Effect of extraction conditions on antioxidant activities of *Xanthium strumarium* L. sprouts extracts.

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Abstract. This research aims to obtain the *Xanthium strumarium* sprouts extract with the most potent antioxidant activities. The single-factor experiments were used to extract. Factors of extraction, including temperatures (40, 50, 60, and 70°C), time (6, 8, 10, 20, and 30 minutes), and % ethanol in solvent (40, 50, 60, and 70 % EtOH), were applied in the ultrasonic assist extraction of 40°C oven-dried *Xanthium strumarium* sprouts 1 gram in 25 mL of solvent. Extraction yield (% yield) and % inhibition of active radical DPPH and ABTS were determined, and the correlation to varied factors was analyzed using a statistical program (SPSS). The results showed that, at low temperatures, it would give a low % yield but not affect antioxidant activities. The longer extraction time did not provide a different % yield than the shorter one. The extraction yield is a little better when 50% ethanol is used in water as a solvent and does not affect antioxidant activities. As they observe, we can conclude that the antioxidant activities of extracts do not depend on temperature, time, or % EtOH in solvents. The % inhibition of ABTS radicals is higher than that of DPPH radicals in all experiments, and it can be relied on that the active components in this extract are more polar and favorable to solute in water than EtOH.

1 Introduction

Xanthium strumarium L. (Family Compositae), or cocklebur, is commonly found as a weed. It is widely distributed in North America, Brazil, China, India, Malaysia, and Thailand. In the Western region, cocklebur is a toxic weed, and animals such as horses, goats, pigs, and sheep die after eating large amounts of this young plant. But nowadays, it has been proved that the toxin in cotyledons or seed leaves will disappear after germination.[1] Farmers in Rayong, Thailand, grow the *Xanthium strumarium* sprout called "Pak Krachap" as a cooking vegetable. This herb is traditionally used for treating many diseases. Extracts of the whole plant, especially leaves, roots, fruit, and seeds, have been applied in traditional medicine.[2] A study reported yield, total phenolic content (TPC), and antioxidant activity of *X. strumarium* leaves extracts were affected by the extraction method, especially by the solvent used, and the best results were obtained with methanol and ethanol.[3] Methanol concentration and solid-to-solvent ratio significantly affected the TPC, DPPH, and FRAP values of *X. strumarium* fruit extracts.[4] Developing Pak Krachap into a more valuable product led us to study the condition of extracting *X. strumarium* sprout, which affects yield and its antioxidant activities. In this work, we do ultrasonic assist extraction with varying factors, including temperature, extraction time, and ethanol concentration in the solvent, and look for the significant effect of antioxidant activities.

2 Methodology

2.1 Plant Material:

Pak Krachap (*X. strumarium* L.) sprout was dried in the oven at 40°C and ground to finely powder. The obtained samples were stored at − 20 °C in a zip lock bag until further use. All chemicals used in this study are in analytical grade.

2.2 Ultrasonic-assisted extraction (UAE):

X. strumarium L. sprout was extracted using UAE with a static power of 215 W and a 35 kHz ultrasonic bath (DT-512 H, Bandelin, Germany). The extraction conditions vary in temperature (40, 50, 60, and 70°C), extraction time (6, 8, 10, 20, and 30 min), and solvent (40, 50, 60, and 70 % EtOH in water). The obtained extracts were filtered and evaporated to dryness and freeze-dried to remove a trace of the remaining solvent. The extraction yield was calculated with Equation 1.

$$\%yield = (g\ extract)/(g\ dried\ plant) \times 100 \qquad (1)$$

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2.3 *In vitro* Antioxidant assays:

The DPPH and ABTS assays were performed according to Wittenauer *et al*. [5] and Payet *et al*. [6] 's methods with some modifications.

2.3.1 Scavenging of DPPH radical: 150 µM DPPH stock solution was prepared by dissolving 3 mg of DPPH in 50 mL EtOH and stored at 8°C until needed. The working solution was diluted with EtOH until the absorption measured at 520 nm was equal to 0.70 ± 0.02. Assays were performed in 96-well flat-bottom microplates. Pipette 20 µL of extract (1 mg/mL in EtOH) or standard (0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mM Trolox in EtOH) or solvent for the blank to individual well, followed by 280 µL of DPPH working solution. The mixture was incubated in the dark at room temperature for 30 minutes, and the absorbance at 520 nm was measured with a microplate reader. The inhibition percentage of the radical scavenging activity was calculated using Equation 2.

$$\% \text{ inhibition} = \frac{|A_0 - A_1|}{A_0} \times 100 \quad (2)$$

Where A_0 is the absorbance of the blank, and A_1 is the absorbance of the sample at 520 nm. All assays were conducted in triplicate.

2.3.2 Scavenging of ABTS radical: $ABTS^{+\bullet}$ stock solution was prepared, dissolving 38.4 mg ABTS diammonium salt and 6.62 mg potassium persulfate in 10 mL water and stored at 8°C until needed. The final radical solution was prepared by diluting the stock solution with water until an initial absorption value (734 nm) of 0.70 ± 0.02 was reached. Assays were performed in 96-well flat-bottom microplates. Pipette 20 µL of extract(1 mg/mL in EtOH) or standard (0.01, 0.02, 0.04, 0.08, 0.16 and 0.32 mM Trolox in EtOH) or solvent for the blank to individual well, followed by 280 µL of ABTS working solution. The mixture was incubated in the dark at room temperature for 5 minutes, and the absorbance at 734 nm was measured with a microplate reader. The inhibition percentage of the radical scavenging activity was calculated using Equation 3.

$$\% \text{ inhibition} = \frac{|A_0 - A_1|}{A_0} \times 100 \quad (3)$$

Where A_0 is the absorbance of the blank, and A_1 is the absorbance of the sample at 734 nm. All assays were conducted in triplicate.

2.4 Statistical analysis:

The observed temperatures, extraction times, and % EtOH values were normal distribution values for statistical analysis. The data were a statistical analysis using one-way ANOVA. We examined the mean difference between the %yield and antioxidant activity (DPPH and ABTS) under the extraction conditions.

3. Results and discussions

The extraction factors of Pak Krachap (*Xanthium strumarium* L.) including temperatures, extraction time and %EtOH in solvent, were related to %yields and % inhibition of DPPH and ABTS radicals as shown in Figure 1 (A-C). We found that the temperature factor did not significantly affect the yields and % inhibition of DPPH and ABTS radicals ($p > 0.05$). This study recommended an extraction temperature of 60 °C due to its ability to extract high levels of yields and % inhibition of DPPH and ABTS radicals (Figure 1(A)). Moreover, the extraction time and %EtOH in solvent were chosen at 8 min and 50% EtOH in water, respectively. Those did not significantly affect the %yields and % inhibition of DPPH and ABTS radicals ($p > 0.05$), as shown in Figure 1 (B) and (C). However, the %yields and %inhibition of DPPH and ABTS radicals in each of the extraction factors were significantly different ($p < 0.05$), and the %inhibition of ABTS was significantly higher than the others ($p < 0.05$). The $ABTS^{+\bullet}$ is soluble in both aqueous and organic solvents, so it can react with both hydrophilic and lipophilic compounds and have the opportunity to react with antioxidants than with DPPH.[7]
Therefore, this study demonstrated that the extraction factors of Pak Krachap (*Xanthium strumarium* L.) were the preparation of 60°C temperatures, 8 min extraction time, and 50% of EtOH in a solvent.
As they observe, we can conclude that the antioxidant activities of extracts do not depend on temperature, time, or % EtOH in solvents. The % inhibition of ABTS radicals is higher than that of DPPH radicals in all experiments, and it can be relied on that the active components in this extract are more polar and favorable to solute in water than EtOH. Furthermore, the scavenging DPPH radicals activity of *X. strumarium* sprout extract (17.2- 31.8%) is lower than present in the report of the extract from fruit (71.4 -93.7 %)[5], in the same way as its detoxified. In other words, *X. strumarium* sprout extract is less active in antioxidant assay but is safer to consume.

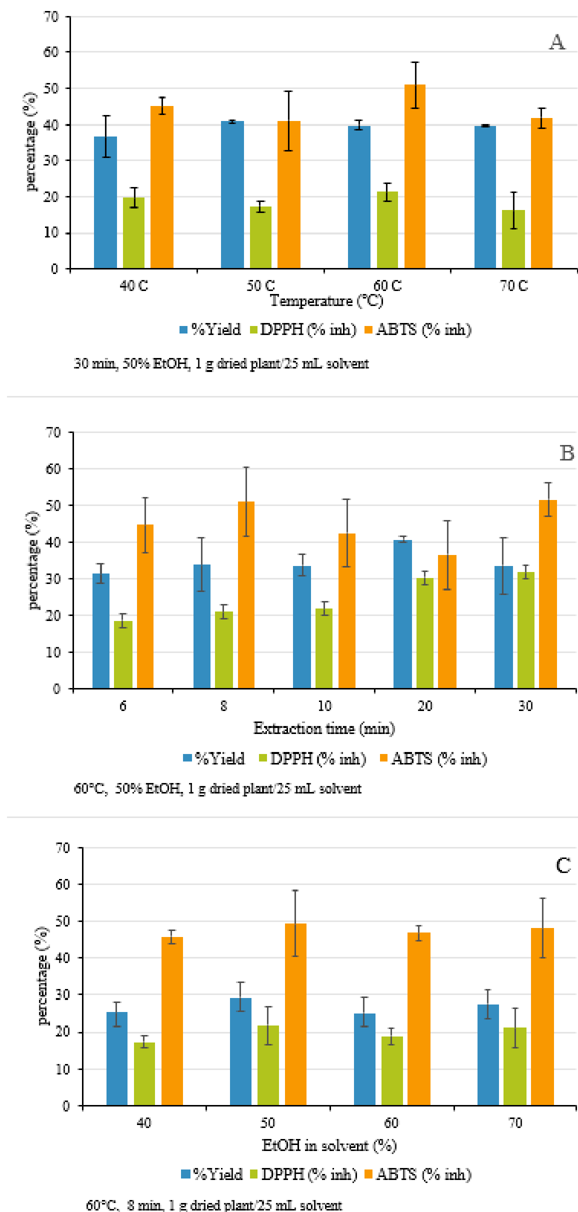

Fig. 1. Response from the extraction, which varies Temperature (A), Extraction time (B), and % EtOH in solvent (C).

4. Conclusion

After varying the single- factor extraction of *X. strumarium* sprout, we discovered that extracts' antioxidant activities do not depend on temperature, time, or % EtOH in solvents. All the tests show that ABTS radicals are more strongly blocked than DPPH radicals. This suggests that the active ingredients in this extract are more polar and dissolve better in water than EtOH. The study found that the best conditions for extracting Pak Krachap (*Xanthium strumarium* L.) were a temperature of 60 °C, an extraction time of 8 minutes, and 50% EtOH as a solvent, since it could get high %yields, % inhibition of DPPH and ABTS radicals from working.

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