Total Phenolic Content, Flavonoid Concentration and Antioxidant Activity of Indigenous Herbs, Physalis Minima Linn

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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 analysed by the use of 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 candidates for natural plant sources of antioxidants with high value of antioxidant activity.

Keywords: *Physalis minima,* total phenolic, total flavonoid, antioxidant

I. 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) [17]. In certain condition, ROS level can be formed at a high level beyond the antioxidant defence system in our body in which it can damage many cellular functions. The unpaired electron of free radical pairs up with an essential cellular components such as DNA molecule or cell membrane and oxidise them. Thus, antioxidant need to be supplied since endogenous antioxidant in our body is not sufficient to neutralize the free radicals [15].

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 these 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 [13]. The presence of phenolic compounds such as flavonoids, anthocyanins etc., are responsible for the antioxidant activity in most plants [6].

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, antitumorial and antimutagenic [10]. 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 [4]. 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 [19]. 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 [11].

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 [8]. 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 [1]. 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 [18]. *Physalis minima linn* is chosen for this research since it is a wild plants 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 unbeneficial 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.*

II. METHODOLOGY

A. Collection of plant materials

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.

Figure 1: *Physalis minima linn* tree

B. Chemical and reagents

Folin-Ciocalteu reagent, aluminum chloride (AlCl3), potassium acetate (C2H3KO2) were purchased from R&M, whereas sodium carbonate (Na2O3) was obtained from Merck. Ethanol, gallic acid and 2,2'-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Systerm and Sigma/Friendman, respectively.

C. The preparation of extracts

Stem, leaves, fruits, roots and whole plant of *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.. Prior to the storage and analysis, the extract was filtered to separate from the filtrate. 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.

D. Total phenolic content

The total phenolic content (TPC) of different extracts were estimated using the Folin-Ciocalteu method [2]. 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 ug/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 mix 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).

E. Total flavonoid content

The total flavonoid content (TFC) of different extracts were estimated using aluminum chloride colorimetric method [2]. 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 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).

F. DPPH assay

The radical scavenging activity assay of different extracts was evaluated using [2] method. 1 ml of extract at five different concentration ranging from 20-500 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 IC50 values were determined as a representation of the ability of plant extract and standard to scavenge DPPH radical. DPPH assay was conducted in triplicate.

$$DPPH\ scavenging\ activity\ (\%) = \left(\frac{A_{control} - A_{sample} - A_{blank}}{A_{control}}\right) \times 100$$

where, Acontrol indicate absorbance of control containing 5 ml of DPPH and 5 ml of ethanol, Asample is the absorbance of the sample while Ablank is the sample blank.

III. RESULTS AND DISCUSSION

A. Total phenolic content

The total phenolic content of roots, fruits, stem, leaves and whole plant of *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 1. 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 [9] also shows that leaves extract of *P. patula, P. subatula, P.solanacea, P. angulate and P. hederifolia var. hederifolia* are higher compared to its fruits extract.

Table 1: Total phenolic and total flavonoid content of *Physalis minima linn*

No	Parts of plant	Equivalents per g dry weight of extract (mgGAE/g)	
		Total phenolic content	Total flavonoid content
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 [5] 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 creates 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 [9]. Different parts of plants give a different value of total phenolic content [7]. 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.

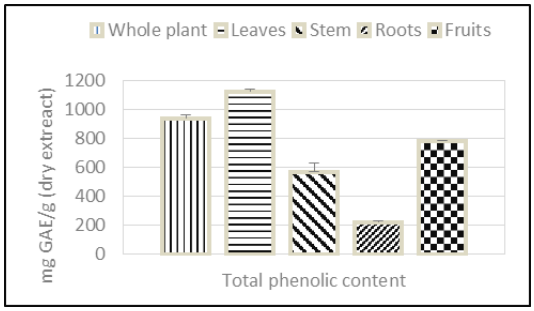

Figure 1: Total phenolic content of different parts extracts of *Physalis minima linn*

B. 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 were 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 were tabulated as in Table 1 and Figure 2. Whole plant extract shows the highest total flavonoid content while roots extract shows the lowest flavonoid content. Study by [9] 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.

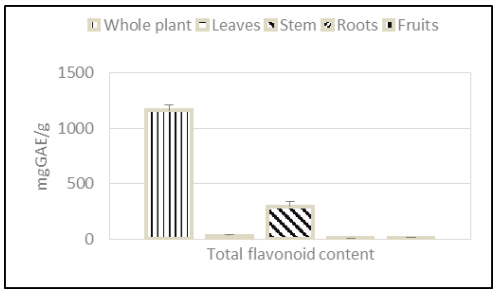

Figure 2: Total flavonoid content of different parts of *Physalis minima linn*

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 [2]. 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 anti-microbial among others [12]. 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.

C. DPPH activity

Antioxidant activity of *Physalis minima linn* extracts were determined by using DPPH assay. The antioxidant activity was shown as percent inhibition (IC50). The data were tabulated as in Table 2.

Table 2: Parts of plants vs percentage inhibition (IC_{50})

No	Parts of plant	% inhibition (IC_{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 [16]. 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 [7]. From the study, the value of IC50 from Table 2 shows that it is complement with the total phenolic and flavonoid content as in Table 1. 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 [7]. The leaves extract and whole plant extract have the lowest IC50 compared with other parts of plants in which due to the high value of total phenolic and total flavonoid content. Low IC50 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 [16]. In the study by [16] shows that the leaves extracts of *Tulbaghia alliacea* and *Tulbaghia violacea* had a much lower IC50 of 0.06 and 0.08 mg/mL, respectively compared to leaves extract *of Physalis minima linn* with IC50 of 1.70 mg/mL.

IV. 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 IC50 whereas the whole plant and stem also shows a significant value of total phenolic, total flavonoid and IC50. This findings revealed that the whole plant including leaves and stem possess as promising as source of antioxidant for pharmaceuticals and nutraceutical industries.

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