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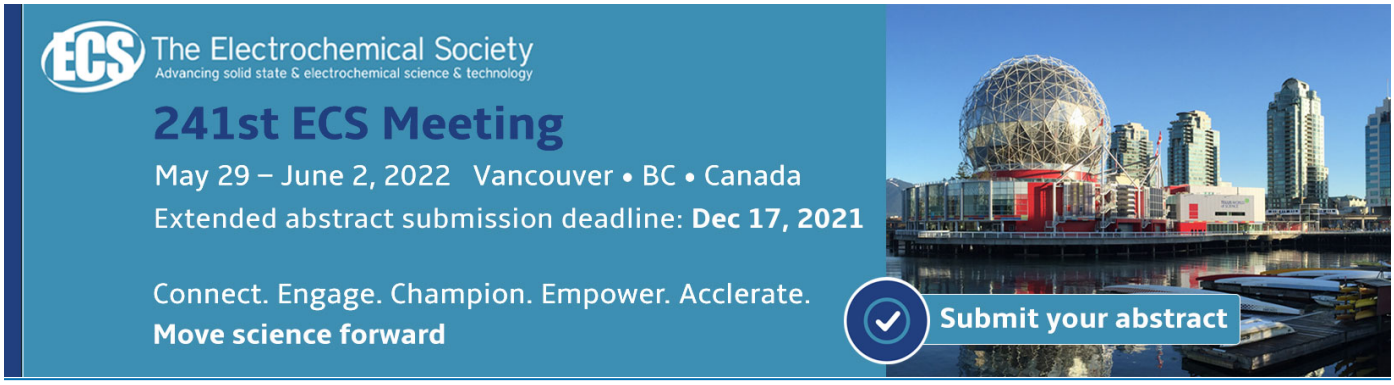


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# Comparison of Ultrasound Assisted Extraction and Conventional Extraction Technique on Recovery of Phenolic and Flavonoid Compounds from Aloe *barbadensis* Miller

**WHW Osman, NL Badri, AT Chong, M Mohamad, and AZ Sulaiman**

Faculty of Bioengineering and Technology, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia

Email: hasnidah.osman@umk.edu.my

**Abstract.** Aloe *barbadensis* Miller (Aloe vera) is useful for skin care and its bioactive compounds could replace the insecure chemical compounds that used in cosmeceutical application. This study was designed to evaluate the effect of solvents and ultrasound assisted extraction (UAE) parameters such as duty cycle and sonication time on the concentration of phenolic and flavonoid compounds from Aloe vera. The extraction efficiencies of the best condition of UAE were compared with that of conventional extraction technique. The Aloe vera gel sample was used in this study. The total phenolic and flavonoid compounds were determined by using UV-Vis spectrophotometer. The result shows that extraction solvents significantly affect extraction yield of phenolic and flavonoid compounds, and it was found that ethanol to be the most suitable solvent. The best condition of UAE was duty cycle of 50% and 40 min of sonication time. The sonication time with the UAE was 40 min, which was six-times shorter than with the conventional extraction technique. The highest concentrations of total phenolic and flavonoid compounds using UAE were found to be 11.41±0.04 and 6.76±0.05 mg/mL, respectively. The UAE was found to be more efficient in extraction of phenolic and flavonoid in comparison with conventional extraction technique.

## 1. Introduction

Aloe *barbadensis* Miller (Aloe vera) is a historical plant that have been used for several application due to its useful advantages affected on the health of population. It consists of three layers which are the outer grind, middle yellowish fluid and the inner transparent mucilage gel. Aloe vera secretes two types of fluid containing proteins and cellular elements. One is a yellowish thick bitter fluid as the presence of aloin that secreted from the pericyclic cells of the plant. The other is a transparent mucilage gel generated by tubular cells located in the central parenchyma zone of the leaf [1].

Aloe vera consists of several bioactive compounds such as phenolic and flavonoid compounds with potential biological and toxicological activities [2]. The natural phenolic and flavonoid compounds are plant secondary metabolites that hold an aromatic ring includes at least one hydroxyl group [3]. Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action. Phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burdens according to previous report [3]. As for Flavonoids, they exhibit many pharmacological activities such as antioxidants, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [4]. Ultraviolet-visible (UV–vis) detection applied for determination of phenolic and flavonoid compounds [5]. Therefore, Aloe vera is often used for the production of cosmetics, drugs and food application [6].

The conventional extraction technique, Soxhlet extraction, has been used for decades for many different purposes. Soxhlet extraction also known as the universal chemical extraction. Several researchers usually used this technique for extracting major and minor compounds [7]. Ultrasound assisted extraction (UAE) has been recognised as modern extraction technique for the phyto-

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pharmaceutical extraction industry for a wide range of herbal extracts [8]. UAE has been reported to be used to increase the extraction yield by disrupting cell tissues [9]. UAE required a moderate use of solvents and energy. In addition, it is easy to handle, safe, economical and reproducible due to the fact that this technology enables its development under atmospheric pressure conditions and at ambient temperatures [10].

To the best of our knowledge, there have been no previous studies on the effect of solvent and ultrasound assisted extraction parameters on extraction yield of phenolic and flavonoid compound of Aloe vera gel; and comparison between modern and conventional extraction techniques for Aloe vera gel. Thus, the aim of this study is to extract the total phenolic and flavonoid compounds from Aloe vera gel using UAE and compare the obtained data with the conventional extraction technique.

## 2. Materials and Methods

### *2.1 Chemicals*

Standard of quercetin was purchased from Sigma-Aldrich Co. Gallic acid, Folin-Ciocalteu's reagent, and Ferric chloride were purchased from R & M Chemicals. Aluminium chloride was purchased from HmBG.

### *2.2. Preparation of Plant Material*

Aloe vera leaves were collected from Universiti Malaysia Kelantan, Jeli Campus. Matured and fresh Aloe vera plant was selected to be harvest. Aloe vera leaves were rinsed with tap water to remove excess soil. The tapering point at the top and the serrated edges were removed from the leaves using sharp cutting blade. The leaves were cut to separate out the outer green rind and inner gel was scraped out. The inner gel of Aloe vera which was the colourless mucilaginous paranchymatous tissue was collected and cut into small pieces. The sample was dried at 50℃ for 24 hours in the oven to devoid of moisture. The sample was grind using heavy duty blender. The sample for UAE was sieved using sieving machine. Average particle sizes of approximately in the range of 355 to 500 μm was used for UAE. Then, the sample was stored in an air-tight clean container.

### *2.3. Ultrasound Assisted Extraction (UAE)*

The extraction was performed using ultrasound homogenizer (Scientz Biotech, Shanghai, China) with the 15 mm ultrasonic probe. The value of operating parameters of UAE are summarized in Table 1. The 200 mL of solvents either ethanol or water were added to the 5g of ground Aloe vera gel sample. The temperature of the UAE was set to be at 50℃ for water and 40℃ for ethanol. The sonication was applied in continuous mode at frequency of 20 kHz and the power of ultrasonic probe of 900W. The probe was directly immersed in about half of the total solvent that contained sample.

**Table 1.** Operating Parameters of UAE

| Parameters | Values |
|---|---|
| Probe tip diameter (mm) | 15 |
| Operating temperature (℃) | 50 (water); 40 (ethanol) |
| Operating frequency (kHz) | 20 |
| Ultrasonic power (W) | 900 |
| Duty cycle (%) | 25-75 |
| Sonication time (min) | 20-60 |

The extraction was performed at different duty cycles which were 25, 50 and 75% and the sonication time was set to be fixed for 40 min. Next, it was further performed at different sonication times of 20, 40, and 60 min and the duty cycle were set to be fixed at 50%. The extraction was performed in triplicate

for each parameter. Then, the extracts were centrifuged at 5800 rpm for 15 minutes at room temperature. Supernatant was removed for the further analysis.

### *2.4. Conventional Extraction*

The conventional extraction was performed using Soxhlet extractor. The 300 ml of solvents either ethanol or water was added to the 40g of ground Aloe vera gel sample. The extraction was performed at 78°C for 4 hours. The obtained extracts were filtered using qualitative filter paper. The excess solvents were removed by using a rotary evaporator.

### *2.5. Phytochemical Screening of Aloe Vera Gel Extracts*

Phytochemical constituents of Aloe vera gel were determined by different qualitative tests such as carbohydrates, steroids, anthraquinones, and tannins. In the test for carbohydrates, 3 mL of aqueous extract was added to 2 mL of Molisch's reagent and the resulting mixture was shake properly. Then, the 2 mL of $H_2SO_4$ was poured carefully to the bottom of the test tube. In the test for steroids, 2 mL of the aqueous extract was dissolved in the 2 mL of $CHCl_3$. Then, 2 mL $H_2SO_4$ was added in the test tube. In the test for anthraquinones, 3 mL of aqueous extract was mixed together with 3 mL of benzene, and then the mixture will be filtered. Then, the 5 mL of 10% $NH_4OH$ was added to the filtrate and the mixture was shake properly. In the test for tannins, 2 mL of the aqueous extract was mixed together with 2 mL of $H_2O$. Then, a few drops of 5% $FeCl_3$ solution was added to the mixture.

### *2.6. Determination of Total Phenolic Compound*

The total phenolic compound of the extract was determined by using Folin-Ciocalteu reagent [11]. 0.2 mL of Aloe vera gel extract was mixed with 0.6 mL of water and 0.2 mL of tenfold Folin–Ciocalteu reagent. 1 mL of 7.5% $Na_2CO_3$ was added to the mixture after 5 min. Then, the mixture was made up to 3 mL with distilled water. The mixture was then kept in the dark condition for 30 min. The absorbance of the standard and sample were measured at 765 nm using UV-Vis spectrophotometer (Shimadzu, USA). The concentration of total phenolic compound was calculated on the basis of a standard curve of gallic acid.

### *2.7. Determination of Total Flavonoid Compound*

The total flavonoid compound of the extract was determined by using the aluminium chloride colorimetric method [11]. 20 mg of quercetin as standard was dissolved in 20 mL of methanol. 0.6 mL diluted standard quercetin solution and the extract were separately mixed with 0.6 mL of 2% $AlCl_3$. The mixture was incubated for 60 minutes at room temperature. The absorbance of the standard and sample were measured at 420 nm using UV-Vis spectrophotometer (Shimadzu, USA). The concentration of total flavonoid compound was calculated on the basis of a standard curve of quercetin.

## 3. Results and Discussion

### *3.1. Phytochemical Screening*

The phytochemical screening of the Aloe vera gel (Table 2) showed the presence of carbohydrates, steroids, tannins, and the absence of anthraquinones. Carbohydrates was presence as violet ring was formed. Steroids was presence as there was a steroidal layer formed. Tannins was presence as green precipitate formed. There was a negative result of free anthraquinones in both Aloe vera extract from Soxhlet Extraction and UAE since the absence of pink, red or violet colour in the bottom phase. Anthraquinones are mostly found in the outer green rind and yellow latex in Aloe vera plant.

The biological activities of carbohydrates included reduces radiation-induced skin reactions, and antibacterial [12] Steroids possess photoprotective and anti-wrinkle efficacy owing to cell division encouragement ability [13]. Tannins are one of the polyphenols components with the function of antioxidant, antibacterial and anti-inflammatory action [14]. Similar results were found in the previous

study conducted by [4]. It is noted that Aloe vera is rich in secondary metabolites. It possesses a broad range of bioactivities such as anti-inflammatory, antimicrobial and antioxidant activities [15].

**Table 2.** Results of phytochemical test

| Phytochemicals | Ethanol | | Water | |
|---|---|---|---|---|
| | UAE | Conventional | UAE | Conventional |
| Carbohydrates | ++ | + | ++ | ++ |
| Steroids | + | + | + | + |
| Anthraquinones | – | – | – | – |
| Tannins | + | ++ | + | ++ |

++, highly present; +, slightly present; –, absent

### *3.2. Effect of Type of Solvent*

Figure 1 shows the effect of type of solvents on the extraction of total phenolic and flavonoid compounds from Aloe vera gel. It was indicated that total phenolic and flavonoid compounds using ethanol were higher than water. The concentration of total phenolic and flavonoid compounds using ethanol as solvent were found to be 11.41±0.04 and 6.76±0.05 mg/mL, respectively. The concentration of total phenolic compound using ethanol as the solvent was found to be similar with that the water used as the solvent. The concentration of total flavonoid compound using ethanol as the solvent was four-times higher than that of the water used as the solvent. By considering that ethanol is environmental friendly and relatively less toxic for human health [16] ethanol was the most suitable solvent and had been chosen as the preferred solvent for extraction in this study.

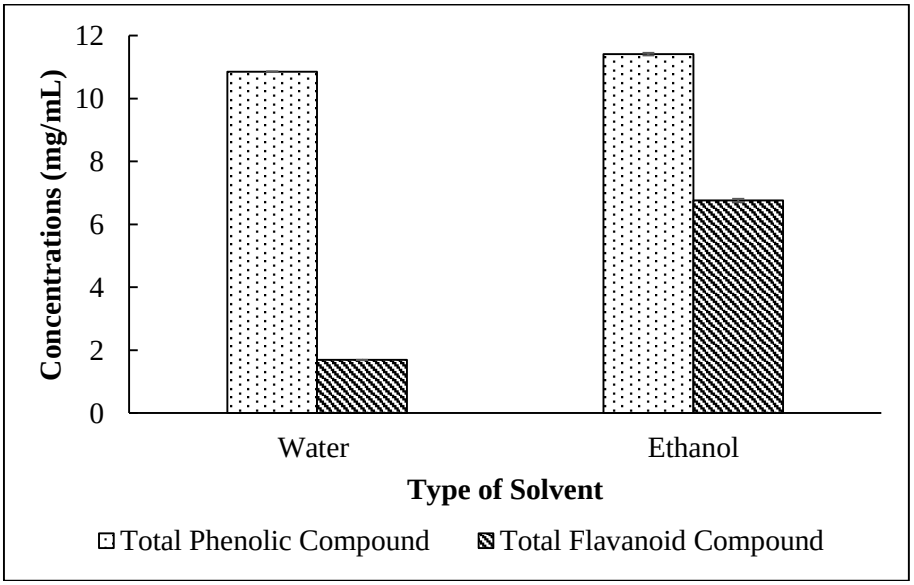


**Figure 1.** The effect of type of solvent on the extraction of total phenolic and flavonoid compounds.

### *3.3. Effect of Duty Cycle*

The effect of duty cycle of UAE to the concentrations of total phenolic and flavonoid compounds from Aloe vera gel is shown in Figure 2. In this study, three different duty cycles were selected to be 25, 50, 75%. The highest concentration of total phenolic compound and flavonoid were found at the duty cycle of 50%. The concentrations of total phenolic and flavonoid compounds were to be the 11.41±0.04 and 6.76±0.05 mg/mL, respectively. Thus, the duty cycle of 50% was the most effective in producing phenolic and flavonoid compounds from Aloe vera gel and the preferred one for extraction in this study.

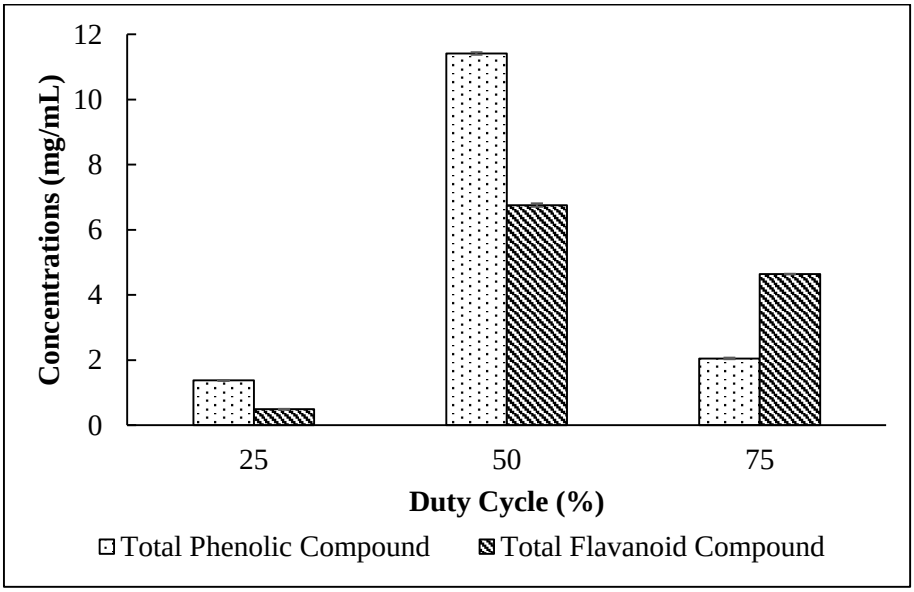


**Figure 2.** The effect of duty cycle on the extraction of total phenolic and flavonoid compounds.

*3.4. Effect of Sonication Time*

The effect of sonication time of UAE to the concentrations of total phenolic and flavonoid compounds from Aloe vera gel is shown in Figure 3. In this study, three different sonication time were selected to be 20, 40 and 60 min. The highest concentration of total phenolic compound and flavonoid were found at the sonication time of 40 min. The concentrations of total phenolic and flavonoid compounds were to be the 11.41±0.04 and 6.76±0.05 mg/mL, respectively.

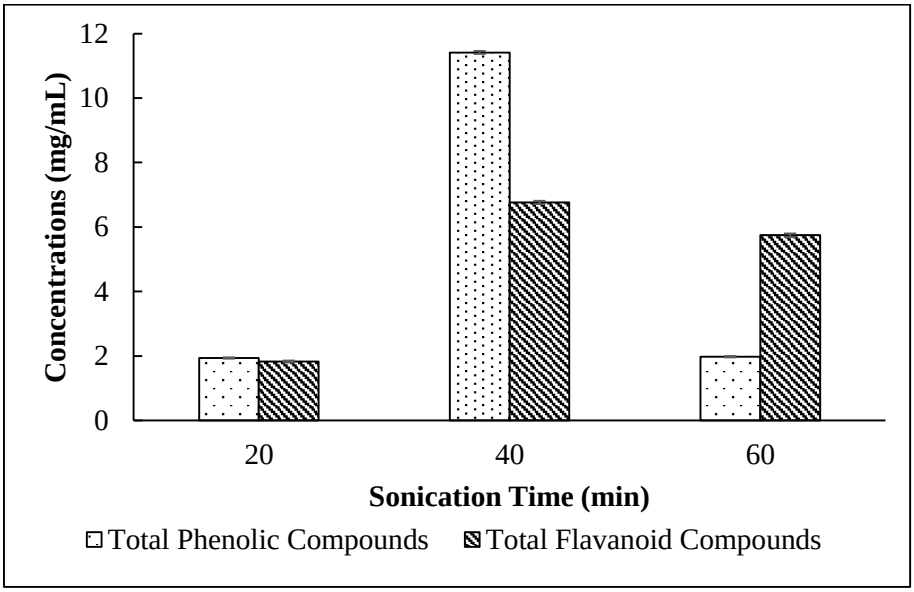


**Figure 3.** The effect of sonication time on the extraction of total phenolic and flavonoid compounds.

Then, the concentrations of total phenolic and flavonoid compounds were decreased as the sonication time increased to 60 min. The decrement is due to the degradation of the compounds that have been

affected by the ultrasonic heat. Thus, the sonication time of 40 min was the most effective in producing phenolic and flavonoid compounds from Aloe vera gel and the preferred one for extraction in this study.

### *3.5. Comparison Between UAE and Conventional Extraction Technique*

In this study, UAE and conventional extraction using Soxhlet extractor were compared for their efficiency on extraction yields of total phenolic and flavonoid compounds as shown in Figure 4. The concentration of total phenolic compound using UAE is significantly higher (63.39%) than the value obtained for the Soxhlet extractor. The concentration of total flavonoid compound using UAE is higher (26%) than the value obtained for the Soxhlet extractor. The concentrations of total phenolic compound and flavonoid using UAE were found to be 11.41±0.04 and 6.76±0.05 mg/mL, respectively. UAE is known as a common advance extraction technique. It is required less time (40 min) for the extraction process. The conventional technique, Soxhlet extraction, was performed for four hours which is six-times longer than with UAE technique.

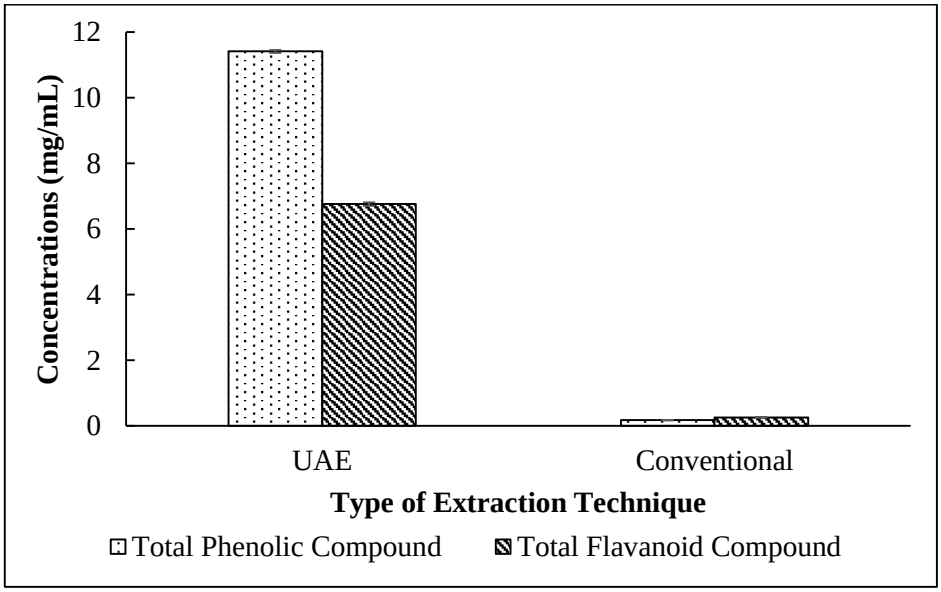


**Figure 4.** Comparison between UAE and conventional extraction technique on the extraction of total phenolic and flavonoid compounds.

## 4. Conclusion

The potential of UAE technique was successfully explored and reported in this paper. The best conditions of UAE were using ethanol as solvent, 40 min of sonication time and duty cycle of 50%. The highest concentrations of total phenolic and flavonoid compound using UAE were found to be 11.41±0.04 and 6.76±0.05 mg/mL, respectively. The sonication time with the UAE was 40 min, which was six-times shorter than with the conventional extraction technique. The UAE best condition is considered as a sustainable green technique for extraction of total phenolic and flavonoid compounds from Aloe vera gel.

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