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

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Abstract. *Physalis angulata* or *ceplukan* is medicinal herb, which grows naturally in Indonesia. It has been used in traditional medicine to treat several diseases. It is also reported to have antimycobacterial, antileukemic, antipyretic. In this research, *Physalis angulata* fruit was investigated for its antioxidant capacity. In order to avoid the toxic organic solvent commonly used in conventional extraction, subcritical water extraction method was used. During drying, filler which is inert was added to the extract. It can absorb water and change the oily and sticky form of extract to powder form. The effects of filler types, concentrations and drying temperatures were investigated to the antioxidant quality covering total phenol, flavonoid and antioxidant activity. The results showed that total phenol, flavonoid and antioxidant activity were improved by addition of filler because the drying time was shorter compared to extract without filler. Filler absorbs water and protects extract from exposure to heat during drying. The combination between high temperature and shorter drying time are beneficial to protect the antioxidant in extract. The type of fillers investigation showed that aerosil gave better performance compared to Microcrystalline Celullose (MCC).

1. Introduction

*Physalis angulata*, well known as *ceplukan*, is a wild medicinal herb found in shaded fields, gardens, wastelands, fallow fields, along roadsides, in open forests, and other places in Indonesia [1]. In folk medicine, all of the parts of *Physalis angulata* plant such as roots, flowers, leaves, stems and fruits have been used to treat some diseases including asthma, diuretic, malaria, hepatitis and cancer treatment [2,3]. There has been significant interest in evaluating the phytochemical and pharmaceutical properties of this plant, including the potential of *Physalis angulata* plant as antimycobacterial, antileukemic, antiseptic, etc [4]. However there were less reports regarding the antioxidant potential of *Physalis angulata*. Our group is the first who investigated the antioxidant extraction from *Physalis angulata* by subcritical water extraction [5,6,7].

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Antioxidant itself is chemical compounds which can prevent oxidation inside our body and protect our body from free radical by neutralizing it. Free radical is very dangerous which can weaken our immune system, damage and kill our cells, also generate diseases in our body [8]. *Physalis angulata* is commonly extracted by organic solvents such as methanol, ethanol, and hexane. However, organic solvents are also well known for its toxicity, carcinogenic, and hazards for environment. It will not simply disappear during extract evaporation and will still remain in the extract. Therefore, pharmaceutical industries tried to exchange the uses of these organic solvents with the more friendly solvents, such as water and supercritical CO$_2$. Water has an unique characteristic. When water is in normal (room) condition, it behaves as a very polar solvent. However, its polarity can be adjusted by changing its temperature and pressure. The condition of water which its temperature is above boiling point and below its critical point, meanwhile its pressure maintains its liquid form is called subcritical water. Subcritical water is chosen as the solvent of this experiment because water is non-toxic, cheap, friendly to environment with adjustable polarity [9,10].

In this experiment, subcritical water was used as a solvent. In previous report, high temperature about 200-250 °C had shown better result [6]. In this research, high temperature of 250 °C, pressure of 100 bar and 15 min of extraction time was utilized to extract *Physalis angulata* fruit. Crude extract which 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, added typically to make consistency of drug. In this research, extract qualities were determined based on total phenol, flavonoid and antioxidant activity.

2. Experimental

2.1. Subcritical Water Extraction

The extraction was conducted in Subcritical Water Extraction batch apparatus (Hanyang Accuracy, Korea). The batch reactor system has volume of 150 mL, is 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 are controlled using control box. Ten grams of the sample was put in the reactor basket. The reactor then was closed and purged with nitrogen for about 15 minutes to remove the oxygen in the reactor and lines. The distilled and deionized water (DDI) water was also purged to remove the dissolved oxygen. Then, DDI water was delivered to the reactor using high pressure metering pump (HKS-600, Hanyang Accuracy, Korea) and bought to the desired pressure. The solution then was heated to the desired extraction temperature and kept for specific time, called as static time. After the time was fulfilled, the reactor then was cooled by opening the insulation and flowing cooling water. The extract was then filtered out with fine grade filter paper (No 393, Sartorius, Germany) to make sure no residue left in the extract. For drying experiment, water was evaporated partially from the extract using rotavapor (RE-3000A, Xi an HEB Biotechnology Co. Ltd, Shanghai, China) until extract volume of 30 ml.

2.2. Drying Experiment

The extract with volume about 30 mL was dried further by vacuum drying oven. Prior to the drying, an amount of filler was added. 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.

2.3. 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.

2.4. 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 Na$_2$CO$_3$ solution (75 g/L). After added with reagent and Na$_2$CO$_3$, the mixture solution was incubated at 40°C for 30 minutes, and the absorbance was measured at 735 nm.

2.5. Total flavonoid
The total flavanoid content of extracts were analyzed using calorimetric method, which used quercetin as the standard. As much as 0.25 mL extracts were mixed with 1.25 mL DDI water and 0.075 mL NaNO$_2$ 5%-wt. After 5 minutes, 0.15 mL AlCl$_3$ 10% was added into the solution. 6 minutes later, 0.5 mL NaOH 4%-wt was added into the solution. Immediately after 10 minutes, water was added to the mixture to bring the final volume of 4 ml. The mixture was thoroughly mixed and allowed to stand for another 10 minutes. Then the absorbance was measured at 510 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.

3. Result and Discussion
3.1. Total Flavanoid
The total flavanoid analysis was conducted by calorimetric method. The total flavanoid was quantified by quercetine as a standard. The effect of MCC addition to the total flavanoid at different drying temperature is illustrated in figure 1 while the effect of aerosil addition is shown in figure 2. It can be shown clearly that the higher amount of filler addition, the better the total flavanoid in extract. It is due to the higher concentration of filler the shorter the drying time, as shown in table 1 for MCC and table 2 for aerosil. For example, by increasing the MCC concentration from 0 wt% to 20 wt%, the length of drying time at 50°C decreased by half and total flavanoid increased by 7 folds. Filler may protect the antioxidant from exposure to heat. It might be also the reason why an increase in drying temperature from 40 to 60°C also increased the total flavanoid, it was due to the time needed for drying became shorter at high temperature.
Figure 1. Total flavonoid as a function of drying temperature and MCC concentration

Figure 2. Total flavonoid as a function of drying temperature and aerosil concentration
Table 1. The length of drying time and water content in the drying of *Physalis angulata* fruit extract in addition of MCC filler.

| MCC Concentration (wt%) | Drying temperature (°C) | Drying time (min) | Water content (wt%) |
|------------------------|-------------------------|-------------------|--------------------|
| 0                      | 40                      | 4330              | 2.65               |
|                        | 50                      | 3645              | 2.52               |
|                        | 60                      | 2680              | 2.44               |
| 10                     | 40                      | 3330              | 3.67               |
|                        | 50                      | 2350              | 2.55               |
|                        | 60                      | 1830              | 3.23               |
| 20                     | 40                      | 3005              | 2.66               |
|                        | 50                      | 1820              | 2.32               |
|                        | 60                      | 1360              | 1.90               |
| 30                     | 40                      | 1990              | 2.52               |
|                        | 50                      | 1340              | 2.84               |
|                        | 60                      | 1050              | 3.35               |

Table 2. The length of drying time and water content in the drying of *Physalis angulata* fruit extract in addition of Aerosil filler.

| Aerosil Concentration (wt%) | Drying temperature (°C) | Drying time (min) | Water content (wt%) |
|-----------------------------|-------------------------|-------------------|--------------------|
| 0                           | 40                      | 4480              | 2.76               |
|                             | 50                      | 3770              | 2.59               |
|                             | 60                      | 2800              | 2.49               |
| 5                           | 40                      | 3830              | 2.33               |
|                             | 50                      | 2755              | 1.77               |
|                             | 60                      | 2450              | 1.44               |
| 10                          | 40                      | 3240              | 1.27               |
|                             | 50                      | 2105              | 1.42               |
|                             | 60                      | 1810              | 1.22               |
| 15                          | 40                      | 2670              | 1.08               |
|                             | 50                      | 1610              | 1.28               |
|                             | 60                      | 1440              | 3.67               |
3.2. Total phenol

The effect of MCC addition to the total phenol at different drying temperatures is illustrated in figure 3 while the effect of aerosil addition is shown in figure 4. The lower the drying temperature, the longer the drying time. For instance, in MCC case, by increasing the temperature from 50 to 60 °C can shorten the drying time till 2 times so that long exposure in heat can be avoided. An increase in filler concentration (0-30% for MCC and 0-15% for aerosil) increases the total phenol in all drying temperatures. It was because the higher the concentration of filler, the shorter the drying time as shown in table 1. For instance, the addition of 30 wt% MCC to sample which dried at 60°C can shorten drying time till 50%. Filler may absorb water and protect the antioxidant from deteriorations because of thermal heat.

![Figure 3. Total phenol as a function of drying temperature and MCC concentration](image)

![Figure 4. Total phenol as a function of drying temperature and aerosil concentration](image)
3.3. Antioxidant Activity

Antioxidant activity is shown by IC50 value. IC50 represents the concentration of extract that is required for 50% inhibition of oxidation. It means the lower the IC50 value, the smallest extract needed for inhibiting the oxidation, means the strongest the antioxidant activity. The IC50 value at different filler concentrations and drying temperatures are shown in figure 5 for MCC and figure 6 for aerosil. For both of filler types, the higher concentration of filler resulted in the lower IC50 or better antioxidant activity. The addition of more filler helps to absorb water, so exposure in high temperature can be avoided. An increase in drying temperature increased the antioxidant activity, shown by a decrease in IC50 value. The higher the drying temperature shortened the drying time as shown in table 1 and 2, so reduced exposure of extract to the heat. The results were confirmed by total phenol and flavonoid which showed the same trends. Started from the additional of 10 wt% filler, antioxidant in Phyalis angulata are categorized as strong antioxidant (IC50<50).

![Figure 5. IC50 as a function of drying temperature and MCC concentration](image-url)
3.4. The effect of filler types to total phenol, flavonoid and antioxidant activity

Table 3. Comparison between MCC and aerosil at the same concentration (10 wt%)

| Drying temperature (°C) | MCC  | Aerosil |
|-------------------------|------|---------|
| Total flavanoid         |      |         |
| 40                      | 42.70| 71.52   |
| 50                      | 55.36| 79.13   |
| 60                      | 91.05| 100.14  |
| Total phenol            |      |         |
| 40                      | 119.02| 146.11 |
| 50                      | 139.47| 166.35 |
| 60                      | 169.43| 183.31 |
| IC50                    |      |         |
| 40                      | 62.54| 51.99   |
| 50                      | 53.83| 26.27   |
| 60                      | 25.50| 19.62   |

The comparison between performance of MCC and aerosil can be shown in table 3. The effect of filler types was observed at drying temperature of 40 - 60°C using 10 wt% of filler. Aerosil showed better total phenol and flavonoid results in all temperatures observed. It may be because aerosil (50 g/L) has much lower density compared to MCC (200-500 g/L) [12, 13], 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

The addition of filler has been proven to improve extract quality in the term of antioxidant capacity. Both of MCC and aerosil gave the same trend for total phenol, flavonoid and antioxidant activity. An increase of filler concentration could enhance the total phenol, flavonoid and antioxidant activity. 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. Comparing aerosil and MCC at the same concentration showed that aerosil gave better performance.

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