may affect the amount of phenolics in herbs (Aryal et al. 2019).

5.0 Conclusion

Total phenolic content of five different type of plants *that is Solanum lasiocarpum* (Terung Asam), *Euodia redlevi* (Tenggek Burung), *Colubrina asiatica* (Peria Pantai), *Ipomoea aquatica s.* (Sudu Itik) *and Amaranthus viridis* (Bayam Hijau) in hexane, DCM, and methanol. Results showed that the highest phenolic content found in methanolic extract of *Ipomoea aquatica s.* was 50.6 mg GAE/g sample followed by *Solanum lasiocarpum* methanolic extract at 43.52 mg GAE/g sample. DCM extract of *Amaranthus viridis* had the lowest phenolic content, which was 2.73±0.16 mg GAE/g sample. When comparing each plant for phenolic content, extracted with their three different solvents, it was clearly showed that there are significant differences for plant *Solanum lasiocarpum* and plant *Euodia Redlevi.* For plant *Colubrina Asiatica,* there are no significant between solvent Hexane and Methanol but there are significant between Hexane and Methanol with DCM. For *Ipomoea Aquatica S.* and *Amaranthus Viridis*, there show the same result where no significant between solvent Hexane and DCM but showed significant different between solvent Hexane and DCM with Methanol. When compare plants in solvent extract, found that hexane extract did not show any significant different between *Solanum lasiocarpum* and *Euodia Redlevi* ; *Colubrina Asiatica* and *Ipomoea Aquatica S.* There were also no significant between *olanum lasiocarpum and Colubrina Asiatica*; *Euodia Redlevi* and *Ipomoea Aquatica S.*; *Colubrina Asiatica* and *Amaranthus Viridis for* DCM extract. Last for methanolic extract of all plants are significantly different ($p<0.05$) with each other.

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