CHARACTERIZATION OF NUTRITION, ANTIOXIDANTPROPERTIES, AND TOXICITY OF PHYSALIS ANGULATA L. PLANT EXTRACT

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ABSTRACT

Objective: This research was to characterize and compare the nutrition, total phenolics (TP) content, antioxidant activity, and toxicity of all part of Physalis angulata L. extract.

Methods: The proximate, minerals, TP content, antioxidant activity, and toxicity of all parts of physalis, namely, stem bark extract of P. angulata L. (ESC), leaf extract of P. angulata L. (ELC), rind extract of P. angulata L. (ERC), unripe fruit extract of P. angulata L. (EFU), and ripe fruit extract of P. angulata L. (EFR) were analyzed. The TP content, total flavonoids (TF), and free radical scavenging activity of ethanolic extract are studied using Folin–Ciocalteu assay, aluminum chloride assay, and 1,1-diphenyl 2-picrylhydrazyl scavenging assay. Brine shrimp lethality bioassay (LC100) was used to measure the toxicity of extract.

Results: The physalis leaves extract (ELC) contains the highest total of phenolics (144.4 mg gallic acid equivalent/g), a total of flavonoids (33.33 mg quercetin equivalent/g), and antioxidant activity (96.97 µg/ml) followed by ERC > EFC > EUP > ESC. Based on the level toxicity of LC100, the ripe fruit extract of P. angulata (EFR) (924.18 µg/ml) valued as cytotoxic.

Conclusion: The data of nutrition, antioxidant properties, and toxicity of all parts of P. angulata extract provide for functional food product uses.

Keywords: Antioxidant, Ciplukan, Physalis angulata, Phytochemical, Toxicity.

INTRODUCTION

In Indonesia, in the past three decades, there has been an increase in the use of herbal medicine products and supplements. To improve the quality of health, 80% of the world population uses plant products [1]. Alternative therapy was developed by taking into bioactive compounds derived from nature. The biodiversity found in Indonesia is the world’s second order. A total of 2500 of 30,000 plants are medicinal plants [2]. With biodiversity, it is potential to develop functional food products. In Indonesia, at 2009–2011, functional food products have increased [3].

Globally, a nutritional transition has occurred from infectious disease patterns toward chronic and degenerative lifestyle-related diseases [4]. In Indonesia, changes in nutritional transition are reinforced by increased non-communicable diseases, such as hypertension from 7.6% in 2007 to 9.5% in 2013; stroke from 8.3/1000 to 12.1/1000 (2013); and diabetes mellitus from 1.1% (2007) to 2.1% (2013) [5]. One of the utilizations of traditional medicine based on local wisdom is using a plant named Physalis angulata Lour.

P. angulata L (Indonesia: “Ciplukan” or Sundanese: “Cecender”), family Solanaceae, empirically already utilized in Sundanese traditional medicine preparation for recovery “kencing manis” (diabetes mellitus). Previous studies have reported that physalis leaves possess an anti-diabetic effect [6]. According to Pinto et al. [7], the ethanolic fruit extract of physalis has given anti-hyperglycemic and anti-hypertension potential. Moreover, the ethanolic crude extract of the fruit of physalis has a role in the immune system (immunomodulation), anti-inflammation effect, and antioxidant activity [8,9].

The utilization of local wisdom, increased demand, and many benefits obtained from physalis make it potentially functional food [9]. However, there are still challenges that must be faced, such as poor quality control of extract (non-standard), the lack of clinical and pharmacological data, and toxicity [10]. Nevertheless, based on our knowledge, the evaluation of characteristics antioxidant activity and toxicity of all parts of physalis remain scarce. The present study focused on evaluation and comparison of the characteristics of the nutrition, total phenolics (TP) content, antioxidant activity, and toxicity of all part of P. angulata Lour. Based on the results of the research, there was an effect of TP content against free radical scavenging activity at physalis extract.

MATERIALS AND METHODS

Reagents and materials
Folin–Ciocalteu’s phenol reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, quercetin was obtained from Sigma-Aldrich (Singapore). Artemia salina L. (Artemia) was purchased from Dohse Aquaristik GmbH and CO, Gelsdorf, Germany. Aluminum chloride, ethanol, sodium hydroxide, and sodium carbonate are obtained from Merck, Tbk. All reagent used is an analytical grade. Fresh P. angulata L (physalis) was collected from Rawale Village – Subang, Indonesia, obtained from January to May 2018 (Fig. 1). Botanical authentication was done by a Botanist from "Herbarium Bogoriense," Research Center for Biology, Indonesian Institute of Sciences (No. 886/IPH.1.01/II/07/IV/2018), with the voucher specimen that has been stored.

Sample preparation
Fresh physalis washed, then dried at 45°C in 3 days and smashed into powder, the powder soaking in ethanol with ratio 1:10 for 24 h by maceration methods (3 times). The filtrates were consolidated and dried by a vacuum evaporator. For analysis, 10 ml of ethanol was added into a centrifugation tube containing 0.06 g of physalis ethanol extract. The samples centrifuged for 10 min after shaking. The resulting
supernatant was inserted into a 10 mL volumetric flask and added ethanol to the limit mark and shaken for 10 min [11].

Procedure analysis
Physical and nutritional composition
Physical composition, namely, yield, pH, total solid, and color was performed in triplicate. The nutrition composition, viz., moisture, ash, carbohydrates, protein, and lipid content, was measured by methods described [12]. Atwater factor used as a direct application in measuring the energy, which 1 g carbohydrate=4 kcal; 1 g lipid=9 kcal; and 1 g protein=4 kcal. Colorimeter 3 nh is needed to determine the total color difference of the three coordinates.

Preliminary phytochemical screening
Physalis powder was the identification of saponin, flavonoid, alkaloids, tannin, glycosides, and sterols or terpenoids [13-15].

Total carotenoids content
Total carotenoid content of the extract studied with methods of Scrob et al. [16]. The extract of physalis was re-extracted with petroleum ether. The total carotenoid content of the samples was analyzed at \(\lambda=450\) nm using a ultraviolet (UV)-VIS spectrophotometer (UV-1700 Shimadzu series) in units of \(\mu g/g\) (the absorbance should be between 0.2 and 0.8).

TP content
The TP content of physalis was analyzed with the Folin-Ciocalteu assay [17]. The 100 \(\mu l\) extract or standard solution of gallic acid or blank (0; 25; 50; 100; 150; and 200 \(\mu g/ml\)) has been added with distilled water (2.8 ml) and sodium carbonate (2 ml and 2%), and allowed to stand for 4 min. The 100 \(\mu l\) of Folin-Ciocalteu solution was added, then silence for 30 min. Measurement of blank solution was carried out at \(\lambda=760\) nm. The extract solution at concentrations is not the same (1 ml) or blank or standard solution has been added with 3 ml of 0.004% DPPH methanolic solution then stored in the dark for 30 min. Measurement of blank solution was carried out at \(\lambda = 517\) nm. Data obtained were calculated by expression (30) and delivered as the concentration of antioxidants needed for 50% DPPH radical scavenger in a defined time period (IC\(_{50}\)). The samples were analyzed in three replications.

\[
%\text{Inhibition} = \frac{(Ac - As)}{Ac} \times 100
\]

Where:
\(Ac = \text{absorbance control or blank, As = absorbance with sample or standard.}\)

Cytotoxicity assay
The cytotoxicity of the ethanolic extract of physalis was investigated by brine shrimp lethality bioassay [20]. Brine shrimp that are hatched is obtained from brine shrimp eggs (Hobby Artemix \(^\circ\) Germany), which is mixed with salt, in a conical shaped vessel, for 48 h they were left in sterile distilled water under constant aeration. Using a capillary glass of ten active nauplii is taken and put into a bottle containing 4.5 ml of brine solution. The 0.5 ml of the ethanolic extract has been added with brine solution (4.5 ml) and stored under light at room temperature for 24 h, and surviving larvae were counted. After incubation, the larvae are counted dead and live in each test. The research was controlled (vehicle-treated) at unequal concentrations (1–1000 \(\mu g/ml\)) with test substances per dose of a set of three tubes. The IC\(_{50}\) values are used to determine the mortality rate of larvae up to 50%, were calculated using probity analysis. Estimated linear correlations were observed when the logarithm of concentration.

Statistical analysis
Data were presented in mean±standard deviation and tested for normality. The differences between treatments were analyzed using ANOVA. Significant differences between mean values were calculated using the Duncan Multiple Range Test (\(\alpha=5\%\)). All statistical analysis was performed using Microsoft Excel 2013.

RESULTS
Nutrition and physicochemical characteristics
The nutrition and physicochemical characteristics of each part of \(P.\) angulata L. are displayed in Table 1 and 2.

Table 2 showed physicochemical characteristics of each part of physalis with the pH value ranging from 5.81 to 6.46, and the total solid ranging from 5.16 to 6.86° Brix. The color of each part of \(P.\) angulata used a colorimeter 3 nh to find out the spectrum of reflection of the sample, so we get the color coordinates of CIE \(L^*a^*b^*\) coordinates and hue (h°) (Table 2). Table also shows that the colors of each part of physalis were darker, greener, and less blue, except for fruit and stem bark powder. The plant cell walls breakdown is related to the extracts obtained. The yields of ethanolic extract of each part of physalis ranged from 2.0 to 3.6% (Fig. 2).

Phytochemical screening
Phenolic compounds in \(P.\) angulata L. ethanolic extracts are found in large quantities in the phytochemical screening process, which proven by the existence of alkaloids terpenoids, tannins, flavonoids, and glycosides. Phytochemical screening is shown in Table 3.

Absorbance = 0.0005 gallic acid \(\mu g/\) mL \(-0.0033\)  

\[
\text{Abosrbanice} = 0.0081 \text{ quercitol } \frac{\mu g}{\text{mL}} + 1.594
\]
Table 1: Nutrition composition of *P. angulata* L. powder

| Constituent    | Part of the plant |
|----------------|-------------------|
|                | ESC               | EFC               | ELC   | EUF   | ERC   |
| Moisture (%)   | 6.04±0.41<sup>a</sup> | 14.31±0.51<sup>a</sup> | 9.30±0.81<sup>b</sup> | 8.60±0.28<sup>d</sup> | 9.38±0.40<sup>e</sup> |
| Ash (%)        | 0.87±0.01         | 0.91±0.00         | 0.90±0.01       | 0.95±0.03       | 0.87±0.01       |
| Protein (%)    | 10.7±0.00<sup>d</sup> | 14.06±0.00<sup>e</sup> | 2.98±0.00<sup>c</sup> | 17.10±0.00<sup>b</sup> | 13.72±0.00<sup>c</sup> |
| Lipid (%)      | 4.10±0.13<sup>a</sup> | 7.39±0.35<sup>c</sup> | 11.28±0.35<sup>c</sup> | 3.65±0.11<sup>ab</sup> | 9.81±0.50<sup>b</sup> |
| Carbohydrates (%) | 70.24±0.14<sup>a</sup> | 63.34±0.22<sup>c</sup> | 75.54±0.23<sup>d</sup> | 69.80±0.10<sup>b</sup> | 66.22±0.23<sup>b</sup> |
| Energy (kcal)  | 392.86±17.3       | 375.99±4.03       | 415.60±4.07     | 380.45±1.39     | 408.05±5.42     |
|               |                   |                   |                   |                   |                   |

Data are expressed as mean±standard deviation (n=3). ESC: Stem bark extract of *Physalis angulata* L., ELC: Leaf extract of *Physalis angulata* L., ERC: Rind extract of *Physalis angulata* L., EUF: Unripe fruit extract of *Physalis angulata* L., EFC: Ripe fruit extract of *Physalis angulata* L. a>b>c>d>e, the existence of the same letter in the same line is expressed as the absence of difference. *P. angulata* L: *Physalis angulata* Lour.

Table 2: Phytochemical characteristics of *P. angulata* L.

| Samples      | pH  | Total solid (Brix) | Color (a) | Preference |
|--------------|-----|--------------------|-----------|------------|
| ESC          | 6.59 | 6.86               | 59.817    | –2.788     | 14.927     | +0.003     | Darker, greener, more yellow |
| ERC          | 6.46 | 5.56               | 53.498    | 3.492      | 15.903     | +0.004     | Darker, less red, more yellow |
| ELC          | 6.33 | 5.16               | 48.410    | –2.168     | 9.593      | +0.003     | Darker, greener, less blue   |
| EUF          | 5.81 | 5.36               | 55.811    | 7.142      | 19.770     | +0.003     | Brighter, less red, more yellow |
| EFC          | 6.01 | 6.56               | 55.811    | 7.142      | 19.770     | +0.003     | Brighter, less red, more yellow |

Data are expressed as mean (n=3). ESC: Stem bark extract of *Physalis angulata* L., ELC: Leaf extract of *Physalis angulata* L., ERC: Rind extract of *Physalis angulata* L., EUF: Unripe fruit extract of *Physalis angulata* L., EFC: Ripe fruit extract of *Physalis angulata* L. a>b>c>d>e, the existence of the same letter in the same column is expressed as the absence of difference. *P. angulata* L: *Physalis angulata* Lour.

Table 3: Phytochemical screening of *P. angulata* L.

| Constituent   | ESC | ERC | ELC | EUF | EFC |
|---------------|-----|-----|-----|-----|-----|
| Alkaloids     | –   | –   | –   | –   | –   |
| Mayer         | –   | –   | –   | –   | –   |
| Terpenoids    | ++  | ++  | +++ | ++  | +++ |
| Saponin       | +   | –   | +   | –   | –   |
| Tannins       | ++  | +++ | +++ | ++  | +++ |
| Flavonoids    | ++  | +++ | +++ | ++  | ++  |
| Glycosides    | –   | ++  | +++ | ++  | +++ |

(+)* means positive; (−)* means negative. ESC: Stem bark extract of *Physalis angulata* L., ELC: Leaf extract of *Physalis angulata* L., ERC: Rind extract of *Physalis angulata* L., EUF: Unripe fruit extract of *Physalis angulata* L., EFC: Ripe fruit extract of *Physalis angulata* L., *P. angulata* L: *Physalis angulata* Lour. a>b>c, same alphabetic in the graphic no difference.

DISCUSSION

This study shows that the ethanolic rind extract of *P. angulata* L. (ERC) had the highest moisture and protein content than other parts (p<0.05) (Table 1). The protein of the fruit of *P. pubescens* L. was 31.8% [21]. Moreover, the fruit of *Physalis peruviana* has better protein contains [22]. The leaf extract of *P. angulata* L. (ELC) contained the highest lipid content (11.28%) followed by EFC>ERC>unripe fruit extract of *P. angulata* L. (EUF) and stem bark extract of *P. angulata* L. (ESC) (p<0.05). According to Ramadan and Mörsel [23], the fruit of *P. peruviana* L. contains 2% lipid content, which is 1.8% (seeds) and 0.2% (fruit skin). The high content of polyunsaturated fatty acids obtained from peruviana, which has been extracted into oil [23]. A phytochemical in extract plant maintained in pH value 3–11 and antioxidant activity influenced by pH [24,25]. ANOVA displayed that the ethanolic extract of the fruit of *P. angulata*, namely, EUF and EFC, with the highest average values in yields is ESC>ERC and ELC (p<0.05) (Table 2).

Table 3 is displayed that leaves and fruit extracts of *P. angulata* have various phenolic compounds. Alkaloids compounds were not in ethanolic extract of physalis. The results of this study are an agreement with methods of Andrianto et al. [26], the ethanolic extract of *P. peruviana* leaves contained phenol, flavonoids, tannins, saponins, steroids, and terpenoids. The stem barks of *P. angulata*, obtained by

Fig. 2: Yields of ethanolic extracts of *Physalis angulata* L. Data were expressed as mean±standard deviation (n=3). ESC: Stem bark extract of *P. angulata* L., ERC: Leaf extract of *P. angulata* L., EUF: Unripe fruit extract of *P. angulata* L., EFC: Ripe fruit extract of *P. angulata* L., *P. angulata* L: *Physalis angulata* Lour.
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AUTHORS’ CONTRIBUTIONS
Ade Chandra Iwansyah (ACI) and Rohmah Luthfiyanti designed and conducted field research; Wahidiyanti P Julianti performed laboratory analysis; ACI conducted statistical analyses; ACI wrote the manuscript with inputs from all coauthors; ACI had final responsibility for the content. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST
There are no conflicts of interest in this paper.

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