

“Emerging Challenges in Chemical Engineering Research, Education, and Industries”



PROCEEDINGS

Regional Symposium on Chemical Engineering (RSCE 2013)

Alona Kew White Beach Resort, Panglao Island, Bohol, Philippines
November 12 – 13, 2013

Organized by the



**Chemical Engineering Department
De La Salle University-Manila**

in cooperation with



**Chemical Engineering Society (CHEN)
De La Salle University-Manila**

**REGIONAL SYMPOSIUM
ON CHEMICAL ENGINEERING 2013
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“Emerging Challenges in Chemical Engineering Research, Education, and Industries”

**20th Regional Symposium on Chemical Engineering
(RSCE 2013)**

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Marthen Luther Doko

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REFERENCE NO. : F2

**Comparison of Conventional Extraction and Subcritical Water Extraction
into the Total Phenol, Flavanoid and Antioxidant Activity of Physalis
Angulata Stem**

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Physalis angulata, a wild plant grow in tropical country such as Indonesia, was used in folk medicine to treat several diseases such as cancer, hepatitis, asthma, malaria, dermatitis, diuretic, rheumatism, etc. Now, it is proved to have extraordinary properties such as antimycobacterial, antileukemic, and antipyretic. Meanwhile, the details of extraction method to obtain the extract were not provided. Most of the method to extract the plant is maceration which is used organic solvent such as methanol. Currently organic solvent is considered not suitable for food and medicine purposes. **The objective** of this research is to prove the effectiveness of subcritical water extraction to extract physalis angulata plant compare to the conventional method such as maceration and hot water extraction.

Subcritical water extraction is wellknown for its unique features and advantages compare to the traditional extraction methods. High difussivity, low viscosity, cheap solvent with adjustable polarity such as temperature and pressure are the advantageous among the others. The extraction was conducted in subcritical water extraction batch apparatus having reactor volume of 150 mL with particle size of -30+40 M. The three variables were observed, temperature (100-250°C), pressure (100-200 bar) and time (15-30 minutes). The maceration was conducted with stirring at F:S=1:24 by varying solvent type (methanol and water) and reaction time (24-48 hr). Hot water extraction was used as a traditional approach to extract physalis angulata in society. The extraction was conducted at 15 minutes. All extracts were analyzed by measuring the total phenol, flavanoid and the antioxidant activity by DPPH Assay

Keywords: subcritical water extraction, antioxidant, maceration, hot water extraction,

INTRODUCTION

Subcritical Water Extraction (SWE) also known as Pressurized Hot Water Extraction (PHWE) is a feasible green solvent extraction method as it is used pressurized water at elevated temperature and controlled pressure condition. (Teo et al., 2010) It has unique features and advantages compare to the traditional extraction methods such as soxhlet extraction, liquid-liquid extraction and sonication. The traditional methods may often be time consuming with low efficiency and utilize large volume of non-environmental friendly organic solvents which has health effect to human such as hexane, methanol, ethanol, benzene, diethyl ether. Then, the utilization of organic solvent in food or medicine is not recommended. The generation of large quantity of organic solvent also could be serious problem to environment. The major advantageous of SWE are the utilization of environmentally benign solvent (water), which are abundant, non toxic and can be recycled or disposed with minimal environmental problem. The term subcritical water refers to water at temperature between temperature 100°C to 374°C under moderate pressure which keep water at liquid state. In this range water behaves as nonpolar to polar depends on operating condition (T, P). The other commercial technique (Supercritical CO₂ extraction), which utilize carbondioxide as a solvent, behaves as nonpolar solvent in all range, then it has very limited power to extract the polar compound. It can be combined with small amount of organic modifier to have polar character meanwhile it still has limited polar range. So, the SWE/PHWE is trully beneficial to extract semi polar or polar substance, better than Supercritical carbondioxide extraction.

The genus *physalis* (Solanaceae) is represented by almost 90 species distributed throughout the tropical and subtropical regions in the world, where it has been widely used in folk medicine. Species such as *P. philadelphica*, *P. peruviana*, *P. grisea*, *P. chenopodifolia*, *P. coztomatl*, and *P. angulata* are cultivated or gathered from wild populations for their edible fruits. *Physalis angulata*, or wellknown as ceplukan (Indonesian) is wild plant in Indonesia, grow well in rice field, garden, forest and other places with less treatment. Its picture is illustrated in figure 1. It is native to temperate, warm and subtropical regions throughout the world. It is a widely-

distributed herbaceous annual plant, naturalized in Australia but has spread over the world. It is known as a Cutleaf groundcherry, Wild tomato, Camapu and Winter cherry. In Colombia, is known as Uchuva, in Japan as Hosuki, in China as Khuzi and in Azores as tomate capucho (tomato hood). In the folk and popular medicine, it is used in the treatment of cancer and other diseases like hepatitis, asthma, malaria, and dermatitis, diuretic, liver problem, rheumatism and has antimycobacterial, antileukemic, antioxidant and antipyretic. There has been significant interest in evaluating the phytochemical and pharmaceutical properties of this plant.

To the best of our knowledge, there are not many reports about the extraction of *physalis angulata*. This is the first report of *physalis angulata* extraction using subcritical Water extraction (SWE). The widely method to extract *physalis angulata* was using methanol and ethanol extraction but no detail report of the extraction method reported. Much studies were reported the activity of bioactive compounds of *physalis angulata* for inhibiting diseases such as tumor, antimycobacterial, immunosuppression, antioxidant, anti-inflammatory and antihepatoma. (Bastos et al., 2006; Bastos et al., 2008; Hseu et al., 2011; Pietro et al., 2000; Pinto et al., 2010; Sun et al., 2011; Wu et al., 2004) There are two groups of working with supercritical carbondioxide (scCO₂) extraction of *physalis angulata* and *peruviana*. One group was working to extract physalin A and B from *physalis angulata* with very small yield (1.62% from aerial part and 1.58% from fruits). (Polezel et al., 2008) The other group was working to extract antioxidant of *physalis peruviana*. (Wu et al., 2009; Wu et al., 2006) Meanwhile from the report, they need modifier to increase the polarity of the solvent. Then subcritical water is potential candidate to extract the semi polar to polar compounds of *physalis angulata* such as phenol and flavanoid by arranging its physical properties

In this research, our aim is to examine a more efficient method to prepare *physalis angulata* extract by subcritical water extraction (SWE). The target compounds are antioxidant in the stem part. Three variables were investigated; temperature, pressure and extraction time. The results will be analyzed from the extract yield,

total phenol, flavanoid and antioxidant activity and further compare with conventional methods including hot water extraction (HWE), maceration and soxhlet.

MATERIAL AND METHODS

Raw material preparation

Before used in experiment, the authenticity of *Physalis angulata* was confirmed. The plant part used in this research is stem part. After harvested, all part were dried and ground to the size of -30+40 M. The water content of raw material are analyzed using moisture analyzer.

SWE/PHWE Extraction

Batch Extraction:

The extraction is conducted in Subcritical Water Extraction batch apparatus (Hanyang Accuracy, Korea). The batch reactor system has 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. Ten gram of the sample is put in the reactor basket. The reactor then is closed and purge with nitrogen for about 15 minutes to remove the oxygen in the reactor and lines. The distilled and deionized water (DDI) water is also purged to remove the dissolved oxygen. Then, DDI water is delivered to the reactor using high pressure metering pump (HKS-600, Hanyang Accuracy, Korea) and bring to the desired pressure. The solution then is heated to the desired extraction temperature and keep for specific time, called as static time. After the time fulfilled, the reactor then is cooled by opening the insulation and flowing cooling water. The extract then is then filtered out with fine grade filter paper (No 393, Sartorius, Germany) to make sure no residue in the extract. For further analysis, water is evaporated from the extract using rotary evaporator (Buchi rotavapor R-205, UK) and the water content is checked by moisture analyzer (Mettler Toledo, HR83, USA)

Conventional Extraction

Maceration

Ten gram of sample is dissolved in 240 mL of solvent (methanol, or DDI water). The extraction is conducted at 25°C with stirring. Maceration is conduction in two different stage; one stage, where the extraction is conducted at

24 hr without renew the solvent. And two stage extraction where the extraction is conducted at 48 hrs totally by changing the fresh solvent after 24 hr. For both of stages, the solvent is evaporated from the extract for then analyzed.

Hot water extraction

Ten gram of sample is extracted by 240 mL of boiled DDI Water for 15 min. The extract is filtered with filter paper to separate the residue which may escape from the basket. The filtrate is concentrated and analyzed.

Soxhlet extraction

Soxhlet extractions were carried out by placing ground of samples (10 gram) in a Sartorius filter paper. The filter paper containing sample was wrapped and placed in a thimble. The thimble was then placed in a Soxhlet extraction apparatus. Approximately 240 ml of extraction solvent (methanol and water) was added into the Soxhlet apparatus. All samples were extracted with hot boiling solvent mixture for 9 h.

Analysis

Phytochemical screening

The phytochemical screening to the *Physalis angulata* extract followed the Raman's procedures. (Raaman, 2006)

Total Flavanoid analysis

The total flavanoid content of extract is analyzed using calorimetric method. As much as 0.25 mL of sample was mixed with 1.25 ml of distilled water and subsequently with 0.075 ml of 5% NaNO₂ solution. After 6 min of incubation, 0.15 ml of 10% AlCl₃ solution was added and allowed to stand for 6 min, followed by adding 0.5 ml of NaOH 1M solution to the mixture. Immediately after water was added to the sample to bring to the final volume of 3 ml, the mixture was thoroughly mixed and allowed to stand for another 15 min. The mixture absorbance was determined at wavelength 510 nm. All values were expressed in milligrams of quercetin equivalents per gram of extract.

Total phenol analysis

The total phenol content of extracts was analyzed by the Folin-Ciocalteu method. In brief, after 0.5 mL *Physalis angulata* extracts (20 mg in 10 mL DDI water) were well mixed with 2.5 ml of the Folin-Ciocalteu stock reagent (Follin : DDI water = 1 : 10 mL), 2 ml of Na₂CO₃ reagent (75 g/l) was added to the

mixture and then incubated at 40°C for 10 min. The mixture absorbance was measured at wavelength 760 nm. The total phenol content was expressed in milligrams of gallic acid equivalents per gram of extract.

Antioxidant activity:

DPPH Assay

The stock solution is prepared by dissolving 3.94 mg DPPH in 50 mL methanol (0.2 mM) and then stored in refrigerator until needed. The working solution is obtained by diluting 40 mg stock solution with 100 mL methanol. It read as 400 ppm. The different concentration of extract (1-400 ppm) were made by diluting the 400 ppm extract with methanol. 4 mL of each extract solution is allowed to react with 1 mL of the DPPH solution for 4 h in the dark. Then the absorbance is taken at 517 nm.

RESULTS AND DISCUSSION

Antioxidant that are present in herbs can be in the form of polyphenol, flavanoid, phenolic compounds and other phytochemicals. In this paper, we quantify the total phenol, flavanoid and antioxidant activity of extract obtained from both Subcritical Water Extraction (SWE) and other conventional methods.

Phytochemical Analysis.

The phytochemical screening was done to all the extract obtained from both of SWE and conventional extraction (maceration, hot water extraction and soxhlet). This analysis revealed that *physalis angulata* stem extract obtained from both SWE and conventional extraction contain phenol, flavanoid, tannin and alkaloid. Of all methods studied, the extract contains steroid except that of obtained from hot water extraction while saponin only found in extract obtained from SWE at 150°C. These phytochemical compounds are known to be responsible for the antioxidant activities of this plant extract used in the study.

Subcritical Water Extraction

The SWE experiment conducted to investigate the temperature, pressure and reaction time effect to the yield, total phenol, flavanoid content and antioxidant activity.

Effect of temperature and pressure

Temperature and pressure are two important variables in SWE. The effect of temperature and pressure were investigated to the extract yield, total phenol, total flavanoid and antioxidant activity. Figure 1 shows the yield of extract obtained by SWE of *physalis angulata* stem as a function of temperature and pressure. Here, the yield is defined as weight of dry extract per weight of dry feed. Yield is increased significantly as temperature. At 200 bar and 15 min extraction time, yield is increased from 19% to 29% when temperature is increased from 100 °C to 250 °C. Generally, more substances are extracted at elevated temperature because of enhancement of solubility. (Ozel et al., 2003)

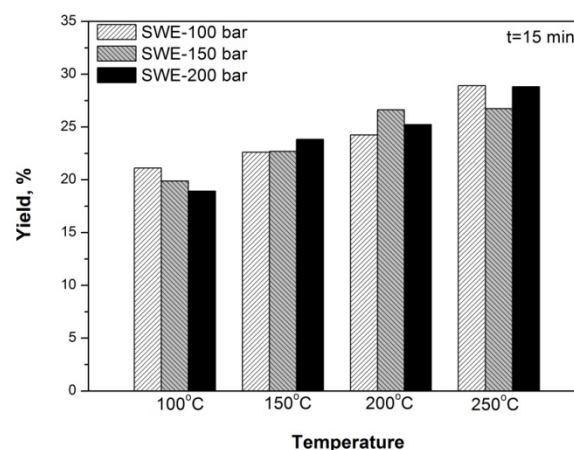


Figure 1. Yield of extract obtained from SWE of *physalis angulata* stem as a function of temperature and pressure at 15 min extraction time

Meanwhile pressure seems to have negligible effect to the extract yield. It is noted that the yield calculated here is based on crude extract only.

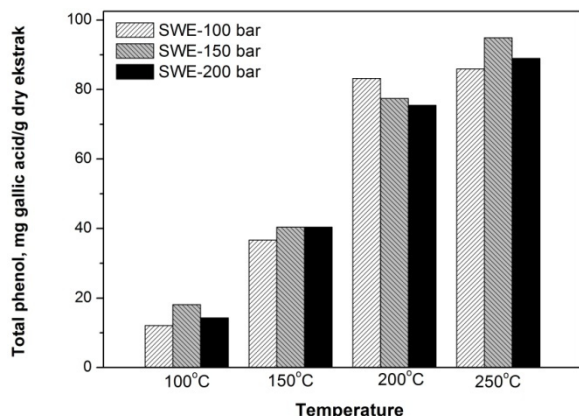


Figure 2. Total phenol analysis of extract obtained from SWE of physalis angulata stem as a function of temperature and pressure at 15 min extraction time

The temperature of 100°C-250°C were studied to investigate the effect of different solvent polarity to the total phenol, flavanoid and antioxidant activity in this plant. The result was summarized in figure 2-4. As yield, total phenol has upward tendencies to temperature. It is related with the polarity of solvent (water). By increasing temperature, the dielectric constant of water decreases from 55 to 27. (Ohmori, 2004) Solvent polarity is decreased, this is the reason for enhancement of total phenol yield due to an increase in solubility of phenolic compounds. The solubility of quercetin dihydrate for instance increase drastically around 1000 folds as as temperature increased from 25°C to 140°C.(Srinivas et al., 2010)

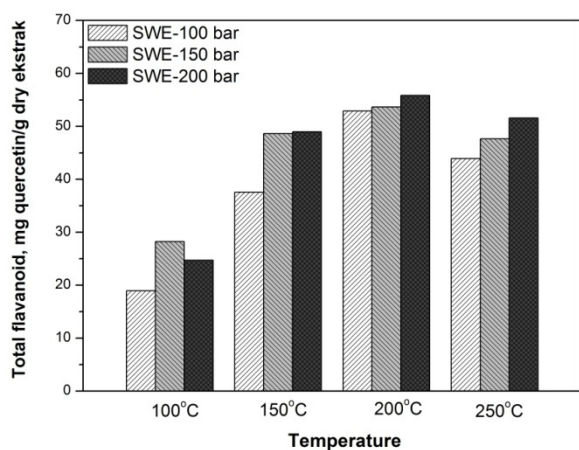


Figure 3. The total flavanoid of extract obtained from SWE of physalis angulata stem as a function of temperature and pressure at 15 min extraction time

As shown in fig 3, the extraction rate of flavanoid increased as temperature increased from 100 to 200 °C at all pressure range (100, 150 and 200 bar) for extraction time of 15 min. For instance the yield of flavanoid increases from 19 to 52 mg quercetin/dry extract as temperature increases from 100 °C to 200 °C, but decrease to 44 mg quercetin/dry extract as temperature increase further to 250 °C. The similar pattern was shown at pressure of 150 bar and 200 bar. The thermal instability may explain why a further increase in temperature can lead to a loss in flavanoid. (Cheigh et al., 2012) The effect of different temperature to the selectivity of flavanoid have been reported by several researchers. (Cheigh et al., 2012; Ko et al., 2010). An increase in temperature shows the same tendency to the antioxidant activity as shown in figure 4. The antioxidant activity was measured by DPPH assay and the results were reported as IC50. The lower the IC50, the stronger the antioxidant activity. The extract obtained at 200 and 250°C, have IC50 less than 100 ppm, it means they are categorized as strong antioxidant.

The effect of pressure to the total phenol, flavanoid and antioxidant activity were less pronounced. It might be due to the changing of pressure does not change the water polarity significantly. For instance, at 100 °C, as pressure increase from 100 bar to 200 bar, the dielectric constant increase slightly from 55.9 to 56.2.(Ohmori, 2004)

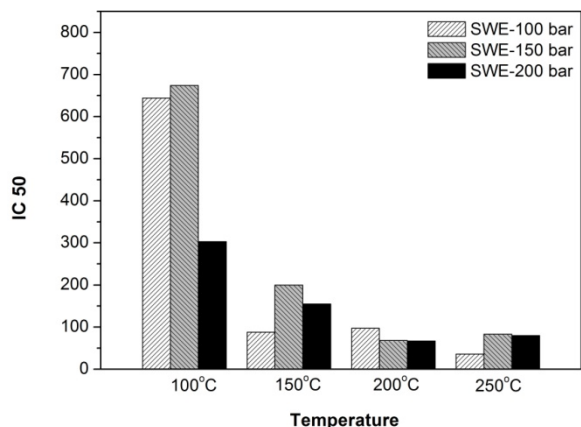


Figure 4. Antioxidant activity of extract obtained from SWE of physalis angulata stem as a function of temperature and pressure for reaction time of 15 min

Time effect

The effect of extraction time on the SWE of physalis angulata stem part was investigated at two different reaction time (15 and 30 min). The experiments were conducted at 100 bar and at temperature of 100-250°C. As shown in fig 5, the effect of extraction time were investigated to the extract yield, total phenol, flavanoid and antioxidant activity. Yield of crude extract is almost similar for both of reaction time, so is the total phenol yield. Meanwhile, longer reaction time are seems needed to complete the extraction of flavanoid, shown by increasing of

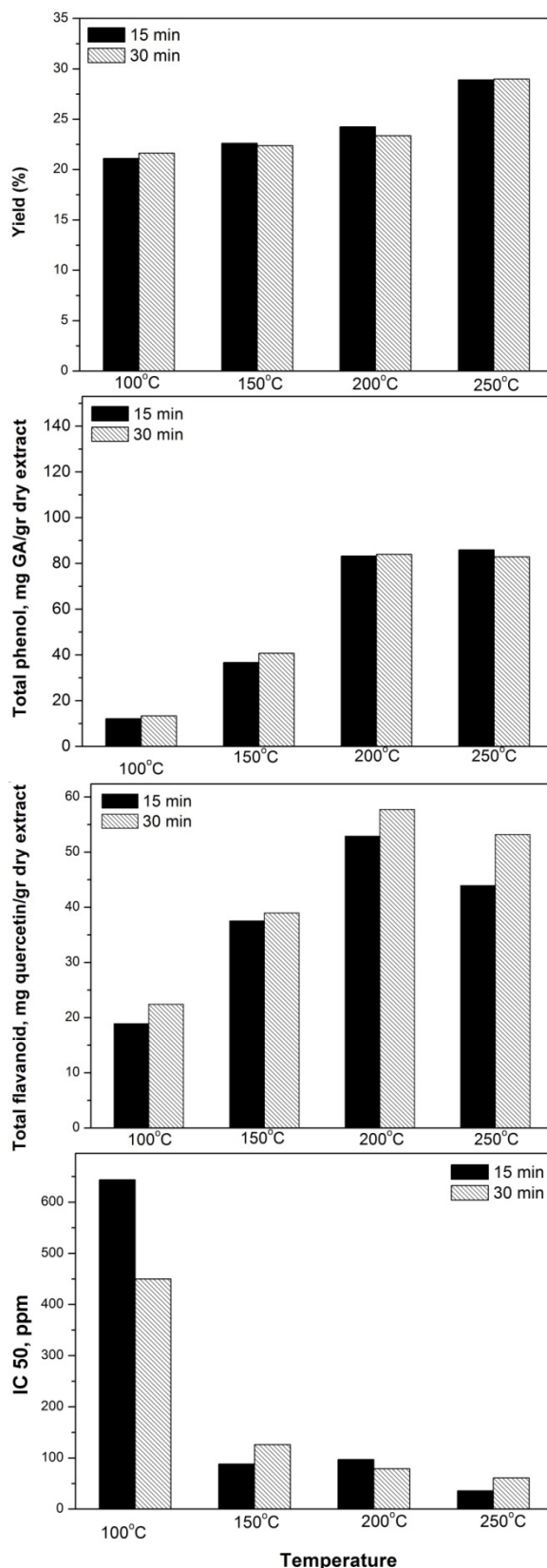


Figure 5. Effect of reaction time into the yield, total phenol, flavanoid and antioxidant activity of extract obtained from SWE of physalis angulata stem

total flavanoid yield by the time. In the case of antioxidant activity, longer reaction time seems contribute largely at low temperature of 100 °C while at higher temperature, the effect was less pronounced.

Comparison with Conventional Methods

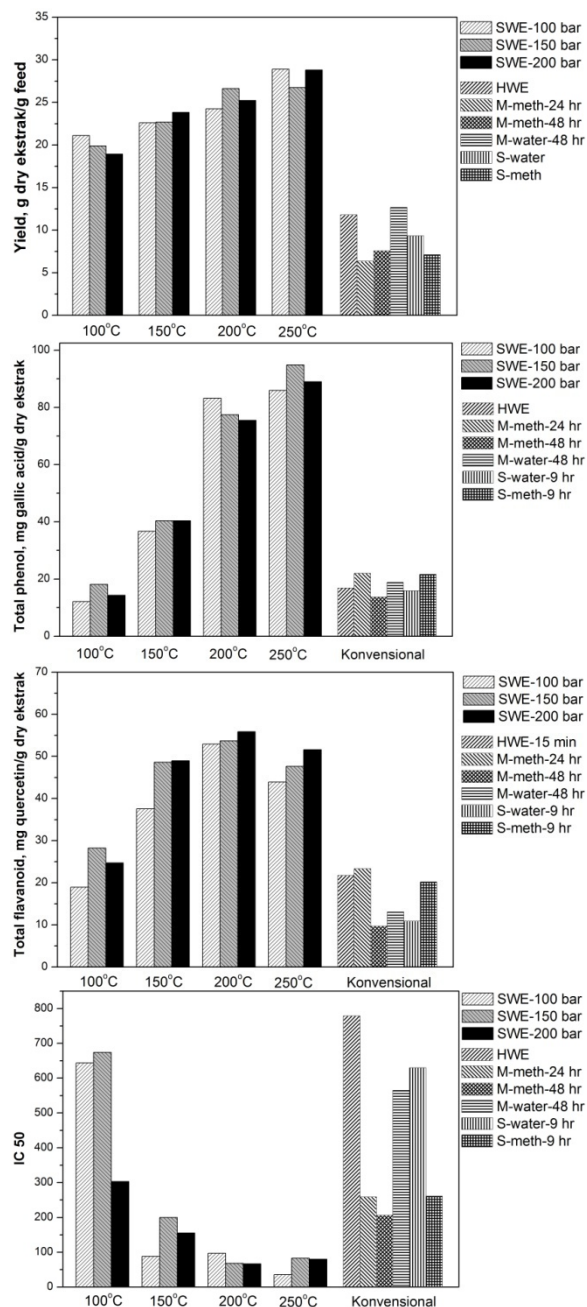


Figure 6. Comparison of yield, total phenol, flavanoid and antioxidant activity of extract obtained from SWE and conventional extraction

To prove the effectiveness of SWE, conventional extraction methods such as hot water extraction (HWE) which is mimic with traditional method to extract *physalis angulata* from ancient; maceration and soxhlet which are the common methods to extract *physalis angulata* currently were done and the result are presented in figure 6.

Extract yield, total phenol and flavanoid of *physalis angulata* stem extract obtained from SWE are shown much larger compare to that of obtained by other methods. Extract obtained from SWE at 200°C shows 2-3 folds better yield, 4-5 folds larger total phenol; 3-6 folds larger total flavanoid and 2-8 times stronger antioxidant activity compare to that of obtained by conventional methods. Among the conventional methods, maceration with water for 48 hr shows better result in term of yield, meanwhile maceration with methanol shows better in the term of total phenol, flavanoid and antioxidant activity.

CONCLUSION

Subcritical Water Extraction of *physalis angulata* stem part was proved to be more effective compare to other conventional methods such as hot water extraction (HWE), maceration and soxhlet in the term of extract yield, total phenol, total flavanoid and antioxidant activity. In SWE, the temperature contributed to the yield, total phenol, flavanoid and antioxidant activity significantly, neither did the pressure. The enough extraction time were needed to complete the extraction process. In addition, extract obtained from SWE at 200 and 250°C show strong antioxidant activity (IC₅₀ < 100 ppm).

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