MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

EXTRACTION OF TOTAL PHENOLIC AND TOTAL FLAVONOID COMPOUNDS FROM *Aloe barbadensis* MILLER USING ULTRASOUND ASSISTED EXTRACTION

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History
International E-Conference of Science and Biosphere Reserve 2021

Keywords:

Aloe vera, Ultrasound-assisted extraction, Phenolic compound, Flavonoid

Abstract

Aloe barbadensis Miller (Aloe vera) is a safe alternative ingredient in nutraceutical and pharmaceutical products due to its bioactive compounds' availability. Ultrasound-assisted extraction (UAE) is the acoustic cavitation principle technique capable of damaging the cell walls of the plant matrix and thus favouring the release of bioactive compounds. This study was performed to extract the total phenolic and total flavonoid compounds using UAE technique. In this study, the inner gel of Aloe vera was used as the sample. The samples were grounded before been extracted with ethanol as the solvent using UAE technique. The UAE was operated at the range of 25-75 % and 20-60 min of duty cycle and sonication time, respectively. The concentrations of the total phenolic and total flavonoid compounds were determined using UV-Vis Spectrophotometer at wavelength of 765 and 420 nm, respectively. The highest concentrations of total phenolic (11.41±0.04 mg/mL) and total flavonoid (6.76±0.05 mg/mL) compounds were obtained at a duty cycle of 50% and 40 min of sonication time. The FTIR spectroscopy results show the presence of functional groups at wavelength range of 640.81 to 3318.64 cm^{-1}. Scanning electron micrograph (SEM) confirmed the efficiency of UAE technique for the bioactive compounds' extraction. This study demonstrates that UAE is a great efficient technique hence feasible for future pharmacological research on plants and herbs.

INTRODUCTION

Aloe barbadensis Miller (Aloe vera) or known as "lidah buaya" is an abundant plant in Malaysia and most of the tropical climate countries. There are three layers of Aloe vera leaf components which are the outer green rind, the middle layer of latex, and the inner colourless parenchyma tissue produces the gel. Parenchyma tissue is abundant in complex carbohydrates, including partially acetylated manans (acemannan and carrysin), most of which are bioactive compounds [1]. Aloe vera contains several biologically active components such as vitamins, minerals, saccharides, amino acids, anthraquinones, enzymes, lignins, saponins, and salicylic acids. They are responsible for the multifunctional activity of Aloe species [2].

Phenolic and flavonoid compounds are both plant secondary metabolites. Phenolic compounds are good electrons donor because their hydroxyl groups and their position in the carboxyl functional group can contribute directly to antioxidant action [3]. Phenolic compound demonstrates free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging, and avoid oxidative disease. Flavonoids protect the plant from UV radiations and fungal infections due to the physiological survival of plant. Besides, flavonoids compound exhibits many pharmacological activities including antioxidants, anti-allergic, anti-inflammatory, antimicrobial and anticancer properties [4].

Ultrasound-assisted extraction (UAE) technique involves ultrasonic irradiation that causes increased mass transfer by

reducing cell and solvent resistance, which facilitates the release of intracellular material to the exterior environment [5]. UAE is an eco-friendly technology due to the decrease of solvent used during extraction process [6,7]. Furthermore, this extraction technique could improve the quality of product by allowing the usage of moderate conditions and process in extraction such as lower extraction temperatures. UAE stands out as a sustainable alternative that required a moderate investment in solvents and energy. UAE is a feasible technological option for industrial scale-up due to the advantage of this extraction method [7].

Thus, the aim of this study is to extract the total phenolic and flavonoid compounds from Aloe vera gel using UAE technique and ethanol as the solvent. This study also conducted to study the effect of UAE parameters such as duty cycle and sonication time on the concentrations of the total phenolic and total flavonoid compounds.

MATERIALS AND METHODS

Materials

Gallic acid, Folin-Ciocalteu's reagent, Dragendoff's reagent, hydrochloric acid, potassium acetate, and ferric chloride were purchased from R & M Chemicals. Quercetin was purchased from Sigma-Aldrich Co. Ethanol was purchased from MERCK. Aluminium chloride was purchased from HmBG.

Methods

Preparation of Plant Sample

Aloe vera plants were collected from the agricultural park located at Universiti Malaysia Kelantan Jeli campus, Jeli, Kelantan, Malaysia. The matured Aloe vera leaves were washed with clean water to discard excess soil. The outer part of the leaves with jagged edges and narrow point at the top were removed. Then, the colourless sticky inner gel was scraped out so it was separated from the outer green rind. After that, the inner gel was collected and sliced into small pieces to increase the surface area of the sample. The gel sample was placed in a drying oven at 50°C for 24 h to eliminate all moisture. After the drying process done, the dried sample was grind using hand blender and sieved with 355 to 500 µm average size particle using sieving machine to obtain the almost uniformly fine texture of the sample. Next, the sample was stored in clean sealed container to avoid any unnecessary moisture from deteriorating the ground Aloe vera sample.

Ultrasound-Assisted Extraction

The extraction process of Aloe vera was performed by using ultrasound homogenizer (Scientz Biotech, Shanghai, China) with the 15 mm tip diameter of ultrasound probe. The operating conditions were set at 40℃ operating temperature, 20 kHz frequency and 900 W ultrasonic power [8]. The 10 g of ground Aloe vera sample was added into 200 mL of in a beaker and stirred using spatula until fully mixed. Then, the probe was directly immersed in about half of the total mixture. The extraction was then performed at different duty cycles which were 25, 50, and 75% and the sonication time was set to be fixed at 40 min. Next, it was further performed at different sonication times of 20, 40, and 60 min and the duty cycle were set fixed at 50%. The sample identification is listed in Table 1. After the sonication process, the extracts were transferred in centrifuge bottle and centrifuged with room temperature for 15 min at 5800 rpm. The supernatant from centrifugation process was removed and collected for further analysis.

Table 1. Sample identification for this study

Sample ID	Description
GE2540	Duty cycle: 25%; Sonication time: 40 min
GE5040	Duty cycle: 50%; Sonication time: 40 min
GE7540	Duty cycle: 75%; Sonication time: 40 min
GE5020	Duty cycle: 50%; Sonication time: 20 min
GE5060	Duty cycle: 50%; Sonication time: 60 min

Determination of Total Phenolic and Total Flavonoid Compounds using UV-Vis Spectroscopy

UV-Vis Spectroscopy analysis was the quantitative analysis of the bioactive compounds of Aloe vera extract. The total phenolic and total flavonoid compounds were analysed using UV-Vis spectrophotometer (Shimadzu, USA) [8]. The total phenolic compound was determined by using Folin-Ciocalteu's phenol reagent. A volume of 0.5 ml of Folin-Ciocalteu's reagent was mixed with 1 ml of sample extract. The mixture was let stand for 7 min, then 7.5 mL of distilled water and 1.5 mL of 20% sodium carbonate solution were added and thoroughly mixed [3]. For an h, the mixture was incubated in the dark. Centrifugation of the mixture was conducted and the sample's blue colour was extracted. The absorbance of the standard and sample were measured at 765 nm. The concentration of total phenolic compound was calculated on the basis of a standard curve of gallic acid [9]. The aluminium chloride colorimetric method was used to determine the total flavonoid compound of the extract. Quercetin was used to make the standard calibration curve of total flavonoid compound determination [3]. 20 mg quercetin was dissolved in 20 mL methanol for the stock quercetin. Then, serial dilution from standard solution was conducted. A volume of 0.6 ml of 2% aluminium chloride, $AlCl_3$ were distinctly mixed with 0.6 ml of diluted standard quercetin and the extract. The incubation of the mixture took 1 h at room temperature. The absorbance of the standard and sample were measured at 420 nm. The concentration of total

flavonoid compound was calculated on the basis of a standard curve of quercetin [9].

Fourier-Transform Infrared Spectroscopy (FTIR) Analysis

Fourier-Transform Infrared spectroscopy (FTIR) analysis was the qualitative analysis of the active components in Aloe vera extract, over a wave number range of 425-4000 cm^{-1}. In the Nicolet™ iS™ 10 FTIR spectrometer (Thermo Scientific, USA), a drop of Aloe vera extract in liquid form was placed. The spectrometer directed IR beams at the sample and measured absorbance by the sample at which frequencies. The sample was then identified using the reference database contains spectra references [6].

Scanning Electron Microscopy (SEM)

The changes of morphological features of the Aloe vera before and after undergo UAE was examined by Scanning Electron Microscope (SEM) (JEOL Ltd., Japan). The samples used had average particle sizes ranging from 355 to 500 µm. The surface morphology of the extracted plant samples was compared with the raw dry plant sample [10].

RESULTS AND DISCUSSION

In this study, three different duty cycles were selected to be 25, 50 and 75%. The highest concentration of total phenolic and total flavonoid was found at the duty cycle of 50 %. The enhanced exposure of acoustic cavitation is affected by duty cycle, which aids to disrupt the cell and separation of intracellular compounds from cell. The enhanced duty cycle increases the extraction of concentration of total phenolic and flavonoid compounds [11]. Thus, the duty cycle of 50% was the most effective in producing total phenolic and flavonoid compounds from Aloe vera gel and the preferred one for extraction in this study. The concentrations of compounds were decreased as the duty was increased to 75% due to high temperature was generated and the phytoconstituents may degrade as a result of continuous exposure of the cell to cavitation [5]. Then, the extraction was operated at different sonication times (20, 40 and 60 min) while the duty cycle was maintained at 50%. The highest concentrations of total phenolic and total flavonoid were found at the sonication time of 40 min. As the sonication time increased, the concentrations of total phenolic and flavonoid compounds were increased. The cavitation caused by ultrasonic waves wherein the disintegration of cell wall due to the abrupt change in shear force and pressure, so releasing the total phenolic and flavonoid compounds and promoting their availability for extraction [12]. However, the concentration of compounds were decreased as the sonication time increased to 60 min. The longer sonication time results the lower concentrations of both compounds due to low stability as the excess heat generated led to compounds deterioration [13]. The concentrations of total phenolic and flavonoid compounds of Aloe vera using UAE technique are shown in Table 2. The highest concentration of total phenolic (11.41±0.04 mg/mL) and total flavonoid (6.76±0.05 mg/mL) compounds was obtained at duty cycle of 50% and 40 min of sonication time.

Table 2. The concentrations of total phenolic and flavonoid compounds of Aloe vera using UAE technique

Sample ID	Phenolic content (mg/mL)	Flavonoid content (mg/mL)
GE2540	1.38 ± 0.01	0.49 ± 0.02
GE5040	11.41 ± 0.04	6.76 ± 0.05
GE7540	2.05 ± 0.02	4.64 ± 0.01
GE5020	1.94 ± 0.02	1.83 ± 0.03
GE5060	1.97 ± 0.02	5.75 ± 0.06

Fourier transform infrared (FTIR) spectrum was applied for the prediction for organic compound present in sample by absorption of ultraviolet light of each wavelength. The functional groups of the active compounds represent in the extract based on the peak's values in the region of IR radiation [6]. In this study, the identification of functional groups was analysed using FTIR involving the Aloe vera gel with ethanol. In this study, the wavelengths ranged from 4000 cm^{-1} to 500 cm^{-1}. FTIR analysis was conducted for Aloe vera gel ethanol extract at 50% duty cycle for 40 min. Figure 1 shows the absorption band occurred resulted from the FTIR spectrum. Based on Figure 1, the absorption band occur at 3318.64 cm^{-1}, 2972.71 cm^{-1}, 2882.17 cm^{-1}, 1379.39 cm^{-1}, 1087.50 cm^{-1}, 1045.45 cm^{-1}, 879.75 cm^{-1} and 640.81 cm^{-1}. Figure 1 was in wave length range of 640.81 cm^{-1} to 3318.64 cm^{-1}. The phenol groups existence was showed by stretching of OH groups attributed from occurrence of broad bands at 3318.64 cm^{-1}. The band at 2882.17 and 2972.71 cm^{-1} would be linked with stretching vibrations of CH_2 or CH_3 group. The ring of aromatic stretch was indicated by the presence of band at 1379.39 cm^{-1}. The C-O-C stretch aliphatic would be linked with bands at 1045.45 cm^{-1} and 1087.50 cm^{-1}. Plus, the band at 879.75 cm^{-1} was associated with aromatic ring vibration.

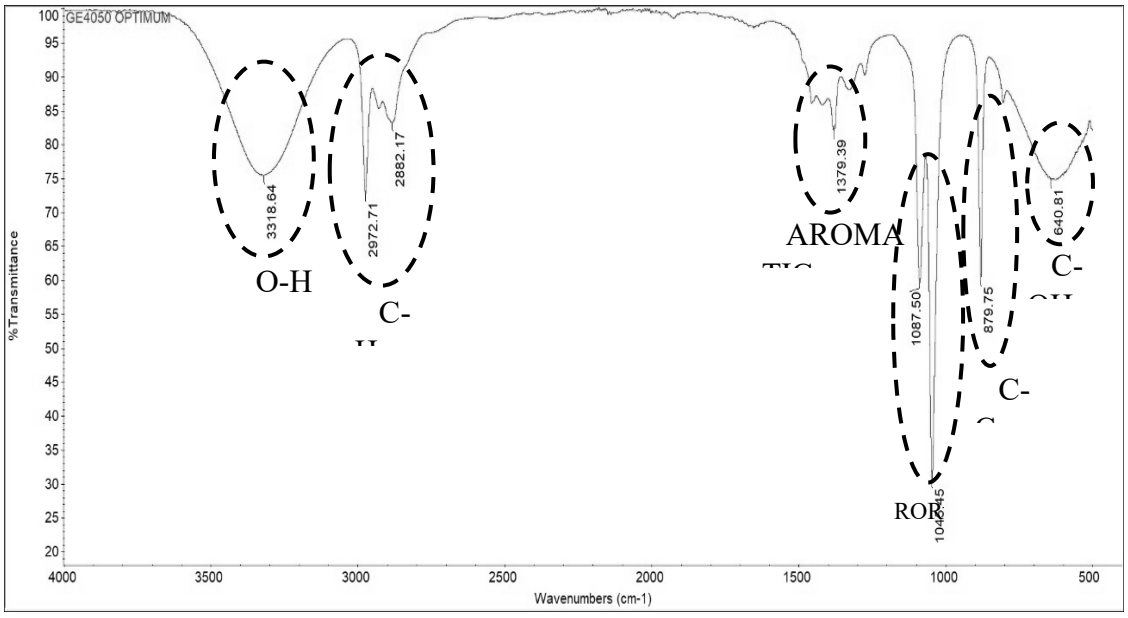

Figure 1. The FTIR spectra of Aloe vera gel extract

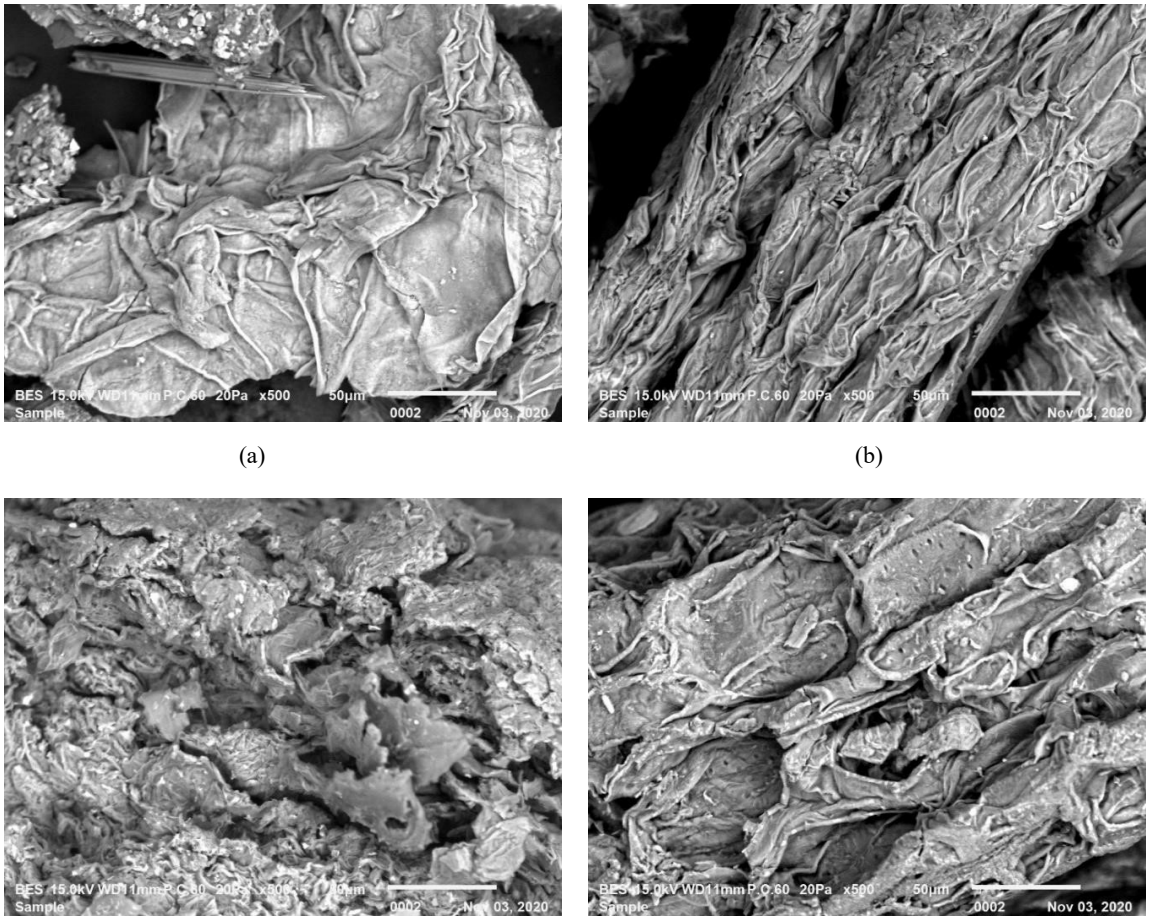

Figure 2. SEM images of (a), (b) Aloe vera samples before extraction and (c), (d) after UAE extraction

The SEM analysis with magnification 500x was used to describe the top surface morphology of sample before extraction (a) (b) and after extraction (b) (c) that represented in Figure 2. The surface of matrix tissues (a) and (b) were shrivelled before extraction. The surface of matrix tissues was slightly damaged (c) and opened stomata (d) after extraction. The cell walls of Aloe vera tissues were ruptured, as showed in (c) and (d), during the ultrasonic irradiation, and a direct contact was established between the intracellular active components and the external solvent, enabling the penetration of cell walls and the freeing of active components [14]. UAE ruptured the tissues aggressively, thus promote the mass transfer, leading to a more and faster release of active components, resulting in a higher extraction yield, shorter extraction time and better product quality [15].

CONCLUSION

The total phenolic and total flavonoid compounds from Aloe vera were successfully extracted using UAE technique. The highest concentration of total phenolic (11.41 ± 0.04 mg/mL) and total flavonoid (6.76 ± 0.05 mg/mL) was found at the duty cycle of 50% and sonication time of 40 min. In addition, SEM analysis confirms that the primary driving force for the efficient extraction by UAE is the structure destruction of the Aloe vera tissues generated by the ultrasonic irradiation. From the data obtained, UAE is a great efficiency technique for extraction of total phenolic and flavonoid compounds from Aloe vera and is substantially feasible extraction technique in the future for commercialize of this highly valuable plant.

ACKNOWLEDGMENT

The authors acknowledge the financial support received in the form of grants from the Ministry of Higher Education Malaysia via Fundamental Research Grant, R/FRGS/A1300/01155A/003/2018/00559.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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