Total phenolic content, flavonoid concentration and antioxidant activity of indigenous herbs, Physalis minima linn

C R Nurhaslina*, H Mealianny¹, A N Mustapa¹ and C Y Mohd Azizi²,³

¹Faculty of Chemical Engineering, Universiti Teknologi Mara, 40450 Shah Alam, Selangor, Malaysia
²Centre of Lipids Engineering and Applied Research (CLEAR), Ibnu Sina Institute for Scientific and Industrial Research, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia
³School of Chemical and Energy Engineering, Universiti Teknologi Malaysia, Skudai, Johor, Malaysia

*nurhaslina483@uitm.edu.my

Abstract. Physalis minima linn is a plant under the Solanaceae family having secondary metabolites with distinct biological activities. The total phenolic, total flavonoid and antioxidant activity on different parts (leaves, whole plant, stem, roots and fruits) of Physalis minima linn were analyzed using Folin-Ciocalteu method, aluminium chloride colorimetric method and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, respectively. Results demonstrated that leaves extract exhibited as the highest value of total phenolic content of 1125.42±14.60 mg of gallic acid (GAE) equivalent per gram of plant (dry extract). The low value of IC₅₀ indicated that leaves, whole plant and fruits can be deemed as a good candidate for natural plant sources of antioxidants with high value of antioxidant activity.

1. Introduction
Many chronic illnesses such as diabetes, cancer or cardiovascular disease among others are results from the oxidation reactions that take place in cell and tissues of human leading to oxidative stress. Oxidative stress is linked to a disproportion in the level of free radical and antioxidant in the human body. Radical is formed when oxygen molecules undergo an incomplete reduction in which it is categorized as reactive oxygen species (ROS) [1]. In certain condition, ROS level can be formed at a high level beyond the antioxidant defense system in our body in which it can damage many cellular functions. The unpaired electron of free radical pairs up with an essential cellular component such as DNA molecule or cell membrane and oxidize them. Thus, antioxidant need to be supplied since endogenous antioxidant in our body is not enough to neutralize the free radicals [2].

In the last century, synthetic antioxidants such as butylated anisole (BHA) and butylated hydroxyltoluene (BHT) have been used widely. However, the use of synthetic antioxidant was reported to have some side effects such as carcinogenesis and related to the possibility of toxicity. Thus, the amount used of this synthetic antioxidant were restricted [3]. The health concerns due to the usage of synthetic antioxidant has caused an increase of interest to produce natural based antioxidant from plant material [4]. The presence of phenolic compounds such as flavonoids, anthocyanins etc., are responsible for the antioxidant activity in most plants [5].
The terms ‘phytochemicals’ is derived from the Greek word ‘phyton’ that means plant. Phytochemicals are compounds that occur naturally in the plant. Phytochemicals can bring benefits to human health since they helps to reduce the possibility to develop the various human disease and also exhibit biological and pharmacological effects such as antimicrobial, antioxidant, antitumoral and antimutagenic [6]. The biggest group of phytochemicals is phenolic compounds in which it can be further divided into several classes of aromatic secondary metabolites and is capable to scavenge many radicals [7]. Meanwhile, the biggest classes of phenolic compounds are flavonoids in which they exhibit chemical and biological activities such as antioxidant and free radical scavenging activity [8]. Among the many biological and pharmacological effect of phytochemical, interest and attention is more focused to the antioxidant as it has a significant role in the prevention of disease stimulating cardiovascular health, decelerates the ageing process in the brain and nervous system, hinders the growth of cancerous tumors and minimize the severity of neurodegenerative disease. The antioxidant is a compound that can scavenge free radicals and inhibit oxidation of molecules in our body [9].

In this work, antioxidant activity of Physalis minima linn extract is presented. In the plant kingdom, Physalis minima linn is considered as the third most economically important families which categorized as Solanaceae. Physalis is part of Solanaceae family alongside with other plants such as S. melongena (brinjal), Capsicum annuum (pepper) and S. tuberosum (potato) among others [10]. Plant in Solanaceae family said to have a high contribution in economic, agricultural and pharmaceutical aspect since it possesses a broad variety of secondary metabolites with distinct biological activities [11]. The common name of Physalis minima linn is native gooseberry. Physalis minima linn is reported to exhibit bioactive steroid derivatives; physalins and withanolides from previous studies [12]. Physalis minima linn is chosen for this research since it is a wild plant that has never been used as food products in Malaysia which helps to reduce the supply competition as source of natural antioxidant besides can be found abundantly throughout Malaysia region. This will also increase the commercial value of Physalis minima linn that is once considered as part of unbenefficial plant. This study aimed to measure the total penolic content, total flavonoid content and antioxidant activity on whole plant, leaves, root, stem and fruit of Physalis minima linn. This study differ from previous study that mostly use Physalis minima linn originated from other country which are India [13], [14].

2. Methodology

2.1. Collection of plant material
Physalis minima linn was gathered from Alor Setar, Kedah. The plants were washed in tap water and air-dried. The stem, leaves, fruits, roots and whole plant were collected in separate plastic bags and dried in shade for several days. The whole plant is needed as part of sample to determine whether a whole plant is much better to be used as an antioxidant rather than parts of plant. Figure 1 shows the tree of Physalis minima linn.

![Figure 1. Physalis minima linn tree](Image)
2.2. Chemical and reagents
Folin-Ciocalteu reagent, aluminum chloride (AlCl\textsubscript{3}), potassium acetate (C\textsubscript{2}H\textsubscript{3}KOH\textsubscript{2}) were purchased from R&M, whereas sodium carbonate (Na\textsubscript{2}O\textsubscript{3}) was obtained from Merck. Ethanol, gallic acid and 2,2’-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Systerm and Sigma/Friendman, respectively.

2.3. The preparation of extracts
Stem, leaves, fruits, roots and whole plant of \textit{Physalis minima linn} were ground into coarse powder in ranges of 1 mm - 8 mm. The powder (2 mg) were extracted twice (20 mL for each) with 95% ethanol, for 24 hours by shaking using shaker (INFORS HT Ecotron) at 100 rpm, at room temperature. The extracts were then centrifuged using centrifuge (SIGMA 3-18K, Sartorius) at 4800 rpm at room temperature for 5 minutes to segregate the supernatant from the solute. Prior to the storage and analysis, the supernatant was further filtered to separate from the filtrate from any remaining traces of the solute. To ensure traces of solvent, the extract was placed in an oven (UFE 500, Saintifik Gemilang) for 6 hours or until most of the solvent has evaporated. The extract was kept in a refrigerator at 8ºC for further analysis.

2.4. Total phenolic content
The total phenolic content (TPC) of different extracts were estimated using the Folin-Ciocalteu method [13]. Gallic acid was used as the standard in which the calibration curve was plotted with different concentration ranged 20-500 µg/ml. A 1 ml of extract (1000 µg/ml) was added to 3 ml of distilled water. Then, 1 ml of Folin-Ciocalteu reagent (previously diluted with distilled water (1:6 v/v)) was added and mixed thoroughly. The mixture was then added with 2 ml of 20% sodium carbonate. The mixture was kept in dark for 30 minutes at room temperature. Absorbance of the sample and standard were taken at wavelength of 765 nm. From the calibration curve of gallic acid, a linear regression equation was used to calculate the TPC. The analysis of TPC was conducted in triplicate and the TPC was presented as mean±SD (n=3) and expressed as milligrams (mg) of gallic acids equivalents (GAE) per gram (g) of the plant (dry extracts).

2.5. Total flavonoid content
The total flavonoid content (TFC) of different extracts were estimated using aluminum chloride colorimetric method [13]. Gallic acid was used as a standard in which the calibration curve was plotted with different ranged 20-500 µg/ml. 1 ml of extract (1000 µg/ml) was added with 0.1 of 10% aluminum chloride followed by 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. The mixture was kept in dark for 30 minutes at room temperature. The absorbance of the sample and standard were taken at wavelength of 415 nm. From the calibration curve of gallic acid, a linear regression equation was used to calculate the TFC. The analysis was conducted in triplicate and the TFC was presented as mean±SD (n=3) and was expressed as milligram (mg) of gallic acid equivalent (GAE) per gram (g) of the plant (dry extract).

2.6. DPPH Assay
The radical scavenging activity assay of different extracts was evaluated using DPPH assay method [13]. 1 ml of extract at five different concentration of 100, 150 200, 250 and 300 µg/ml were added with 1 ml of 0.2 mmol DPPH (2,2’-diphenyl-1-picrylhydrazyl) in ethanol solution. The mixture was allowed to stand in dark for 30 minutes at room temperature. The absorbance of extract and standard were taken at 517 nm. The percentage inhibition of absorbance was calculated and IC\textsubscript{50} values were determined as a representation of the ability of plant extract and standard to scavenge DPPH radical. DPPH assay was conducted in triplicate.
where, \( A_{\text{control}} \) indicates absorbance of control containing 5 ml of DPPH and 5 ml of ethanol, \( A_{\text{sample}} \) is the absorbance of the sample while \( A_{\text{blank}} \) is the sample blank.

3. Results and Discussion

3.1. Calibration Curve
The gallic acid standard calibration curve is plotted as in Figure 2 by using five different concentration of 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml.

![Gallic acid standard calibration curve](image)

Figure 2. Gallic acid standard calibration curve

3.2. Total phenolic content
The total phenolic content of roots, fruits, stem, leaves and whole plant of \textit{Physalis minima linn} extract were calculated from the linear regression equation of gallic acid standard calibration curve. The total phenolic content was expressed in terms of milligram gallic acid equivalent per gram dry weight of plant extract. The total phenolic content of different part of plant extract ranged from 223.08±12.73 and 1125.42±14.60 mgGAE/g. The results are tabulated as in Table 1 and Figure 3. Leaves extract shows the highest value of total phenolic content while roots extract shows the lowest value of total phenolic content. For comparison with the total phenolic content in [15] also shows that leaves extract of \textit{P. patula}, \textit{P. subatula}, \textit{P. solanacea}, \textit{P. angulate} and \textit{P. hederifolia var. hederifolia} are higher compared to its fruits extract.

| No | Parts of plant | Total phenolic content (mgGAE/g) | Total flavonoid content (mgGAE/g) |
|----|----------------|----------------------------------|----------------------------------|
| 1  | Whole plant    | 941.61±24.50                    | 1161.03±52.90                   |
| 2  | Leaves         | 1125.42±14.60                   | 28.92±14.24                     |
| 3  | Stem           | 569.97±62.98                    | 294.46±48.75                    |
| 4  | Roots          | 223.08±12.73                    | 8.42±1.37                       |
| 5  | Fruits         | 784.70±3.43                     | 11.90±0.98                      |

The data represent the mean (n=3)±SD

Phenolic compounds present in plants are a big secondary metabolite and is very important. It is found that phenolic compounds have many health benefits potentials [16] besides having the
importance in fruit maturation and food preservation. In food, it’s organoleptic and antioxidant properties are due to the presence of phenolic compounds in which they create an interest in the food industry. The study of total phenolic in plants in which it varies in distribution helps to improve human health through the making of new drugs [15]. Different parts of plants give a different value of total phenolic content [17]. In this study, the leaves extract gives the highest value of total phenolic content followed by whole plant extract, fruits extract, stem extract and finally, roots extract. According to [18], the genetic factors, environmental effects and their interaction can be factors that caused the quantity of phenolic to be different amid the plant species. Light, nitrogen (N) availability and temperature of surrounding were the environmental aspects that cause the difference on total phenolic content at different parts of plant particularly. Each parts of plant had different total phenolic content in a condition when the nitrogen was insufficient [19]. Study by [15] using plant of Solanaceae family shows the same results in which the leaves of Physalis subulata has the highest total phenolic content.

![Figure 3. Total phenolic content on different parts extracts of Physalis minima linn](image-url)

3.3. Total flavonoid content
Total flavonoid content of plant extracts of Physalis minima linn was determined by using the aluminum chloride colorimetric method. The total flavonoid content was expressed as in terms of milligram gallic acid per gram dry weight of plant extract. The total flavonoid content of roots, stem, fruits, leaves and whole plant of Physalis extract ranged from 8.42±1.37 to 1161.03±52.90 mgGAE/g. The results are tabulated as in Table 1 and Figure 4. Whole plant extract shows the highest total flavonoid content while roots extract shows the lowest flavonoid content. Study by [15] shows that leaves extract of P. patula, P. subatula, P.solanacea, P. angulate and P. hederifolia var. hederifolia had higher total flavonoid content compared to its respective fruit extracts.

![Figure 4. Total flavonoid content on different parts extracts of Physalis minima linn](image-url)
The antioxidant enzyme activity in our body can be improved through the scavenging of reactive oxygen species of flavonoid [20]. Due to microbial infection, the flavonoid is being synthesized by plants and is known as hydroxylated phenolic substance. Different mechanisms which include scavenging of free radicals, chelation of metal ions and inhibition if enzymes exert the antioxidant properties of flavonoid [13]. Flavonoid can be found in the plant in which it is distributed generally. Flavonoid possesses many health benefits which includes anti-cancer, anti-inflammatory and antimicrobial among others [21]. In this study, the amount of total flavonoid content in different parts of the plant are calculated. The total flavonoid content is found to be highly abundant at the whole plant extract followed by stem extract, leaves extract, fruits extract while the roots extract has the lowest value of total flavonoid content. [22] report in their study that the discrepancy of total flavonoid content on different part of plant is due to the changes in environmental condition. The ultraviolet ray radiation intensity affects the weight and transpiration rate on different part of plant in which it is possible that the flavonoids are affected as well [23]. However, to my best knowledge, no scientific reason is presented to explain why whole plant the highest total flavonoid content has compared to other part of plant.

3.4. DPPH Assay
Antioxidant activity of Physalis minima linn extracts were determined by using DPPH assay. The antioxidant activity was shown as percent inhibition (IC\textsubscript{50}). The data are tabulated as in Table 2.

| No | Parts of plant | % inhibition (IC\textsubscript{50}) |
|----|----------------|----------------------------------|
| 1  | Whole plant    | 1.28                             |
| 2  | Leaves         | 1.70                             |
| 3  | Stem           | 150.99                           |
| 4  | Roots          | 825.86                           |
| 5  | Fruits         | 3.46                             |

The maintenance of cell structure and function by bioactive compound through scavenging of free radicals, lipid peroxides inhibitions and reduces damages of other oxidative indicates an antioxidant activity [24]. The complementary of DPPH radical scavenging activity with total phenolic and total flavonoid makes DPPH assay is used to determine the antioxidant activity of plants [17]. From the study, the value of IC\textsubscript{50} from Table 2 shows that it complements with the total phenolic and flavonoid content as in Table 1. However, this statement is only applied to whole plant extract and leaves extract since other parts of plant does not shows a complementary between total phenolic content, total flavonoid content and IC\textsubscript{50}. The high association of antioxidant activities with total phenolic and total flavonoid content found in the present study agrees with the report of other authors [17]. The leaves extract and whole plant extract have the lowest IC\textsubscript{50} compared with other parts of plants in which due to the high value of total phenolic and total flavonoid content. Low IC\textsubscript{50} indicates high antioxidant activity. The antioxidant activities of the plant are usually associated with the value of total phenolic. Phenols as the main group of primary antioxidant in which phenols and flavonoid possess antioxidant activities [24]. In the study by [24] shows that the leaves extracts of Tulbaghia alliacea and Tulbaghia violacea had a much lower IC\textsubscript{50} of 0.06 and 0.08 mg/mL, respectively compared to leaves extract of Physalis minima linn with IC\textsubscript{50} of 1.70 mg/mL.

4. Conclusion
Total phenolic and total flavonoid content in a plant were distributed generally in different part of Physalis minima linn and had a different value of total phenolic and total flavonoid. Leaves extract demonstrated as the highest value of total phenolic, total flavonoid and IC\textsubscript{50} whereas the whole plant
and stem also shows a significant value of total phenolic, total flavonoid and IC$_{50}$. This finding revealed that the whole plant including leaves and stem possess as promising as source of antioxidant for pharmaceuticals and nutraceutical industries.

Acknowledgement
The authors acknowledge the financial support provided by RMI, Universiti Teknologi MARA under Lestari Grant [600-IRMI/ MYRA 5/3/LESTARI (022/2017)]. Special thanks to the Department of Chemical Engineering, Shah Alam, Malaysia for providing the materials and chemicals used to carry out the research. Special thanks to the staff laboratories for their assistance.

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