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ULTRASOUND-ASSISTED EXTRACTION OF PHENOLIC COMPOUNDS FROM BANANA (*MUSA BALBISIANA)* SEEDS

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Abstract: *The aim of this study is to determine the influence of factors such as types of solvents (acetone, ethanol, methanol and distilled water), material/solvent ratio (1/30-1/70), solvent concentration (30%-70%, v/v), time (10-30 minutes) and temperature extraction (30°C-70°C) which affect total polyphenol content (TPC) and antioxidant capacity (AC) of banana seed extract. The optimal conditions for the extraction process were aqueous acetone concentration of 40%, material/solvent ratio of 1/50, extraction temperature of 50°C in 15 minutes. The TPC and AC obtained were approximatively 45.41 mg GAE/g DW and 167.10 µmol TE/g DW, respectively. The effect of ultrasound-assisted extraction (UAE) on the structure of the materials of banana seed was observed by scanning with the electron microscopy (SEM).*

Key words: *Antioxidant, banana seed, extraction, solvent, ultrasound.*

1. Introduction

Musa balbisiana is a species of wild banana native to eastern South Asia, northern Southeast Asia, and southern China. It is one of the ancestors of modern cultivated bananas, along with *Musa acuminata*. In India, some parts of *Musa balbisiana* were used as food or traditional medicine, for instance leaf, fruit, ash of fruit bark, ash of dried peel which may prevent some diseases as pinworm infection, cough, infertility in women, jaundice, gout and gastritis [2]. However, seed has not been used but it was widely known for its ability to cure kidney stones in Vietnam. It also cures many other symptoms such as back pain, joint aches, joint pain, menstrual hypertrophy, hypertension, etc. The healing power of banana seeds is due to the biologically active compounds, especially polyphenol. Polyphenols are antioxidants that can prevent many diseases and commonly found in many part of plants such as pomegranates peels [22], black tea [20], wheat bran [28], *Polygonum multiflorum* Thunb. root [12] etc.

Currently, polyphenol extraction attracted the attention of scientists. Many methods can be used for extracting these compounds from plants, for instance accelerated solvent extraction, convectional extraction, microwave-assisted extraction (MAE), supercritical fluid extraction and ultrasound-assisted

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extraction (UAE) [21]. Among these, UAE is also a new method, high effect and the lowest instrumental requirements. The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the phenomenon of cavitation produced in the solvent by the passage of an ultrasonic wave [13]. However, according to [16], the various extraction methods of each material must be modified and optimized.

Until now, no studies have been carried out on UEA method for the extraction of polyphenol from banana seeds. Hence, the objective of the study was to evaluate the effect of the type of solvents, solvent concentrations, material/solvent ratios as well as temperature and extraction time on total phenolic content and antioxidant capacity from banana seeds. In addition, the structure of materials was observed by a scanning electron microscope (SEM).

2. Materials and Methods

2.1. Sample Preparation

Banana seeds (*Musa balbisiana*) were collected from the Vung Tau province (Vietnam). Fresh seeds were separated from bananas (no diseased or physically compromised fruits) and dried by sun until moisture was under 14%. The dried samples were ground into a fine powder (<0.5 mm), packaged in vacuum conditions and stored at room temperature for further use.

2.2. Chemicals and Reagents

The Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) reagent was purchased from Sigma-Aldrich (USA). DPPH (2.2-diphenyl-1-picrylhydrazyl) and the Folin–Ciocalteu reagent was purchased from Merck (Germany). All organic solvents and other chemicals were of analytical reagent grade.

2.3. Extraction Process

Dried sample was extracted with various solvents (distilled water, ethanol, methanol and acetone) in an ultrasonic bath (ELMA–S60H type, 37 kHz, 550W, Germany) for different times (10-30 minutes) at required temperatures (30-70^{o}C), the material/solvent ratio (1/30-1/70, v/w) and solvent concentration (30%-70%, v/v). The mixture was filtered for removal of residue by means of the vacuum filtration system, and then TPC and AC were analyzed. Water extraction samples were control samples for the evaluation of all experiments

2.4. Determination of Total Polyphenol Content (TPC)

The TPC was determined and slightly modified by the Folin-Ciocalteu method with some slight modifications. Gallic acid was used as the standard. TPC were expressed as mg of gallic acid equivalents per g of dry weight (mg GAE/g DW) [15].

2.5. Determination of Antioxidant Capacity (AC)

The AC was determined by DPPH assay which was described by [17], with some slight modifications. Trolox was used as the standard. AC was expressed in TEAC (Trolox equivalent antioxidant capacity) determined as µmol of Trolox per g of dry weight (µmol TE/g DW).

2.6. Scanning Electron Micrographs (SEM)

Using the scanning electron microscope system (Jeol JSM-7401F, USA) the

morphological alterations of materials and residues after extraction were examined.

2.7. Data Analysis

All assay results were performed in triplicates, the obtained values were expressed in the form of mean±standard deviation (SD) and analyzed by the Statgraphics software (Centurion XV). The one-way analysis of variance (ANOVA) at $p<0.05$ was used to determine significant differences between the means.

3. Results and Discussions

3.1. The effect of Solvent on the TPC and AC of Extract

Dried samples were extracted with distilled water and solvent concentration of 50% (v/v) (methanol, ethanol, acetone) under the same extraction conditions as the material/solvent ratio of 1/50, 50°C and 20 minutes. Water extraction samples were control samples for the evaluation of the following experiments. Figure 1 shows that all samples displayed significant differences ($p<0.05$). The use of 50% acetone as solvent has the TPC and AC values obtained were 42.43±0.71 mg GAE/g DW and 165.74±4.05 µmol TE/g DW, respectively. Extracting by distilled water has the worst results (TPC: 15.09±0.42 mg GAE/g DW; AC: 4.16±1.88 µmol TE/g DW). In this case, polyphenol in materials consist of average polarity compounds and quite differently dissolve in the highest polarity solvent namely water. In addition, water can dissolve some impurities for instance soluble protein, organic acids and sugars) which strongly affect the obtained results [7].

Besides, the yield of polyphenol also depends on many factors such as viscosity, dimension of particles, molecular structure of molecular [25] or the presence of enzyme polyphenol oxidase in extracts [9] which are active and degrade phenolic compounds. Hence, the choice of solvents is very important for extracting the bioactive compounds from the plant. When using aqueous acetone as solvent the result was similar with studies conducted by [10], that extracted polyphenol from soybean or the study of [12] who extracted epolyphenol from the *Polygonum multiflorum* Thunb root etc.

Based on the above results, an aqueous acetone concentration of 50% was chosen for further experiments.

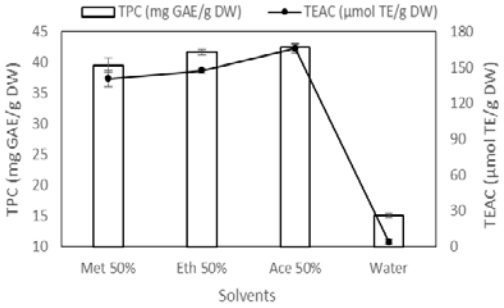

Fig. 1. *TPC and AC of extracts at various solvents*

3.2. Effect of material/solvent ratio on the TPC and AC of extract

The effect of the material/solvent ratios on TPC and AC is shown in Fig. 2. TPC and AC at various material/solvent ratios have significant differences ($p<0.05$). The material/solvent ratio of 1/50 was the optimal result, the TPC and AC of the extract obtained the best values with 41.84±1.21 mg GAE/g DW and 167.78±4.54 µmol TE/g DW, this result was higher than the control sample. The decrease of the amount of solvent caused difficulties in the extraction process. Conversely, a considerable amount of solvent may promote the phenolic compounds that diffuse easily into the solvent [3]. However, these components increase rapidly once equilibrium is

reached [6]. This is evident from the material/solvent ratio of 1/30 to 1/60, the increase of acetone volume increases the TPC and AC. In addition, increasing the solvent in this case is unnecessary because of the increase of cost, time and energy to search for a volume solvent, while the prolonged pursuit of a high temperature solvent would significantly reduce polyphenol antioxidant activity. From a material/solvent ratio of 1/60 to 1/70, the amount of acetone increased whereas the TPC and AC of the extract decreased.

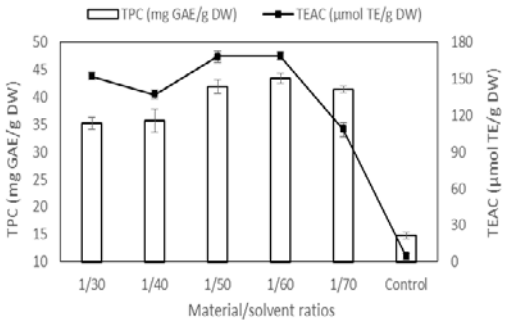

Fig. 2. *TPC and AC of extracts at various material/solvent ratios*

Determining the suitable material/solvent ratios for extraction to achieve the highest economic efficiency is quite necessary and important, to ensure no shortage, and wastage that may occur when using less or too much extracting solvent. Besides, changes of material/solvent ratios also depend on the extraction method and initial material. For instance [5]) extracted phenolic compounds from *Pistacia lentiscus* L. leaves with ethanol as solvent by three extraction methods. The optimal material/solvent ratios were 1/50 (ultrasound-assisted extraction and traditional solvent extraction method) and 1/30 (microwave-assisted extraction) [5].

Based on the above results, the material/solvent ratio of 1/50 was chosen for the evaluation of the next steps.

3.3. The effect of Acetone Concentrations on the TPC and AC of the Extract

The extraction of phenolic compounds from plant material is directly related to the compatibility of the phenolic compounds to the solvent and thus, when the compounds are well matched in polarity with the solvent they will be easily extracted. The effect of the solvent concentrations on TPC and AC is shown in Fig. 3 and have a significant difference ($p<0.05$). The best results peak at an acetone concentration of 40%, TPC value is 43.28±0.82 mg GAE/g DW and AC is 162.98±3.66 µmol TE/g DW. In the AC of acetone concentrations of 40% and 50% there was no statistically significant difference whereas the TPC of acetone concentrations of 40% was higher than that of acetone concentrations of 50% (approximately 10%).

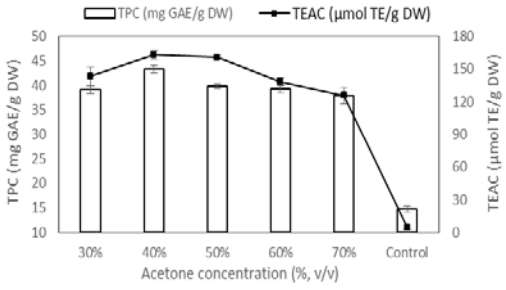

Fig. 3. *TPC and AC of extracts at various solvent concentrations*

During the extraction process, changes in TPC and TEAC may not correspond because of the number of the hydroxyl group, the length of the hydrocarbon branch and the molecular size of a phenolic compound that affect the solubility in the solvent. Acetone is a low-polar solvent while water is a strong polar solvent, and they can dissolve each other in any proportion. Hence, adding water into

an acetone solution leads to the increase of the polarity of the solvent [8]. The TPC of polar phenolic compounds in banana seeds increase with the increase of the water content according to the "like dissolves like" principle [24]. Besides, the yield of the extraction process may depend on the swelling of cell plant by water, which increases the surface area of contact between the plant matrix and the solvent [18]. However, the large amounts of water can dissolve many different organic compounds such as sugar, protein, etc. which affects the accuracy of measuring the TPC and AC. Accordingly, the suitable acetone concentration for the next experiment was 40%.

3.4. The effect of Extraction Temperature on the TPC and AC of the Extract

Fig. 4 shows that the TPC and AC of various extraction temperatures have significant differences ($p<0.05$). The yield of the extraction process reached the highest value at 50ºC, TPC and AC were 45.28±0.99 mg GAE/g DW and 168.85±5.75 µmol TE/g DW, respectively. As the extraction temperature increases from 30ºC to 50ºC, the values of TPC and AC increase rapidly. The extraction temperature increases with the increased diffusion of phenolic compounds and decreasing viscosity of the solvent [23]. In addition, it may open the cell matrix and release phenolic compounds [26]. However, the TPC and AC decline rapidly because they are quite sensitive to the with heat treatment applied in this study and some phenolic compounds were destroyed by high temperature extraction (>50ºC).

In addition, high temperatures lead to the loss of solvent through evaporation thereby increasing the cost of extraction from the point of view on industrialization. Furthermore, rising temperatures also lead to excessive consumption of energy and the bioactive compound may change or be oxidized [19], [25] whereas low extraction temperatures can not be achieved an effective extraction as desired. Extraction temperature depends on many factors such as initial material or extraction methods. For instance, the optimal extraction temperature in this study was 50ºC whereas that of the study conducted by [14] was from 64ºC to 70ºC, they used UAE for extracting phenolic compounds from *Artemisia absinthium* or [10] used the maceration method for extracting phenolic compounds from soy beans, the optimal extraction temperature being 40ºC [10]. Based on the achieved results, the optimal extraction temperature was 50ºC.

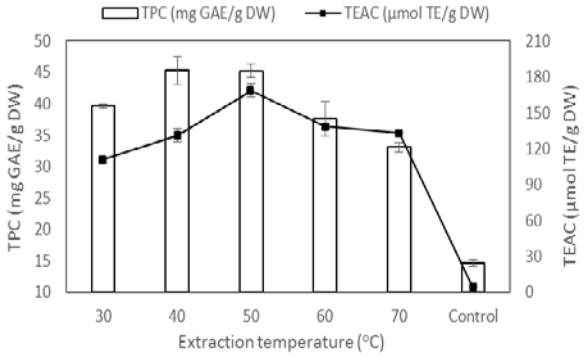

Fig. 4. *TPC and AC of extracts at various extraction temperatures*

3.5. The effect of Extraction Time on the TPC and AC of the Extract

Based on Fig. 5, all samples have a significant difference ($p<0.05$). The best TPC and AC values reached after an extraction time of 15 minutes were 45.41±0.77 mg GAE/g DW and 167.10±2.07 µmol TE/g DW, respectively. The difference of the TPC and AC values of the extraction times of 15 and 20 minutes, were negligible.

The TPC and AC value increase with the increase of extraction time from 10 to 15 minutes, remain from at 15 to 20 minute and then drop steadily from 20 to 30 minutes. These phenomena can be accurately explained explicitly by Fick's second law of diffusion, the final balance between the solute concentration in solids (plant cells) achieved after a certain time. Excessive extraction time is not useful to extract more bioactive compounds, especially polyphenols [16]. Furthermore, the extraction time increases with increasing the phenolic degradation and oxidation due to prolonged exposure to unfavorable environmental factors such as temperature, light and oxygen [11]. Therefore, determining extraction time is very important in the extraction of phenolic compounds and also depends on initial material, extraction methods, component of phenolic compounds, etc. For instance, the study of [14] used UAE for extracting phenolic compounds from *Artemisia absinthium*, the best extraction temperature was from 101 to 107 minutes whereas [12] used MAE for extracting phenolic compounds from *Polygonum multiflorum* Thunb. root, the optimal extraction temperature was 5 minutes. Based on the obtained results, the optimal extraction temperature was 15 minutes for this study.

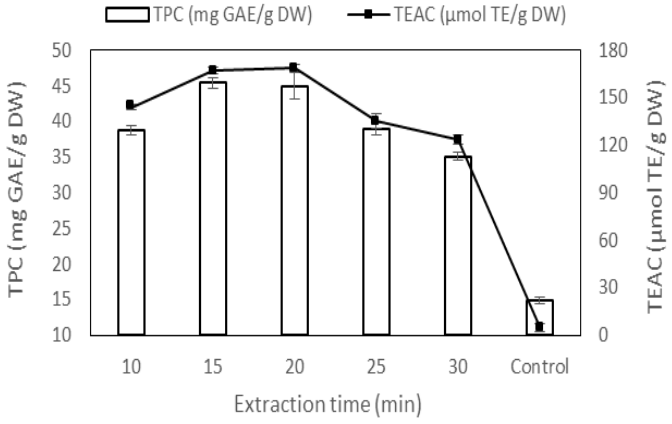

Fig. 5. *TPC and AC of extracts at various extraction times*

3.6. Scanning Electron Micrographs (SEM)

Figure 6 and 7 show the effect of the ultrasonic method on the physical structure of fine powder of banana seeds. Surface morphology of the initial material changes greatly strongly after 15 minutes with the UAE.

The shape of the fine powder before treatment UEA is round, smooth and has different diameters ranging between 20-25 µm. The structure of the material is intact and there is no cohesion between the particles. After treatment with UAE, the

structure of particles is broken and they are attached to each other with many cracks on the surface. This suggests that the ultrasound can increase the breakdown of plant tissue, causing expansion in plant cell walls, diffusion, and increased volume transfer. Because thin cell walls can not withstand the localized pressure and high temperatures created by cavitation bubbles. However, these changes in plant cell might allow the solvent to enter the cellular channels easily [1]. This result is similar to the study of [26] that extracted polyphenol from *Semen Astragali Complanati* seed by means of UAE or [4] who also extracted carvone and limonene from caraway seeds using UAE.

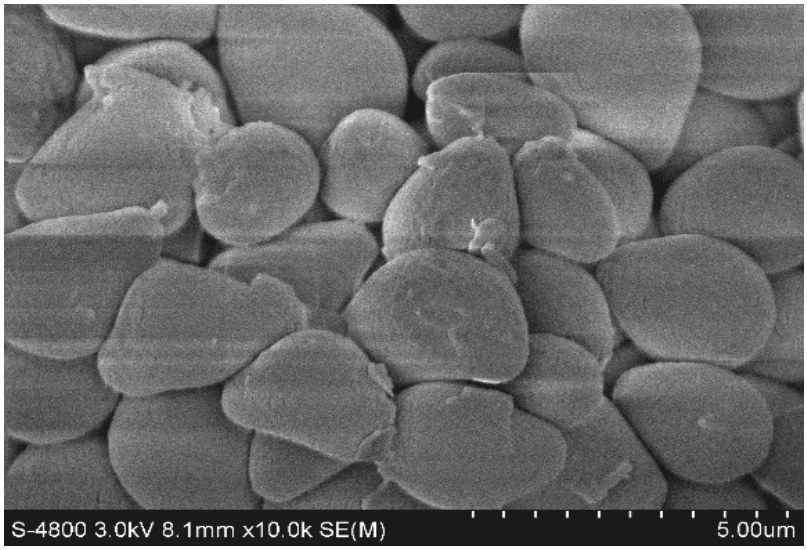

Fig. 6. The *structure of the material before treatment with UAE*

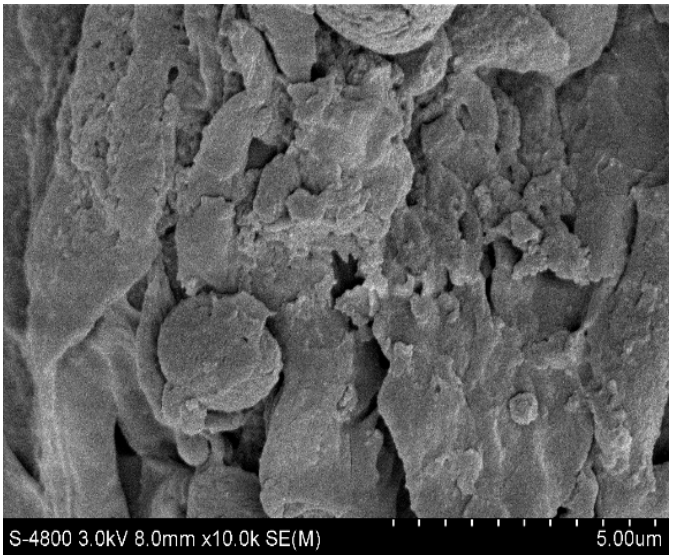

Fig. 7. *Structure of material after treatment with UAE*

4. Conclusions

The result obtained showed that acetone was the optimal solvent for the phenolic compounds with ultrasound-assisted extraction from banana seed. The highest TPC and AC in the extract peaked approximately at 45.41 mg GAE/g DW and 167.10 μmol TE/g DW, respectively. The optimal conditions were the aqueous acetone concentration of 40%, material/solvent ratio of 1/50, extraction time of 15 minutes and extraction temperature of 50ºC. The cells of the material were destroyed by UAE, had wrinkles on their surface and the structure changed greatly.

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