The effect of filler addition and oven temperature to the antioxidant quality in the drying of *Physalis angulata* leaf extract obtained by subcritical water extraction

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**Abstract.** In traditional medicine, *Physalis angulata* which is well known as cplukan in Indonesia, has been utilized to cure several diseases by conventional extraction in hot water. The investigation of the *Physalis angulata* extract activity in modern medicine typically utilized organic solvents such as ethanol, methanol, chloroform and hexane in extraction. In this research, subcritical water was used as a solvent instead of organic solvent to extract the *Pysalis angulata* leaf part. The focus of this research was the investigation of extract drying condition in the presence of filler to preserve the quality of antioxidant in *Physalis angulata* extract. Filler, which is inert, was added to the extract during drying to help absorb the water while protect the extract from exposure in heat during drying. The effects of filler types, concentrations and oven drying temperatures were investigated to the antioxidant quality covering total phenol and antioxidant activity. Aerosil and microcrystalline cellulose (MCC) were utilized as fillers with concentration varied from 0-30 wt% for MCC and 0-15 wt% for aerosil. The oven drying temperature was varied from 40-60 °C. The results showed that compare to extract dried without filler, total phenol and antioxidant activity were improved upon addition of filler. The higher the concentration of filler, the better the antioxidant; however it was limited by the homogeneity of filler in the extract. Both of the variables (oven temperature and concentration) played an important role in the improvement of extract quality of *Physalis angulata* leaf. It was related to the drying time which can be minimized to protect the deterioration of extract from heat. In addition, filler help to provide the powder form of extract instead of the typical extract form which is sticky and oily.

1. **Introduction**

*Physalis angulata* is a widespread pristine herb found in tropical and subtropical areas of Asia, Afrika and America.[1] It is belongs to Solanaceae family and well known as *ciplukan* or *ceplukan* in Indonesia. It has been utilized in traditional medicine however it is not cultivated well. It grows wildly in yard or rice field without special treatment. Sometimes farmer exterminates those plants before planting season because they considered as weeds. In such a traditional way, *Physalis angulata* is utilized by boiling all plant part to obtain the extract while in modern medicine typical organic solvents such as methanol, ethanol and even hexane and chloroform were used.[2-4] However, those organic solvents are classified as Volatile Organic Solvents (VOC) which the exposure to human body should be limited strictly. Water is considered as a safer solvent, however at normal temperature until around its boiling point, it still behaves as polar solvent. Therefore it is not suitable to draw the less polar substances from *Physalis angulata*. In this research, water at subcritical state was utilized as a solvent. It is because water polarity under pressure and temperature can be adjusted to mimic the organic solvents commonly used in extraction. Subcritical water is water at elevated temperature

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(between its boiling point to supercritical state) under pressure to maintain its liquid state [5]. The fixed extraction condition was used to extract Physalis angulata leaf (250°C, 100 bar and 15 minutes).

The Physalis angulata extract itself has been proven to have activities as antimicrobial [3], antinociceptive [6], antihepatoma [4], immunosuppression [7], anti-inflammatory [1] etc. Our group was the first who investigate the antioxidant activities in Physalis angulata extract based on each plant part individually [8-11]. Antioxidant is any substance which if present in low concentration can inhibit or delay the oxidation of the cell. It can prevent damage of cell in our body arising from chemical reaction involving free radical reaction. Free radical is very dangerous which can weaken our immune system, damage and kill our cells, also generate diseases [12]. In this research, the antioxidant potential of Physalis angulata was investigated in related with extract drying condition.

Crude extract which is commonly obtained from extraction of plant material contains oleoresin (oily and solid form at once) often causes difficulty in the determination of drug dosage. The treatment of crude extract into dry extract can be obtained by drying in dryer. However, the bioactive compound in crude extract which contains mostly antioxidant may damage during exposure in heat. In addition just simply drying resulted in a sticky form of extract. The addition of filler was intended to make the drying process faster, to keep the flavor, to add the quantity, and to make the powder form of extract [11]. It is inert and typically added to add the consistency of drugs. The fillers commonly used in pharmacy are sucrose, lactose, dextrose, cellulose, sorbitol etc. [13, 14] Therefore the best filler type to preserve the antioxidant in Physalis angulata extract and the proper concentration of filler should be investigated. In this research, extract qualities were determined based on total phenol and antioxidant activity. The effects of filler types (Microcrystalline Cellulose (MCC) and aerosil), filler concentrations (0-30 wt% for MCC and 1-15 wt% for aerosil) and drying temperatures (40-60°C) were investigated to the extract obtained from Physalis angulata leaf which were dried in vacuum drying oven.

2. Experimental
The experimental procedure consists of pretreatment, extraction by subcritical water, drying in vacuum drying oven and extract characterization.

2.1 Pretreatment
Physalis angulata plants were collected from several rice fields and yards in Yogyakarta. After identified at Faculty of Pharmacy, Gadjah Mada University, each plant part was separated and investigated individually. In this experiment, leaf part was investigated separately while fruit part has been reported previously [11]. After collection, leaf were washed and dried in tray dryer for 24 hours for then ground and classified into size of -20+30 mesh. The water content was analyzed using moisture analyzer (Mettler Toledo, HR83, USA)

2.2 Subcritical Water Extraction
The reactor system was fabricated from SUS316 with volume of 150 ml, equipped with electric heating for heating the reaction to the desired temperature and cooling jacket for decreasing the reactor temperature once the reaction finished. The temperature and pressure of the system is controlled using control box. The extraction procedure has been described elsewhere [11].

2.3 Drying Experiment
The drying experiments were conducted in vacuum drying oven (Jeio Tech, Korea). The extract with volume about 30 mL was dried upon an addition of filler. The mixture of extract and filler was stirred carefully to obtain a homogenous solution. The drying temperature was set and the extract was dried and weighted every hour. The drying was stopped if the constant weight of extract was observed. The
water content then was checked by moisture analyzer (Mettler Toledo, HR83, USA). The dry extracts were stored at refrigerator prior used.

2.4 Analysis
Prior used in analysis, extract powder was dissolved in methanol p.a to separate the extract from filler. The solution then was filtered using filter paper Sartorius 388 (Sartorius Stedim Biotech S.A.), the filtrate was used for analysis while the filler was dried and weighted for calculation purpose. Total phenol and antioxidant activity were quantified according to the method which has been reported in previous publication[11].

2.5 Total phenol
The total phenols of the extracts were analyzed by Follin-Ciocalteu method, which used gallic acid as the standard solution. Accurately, 0.5 mL extracts 500 ppm (5 mg in 10 ml DDI water) were mixed with 2.5 mg Follin Ciocalteu reagent (1:10) and 2 mL of Na₂CO₃ solution (75 g/L). After added with reagent and Na₂CO₃, the mixture solution was incubated at 40°C for 30 minutes, and the absorbance was measured at 735 nm.

2.6 Antioxidant activity
The stock DPPH solution was prepared by dissolving 7.9 mg DPPH in 100 ml methanol p.a. (0.2 mM). The solution was obtained by diluting a specific amount of dry extracts with 100 ml methanol p.a. The different concentration of solutions (20-400 ppm) were made by diluting the extract mother liquor. Then, 2 ml of this solution was allowed to react with 2 ml of the DPPH solution for 24 h and the condition was kept away from any sources of light. Then the absorbance was measured at 517 nm. The IC50 value was calculated by making a plot of % inhibition (ordinat) versus extract concentration (absis) at different extract concentration, then calculating the linear trendline (y=ax+b). From the trend line equation, IC50 is determined by replacing y value with 50 and calculating the x value, which later considered as IC50.

% inhibition was calculated by formula:

\[ \% \text{ Inhibition} = \frac{A_0-A_a}{A_0} \times 100\% \]  

(2.1)

With \( A_0 = \) absorbance in the absence of extract (blanko)
\( A_a = \) absorbance in the presence of extract

3. Result and discussion
The extraction was conducted by subcritical water extraction at temperature of 250°C, 100 bar and 15 min. The extraction condition was not varied since it is not the focus of this research. Two kinds of filler were used in drying, microcrystalline cellulose (MCC) and aerosil. Both of fillers are typical filler used in pharmaceutical. The affectivity of drying then characterized by measuring total phenol and antioxidant activity since they are representative variables to characterize antioxidant.

1.1. The effect of drying temperature and filler concentration on the total phenol
The content of total phenolic in the extract was quantified based on absorbance values of Physalis angulata leaf extract reacted with Folin-Ciocalteu reagent and compared with standard solution of gallic acid equivalent as describe in the procedure. The total phenol as a function of filler concentration and drying temperature is illustrated in Figure 1. An increase in the filler concentration leads to an increase in total phenol in the extract in the increment of drying temperature. It can be explained in relation with drying time which presented in table 1. An increase in the filler concentration showed shortening the drying time, therefore decreasing the exposure of extract in heat. For instance, in the absence of filler at 40°C, it is needed 6030 minute to dry 30 mL of extract, however in the presence of aerosil 15 wt%, it only need around 2340 minutes. A significant increase in
total phenol at drying temperature of 60°C was observed when concentration of filler was above 10 wt%. That tendency was observed at both of filler type, MCC and aerosil, even though the investigation of aerosil concentration was only observed till 15 wt%. While the trend of total phenol at 40-50°C was similar. It might be because a significant decrease of drying time was observed when drying temperature increased from 50 to 60°C compared to 40 to 50°C. In MCC case, an increase in drying temperature from 50 to 60°C was decrease the drying time around 40-60% while an increase in the drying temperature from 40 to 50°C only decreased drying time in the range of 10-20%. At the drying temperature of 50°C, the total phenol in all samples was lower compare to that of dried at 40°C and 60°C. It is not clear what causes the trend; however the combination between drying temperature and length of drying time might be one of the reason.

![Figure 1](image)

**Figure 1.** Total phenol as a function of filler (MCC (a) and aerosil (b)) concentration under different oven drying temperature

**Table 1.** The length of drying time and water content in the drying of *Physalis angulata* leaf extract in addition of MCC filler

| Drying temperature (°C) | MCC Concentration (wt%) | Drying time (min) | Water content (wt%) |
|-------------------------|-------------------------|------------------|--------------------|
| 40                      | 0                       | 6820             | 2.39               |
|                         | 10                      | 5710             | 6.56               |
|                         | 20                      | 5455             | 2.66               |
|                         | 30                      | 5215             | 3.01               |
|                         | 0                       | 5505             | 2.37               |
| 50                      | 10                      | 5160             | 1.80               |
|                         | 20                      | 4620             | 3.28               |
|                         | 30                      | 4335             | 2.84               |
|                         | 0                       | 3250             | 2.42               |
| 60                      | 10                      | 2535             | 5.36               |
|                         | 20                      | 2050             | 4.27               |
|                         | 30                      | 1700             | 2.98               |
Table 2. The length of drying time and water content in the drying of Physalis angulata leaf extract in addition of Aerosil filler.

| Drying temperature (°C) | Aerosil Concentration (wt%) | Drying time (min) | Water content (wt%) |
|-------------------------|-----------------------------|-------------------|--------------------|
|                         | 0                           | 6030              | 2.66               |
|                         | 40                          |                   |                    |
|                         | 5                           | 3090              | 2.23               |
|                         | 10                          | 2610              | 1.17               |
|                         | 15                          | 2340              | 0.98               |
|                         | 0                           | 4980              | 2.56               |
|                         | 50                          |                   |                    |
|                         | 5                           | 2340              | 3.12               |
|                         | 10                          | 2130              | 1.34               |
|                         | 15                          | 1800              | 2.84               |
|                         | 0                           | 3080              | 2.39               |
|                         | 60                          |                   |                    |
|                         | 5                           | 2220              | 1.34               |
|                         | 10                          | 1470              | 1.12               |
|                         | 15                          | 1320              | 9.76               |

3.2 The effect of drying temperature and filler concentration on antioxidant activity

Antioxidant activity which is presented as Inhibition Concentration 50 (IC50) is defined as the concentration of extract which is required for 50% inhibition of oxidation. It means the lower the IC50 value, the smaller extract needed for inhibiting the oxidation, means the stronger the antioxidant activity. The dependence of IC50 to the filler concentration and drying temperature can be seen in figure 2. An increase in filler concentration decreases the IC50 value that means higher the antioxidant activity. It is also in agreement with total phenol value which also showed the similar trend. The temperature of 60°C gave the best results among all with IC50 value of 50, which then considered as strong antioxidant.[15, 16] Total phenol value and antioxidant activity trend is similar in both of variables, concentration and oven drying temperature.

Figure 2. IC50 as a function of filler (MCC (a) and aerosil (b)) concentration under different oven drying temperature.
3.3 Effect of filler type on the total phenol and antioxidant activity

Due to the different order of filler density, we investigated the different concentration range for MCC and aerosil. Aerosil has much lower density compare to MCC, therefore for the same volume of extract, aerosil volume is much more compare to MCC. In this experiment, the maximum concentration for homogenous solution of extract, aerosil was 15 wt% while MCC was 30 wt%. Therefore, we can only compare the performance of 10 wt% of MCC and aerosil in the extract under different oven temperature as can be seen in figure 3.

![Figure 3. IC50 and total phenol as a function of filler type and oven drying temperature](image)

Aerosil showed better total phenol and antioxidant activity results in all temperatures observed. It may be because aerosil (50 g/L) has much lower density compared to MCC (320 g/L) [14] so that for the same weight, the volume of aerosil was more than MCC. The impact would be in a better ability of aerosil to absorb water compared to MCC for the same quantity, giving faster drying time.

4. Conclusion

Total phenol and antioxidant activity can be improved in addition of filler. Increasing of filler concentration for both of microcrystalline cellulose (MCC) and aerosil as well as increasing drying temperature were proven to give positive effect in the total phenol and antioxidant activity. It is because prolong exposure in heat could deteriorate the extract, however addition of filler could shorten the drying time so the antioxidant in the extract could be maintained. An increase in filler concentration from 0 wt% to 30 wt% for MCC and 0 wt% to 15 wt% for aerosil and oven drying temperature from 40-60°C has been proven to improve the antioxidant quality because of reduction in drying time. Extract was protected from long exposure to heat. At 10 wt%, aerosil showed better performance compare to MCC.

Acknowledgments

The author gratefully appreciate the financial support from Korea Institute of Science and Technology (KIST), Korea through KIST Collaboration Alumni Project and Lembaga Penelitian dan Pengabdian Masyarakat (LPPM) UNPAR through Internal Funding.
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