Extraction of Tannic Acid from Macassar Kernels (Brucea Javanica) using Ultrasonic-Assisted Extraction

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Abstract. In this study, the tannic acid compound from *Brucea javanica sp* was extracted by using conventional and non-conventional method, ultrasonic-assisted extraction (UAE). Tannic acids are based of flavonoid compound with antimicrobial properties that usually used as astringent to protect irritation effect on the skin. In conventional extraction, there are two parameters studied namely; extraction time (4, 6, and 8 hours) and temperature (30℃, 40℃, 50℃ and 60℃). From the obtained results of conventional extraction, the optimum yield of tannic acid was found at 6 hours of extraction time with temperature of 50℃ was 0.1527 mg/mL. Ultrasonic-assisted extraction is a green technology, which expected to extract tannic acid in shorter time, high extract yield and eco-friendly extraction technologies. Two parameters studied namely; sonication time (15, 30 and 45 minutes) and duty cycle (25, 50 and 75 %). For the extraction of tannic acid using ultrasonic-assisted extraction method (UAE), the optimum conditions obtained are 30 minutes of sonication time and 25% duty cycle for ultrasonic-assisted extraction method (UAE) with the concentration of 0.2313 mg/mL. The band spectrum for FTIR analysis that presented in pure sample and extracted sample show the similar pattern that represented by the functional group of anhydride and carbonyl stretching vibration. In conclusion, the extraction yields of ultrasonic-assisted extraction method (UAE) are higher than conventional extraction method. From the findings, it was proven that UAE is a significant method for tannic acid extraction from *Brucea javanica sp*.

INTRODUCTION

Brucea javanica also known as Melada pahit is also widely distributed in Asia Pacific regions such as Indonesia, Thailand and Malaysia. In Malaysia, this plant is known as "melada pahit" due to its bitter taste. The seeds from *Brucea javanica* have been used traditionally for treating diabetes mellitus [1]. Tannic acid is secondary metabolites that could form complex compound with other macromolecules. Tannic acid is a naturally existing plant polyphenol that can be present in almost all aerial plant tissues. Tannic acid has traditionally been used for the prevention of diarrhea, the topical treatment of skin burns and the treatment of unidentified rectal disorders [2].

The conventional methods that have been used to conduct the extraction before, consuming longer extraction time and the production of yield was low. Usually, the conventional method was solvent-based extraction required solvent and high temperature. These conditions effect the quality and yield from Brucea javanica plant. The organic solvent used might have potential to produce toxic during extraction and environmental pollutant. To create green extraction, processing method used for obtaining plant extracts need to bring the least impact on the environment. To main goal of extraction methods was to perform in faster extraction speed, more energy efficiency, improved mass and heat transfer, simpler processing step and small equipment size. The waste by-product of non-conventional method was non-

pollutant and could be degrade easily. Example of green extraction method were ultrasonic-assisted extraction (UAE), microwave-assisted extraction (MAE), enzymatic extraction (EE) and ultrasonic-assisted enzymatic extraction (UAEE) [3]. Ultrasonic-assisted extraction (UAE) is a new extraction method which is more environmentally friendly. Ultrasound has a mechanical effect that can facilitate eddy and internal diffusion, thereby increasing the transfer of mass and the solvent's penetration into the sample matrix. Ultrasound-induced cavitation capable to split the cell walls structure, speeding up the release of contents. In addition, the UAE is simple procedure and inexpensive [4].

In this study, ultrasonic-assisted extraction (UAE) was employed for the extraction of phenolic compounds from Brucea javanica plant. Several parameters were studied to determine the optimum conditions for this extraction method. Physical characterization such as FTIR was performed for the identification of functional groups and HPLC analysis was used to determine the concentration of tannic acid. Scanning Electronic Microscope (SEM) analysis was used to observe the surface morphology and microstructure changes of the plant samples before and after each extraction method. The efficiency for UAE method and conventional method also was compared and analysed.

MATERIALS AND METHODS

Brucea javanica plant

Brucea javanica was collected from Pekan Nanas and Batu Pahat, Johor, Malaysia. The fruit that was collected must be matured and the color of fruit is about reddish-purple color.

Preparation of sample powder

Brucea javanica kernels was collected, cleaned and washed with tap water. *Brucea javanica* was dried in air dryer for 4 to 5 days. Dried kernels were grinded by milling machines into powder form before extraction process. After that, the powder was sieved using sieving machine to constant the mesh size of particle (500 µm). The sample powder was stored under room temperature in a zip-lock bags and keep away from the sunlight.

Extraction process

For conventional extraction using maceration extraction method, 5g of the dried Brucea sp seeds was soaked in the 100 mL water and heated. The ratio used was 1:20. The extraction time used were 4, 6 and 8 hours with various temperature; 30℃, 40℃, 50℃ and 60℃ as stated by previous study [5]. By using solid -solvent ratio of 1:20, 5g of dried Brucea sp seeds powder was dissolved in 100mL of water and sample were extracted using ultrasonic-assisted extraction (UAE) with different parameters, which are sonication time and duty cycle. Temperature was set constant temperature at 50℃. The results that obtained from conventional method were compared with the results from UAE. The samples were undergoing ultrasonic extraction of 50% duty cycle and 50℃ temperature in different time of sonication which are 15, 30 and 45 minutes. For the effect of sonication time for UAE, Table 1 shows the parameters of extraction process that are designed with these corresponding conditions. The sample was undergone ultrasonic extraction with optimum sonication time and temperature that was obtained from previous experiment in different duty cycle which are 25%, 50% and 75%. Table 2 shows the parameters of extraction process that are designed with these corresponding conditions for duty cycle parameter of UAE. The sample solution was undergone centrifuged at 5800 rpm for 15 minutes to collect the supernatant. All the supernatant was filtered by using filter paper. The sample was undergoing phytochemical screening. Lastly, the supernatant was characterized by using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Scanning Electronic Microscope (SEM). ATR-FTIR was used to determine the functional group of tannic acid compound in the extract. Surface morphology of the extracted plant samples was compared to the raw dry powder plant sample using SEM analysis.

TABLE 1. Parameter for the effect of sonication time for UAE

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Constant parameters	Duty cycle	50%
	Temperature	50℃
Manipulated parameters	Sonication time	15, 30, 45 minutes

TABLE 2. Parameter for the effect of duty cycle for UAE

Constant parameters	Temperature	50%
	Sonication time	Optimum value that obtained from effect of sonication time
Manipulated parameters	Duty cycle	25%, 50%, 75%

Phytochemical screening

1 mL of extract was dissolved in 1 mL of distilled water. Next, 2 mL of 5% ferric chloride solution was added. Formation of blackish green color indicated the presence of phenolic compound [6].

HPLC Method for detection of tannic acid

20 mg of tannic acid standard was diluted into 20 mL of methanol (HPLC grade) as a stock dilution. The stock solutions were then diluted to appropriate concentration ranges and used to establish calibration curves. For the preparation of sample for HPLC analysis, the sample was diluted to 10 mL with deionized water. After dilution, the sample was filtered through 0.45 µm syringe filter before injected into HPLC system. HPLC analysis was performed with MetaChem C18-A (250 x 4.6 mm, 5 µm) column. The mobile phase consisting of solvent A (HPLC Water/ acetic acid (99:1)) and solvent B (Methanol (HPLC grade)) at a flow rate of 1.2 mL/min. The injection volume fixed at 50 µL and running time of each sample was 7 minutes.

RESULTS AND DISCUSSION

Phytochemical screening

The purpose of phytochemical screening was to ensure the presence of tannic acid after the ultrasonic assisted extraction (UAE). 5% of ferric chloride was used to identify the presence of tannic acid after UAE extraction from *Brucea javanica* sp. A few drops of 5% ferric chloride were added to the extract and it gave blackish green colour. Table 4.1 shows the phytochemical results for the conventional extraction. From Table 3, it was found that the temperature 50℃ and 60℃ for all duration time showed darkest blackish green appeared, which means it contained most bioactive compound detected. However, there were only light blackish green appeared for 30℃ and 40℃ for all duration time. This means that the low temperature level was not enough to disrupt the structural integrity of the plant cell wall. Meanwhile, the yield of bioactive compound that used high 60℃ was lower than 50℃.

TABLE 3. Phytochemical results for conventional extraction

Time / Temperature (℃)	4 hours	6 hours	8 hours
30	+	+	+
40	+	+	+
50	++	++	++

60	++	++	++

Key: + (indicate presence of tannic acid)

Table 4 shows the phytochemical results for effect sonication time using UAE. The effect of sonication time on the extraction efficiency was studied by setting the constant parameters, temperature at 50℃ and duty cycle of 50%. From the table, it could be observed that 30 minutes and 45 minutes of sonication time showed darkest blackish green colour appeared, which means it contained most bioactive compound detected. However, there was only showing light blackish green colour for 15 minutes of sonication time. This means that the low sonication time was not enough for disruption the structural integrity of the plant cell wall. The optimum sonication time could enhance the sonication process in disrupts the cell wall structure and accelerates diffusion through membranes. Hence, the plant cell lyses and release of bioactive compound [5]. Table 5 shows the phytochemical results for effect of duty cycle. The effect of the duty cycle on the extraction efficiency was studied by fixing sonication time at 30 minutes as optimum sonication time from the previous analysis with temperature at 50℃. It could be observed that all duty cycle had showing darkest blackish green colour appeared, which means it contained most bioactive compound detected at all various duty cycle.

TABLE 4. Phytochemical results for the effect of sonication time

Sonication time (minute)	Tannins compound detected
15	+
30	++
45	++

Key: + (indicate presence of tannic acid)

TABLE 5. Phytochemical results for the effect of duty cycle

Duty cycle (%)	Tannins compound detected
25	++
50	++
75	++

Key: + (indicate presence of tannic acid)

HPLC analysis for conventional extraction

In conventional extraction, the effect of extraction time was studied against effect of extraction temperature. Each temperature of sample was undergoing varying extraction time of 4, 6 and 8 hours. Fig. 1 shows that concentration yield of tannic acid versus temperature for different extraction time. From Fig. 1, the highest concentration of tannic acid was obtained at 50℃ with 6 hours extraction time with 0.1527 mg/mL. In this present finding, there are a significant change in the yield was observed between 30℃ to 60℃ at 6 hours, the extraction yields were fluctuation trend between 30℃ to 50℃ and reached the maximum value of 0.1527 mg/mL at 50℃ and then it gradually decreased in the extraction time of 8 hours. Compared to the other condition between 30℃ to 60℃ at 8 hours, the trend was significant similar as 6 hours and the yield of concentration of tannic acid at 50℃ also the highest at this extraction time with the value of 0.1257 mg/mL. Same temperature level at 50℃ but different extraction time between 6 hours and 8 hours obtained different concentration of tannic acid which were 0.1527 mg/mL and 0.1257 mg/mL. It could be because the duration time was longer compared to 6 hours, because the tannic acid in the sample starting to degrade [5]. The causes the lower concentration of tannic acid obtained compared to 6 hours of extraction time. Based on the obtained result, the temperature was fixed at 50℃ for UAE extraction to avoid degradation of tannic acid in the extracts.

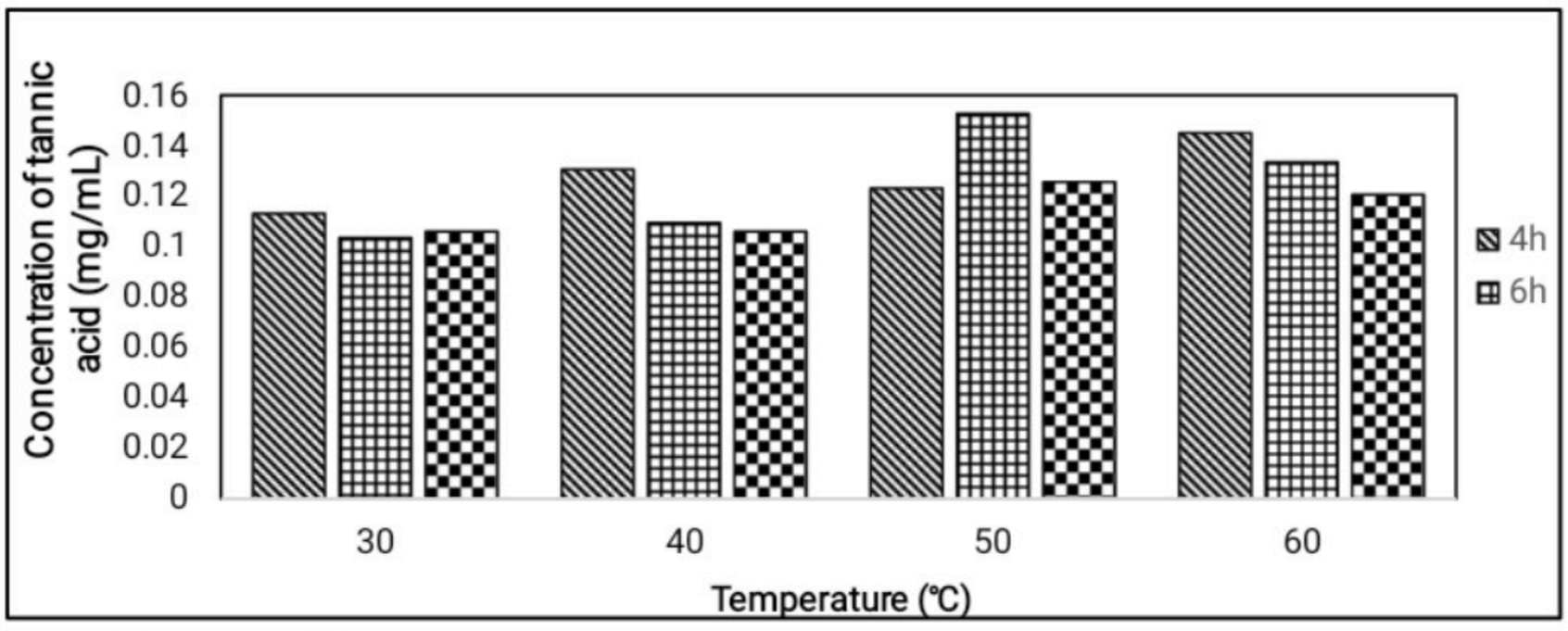

FIGURE 1. Effect of extraction time and temperature for the conventional method.

HPLC analysis of ultrasonic -assisted extraction for the effect of sonication time

The effect of sonication time on the extraction efficiency was examined by fixing the temperature at 50℃, duty cycle at 50% and the solid to liquid ratio of 1:20 while varying the sonication time from 15 to 45 min as shown in Fig. 2. The graph are showing a fluctuation trend between 15 minutes to 45 minutes. The trend of the graph shows increasing when sonication time from 15 minutes to 30 minutes and reached the maximum value of 0.1502 ± 0.00075 mg/mL at 30 minutes and then it decreased gradually in sonication time 45 minutes. Based on Fig. 2, 30 minutes of sonication time showed the highest concentration of tannic acid in 0.1502 mg/mL that was determined as the optimum sonication time by using UAE extraction methods in extracting tannic acid from *Brucea javanica* sp. This was because the time for the vibration to break down the cell was enough and did not destroy the active compound from the cell [7]. When the sonication time was higher than 30 minutes, the concentration of tannic acid that extracted was decreases. This could be explained because sonication process would produce heat and cause the increase in temperature. Thus, the sonication of 45 minutes causes the heat that produce was high and cause the tannic acid in the sample degraded. The causes the lower concentration of tannic acid obtained compared to 30 minutes of sonication time. This implies that the sonication time for 30 minutes was suitable for enhance and increase the concentration of tannic acid compound.

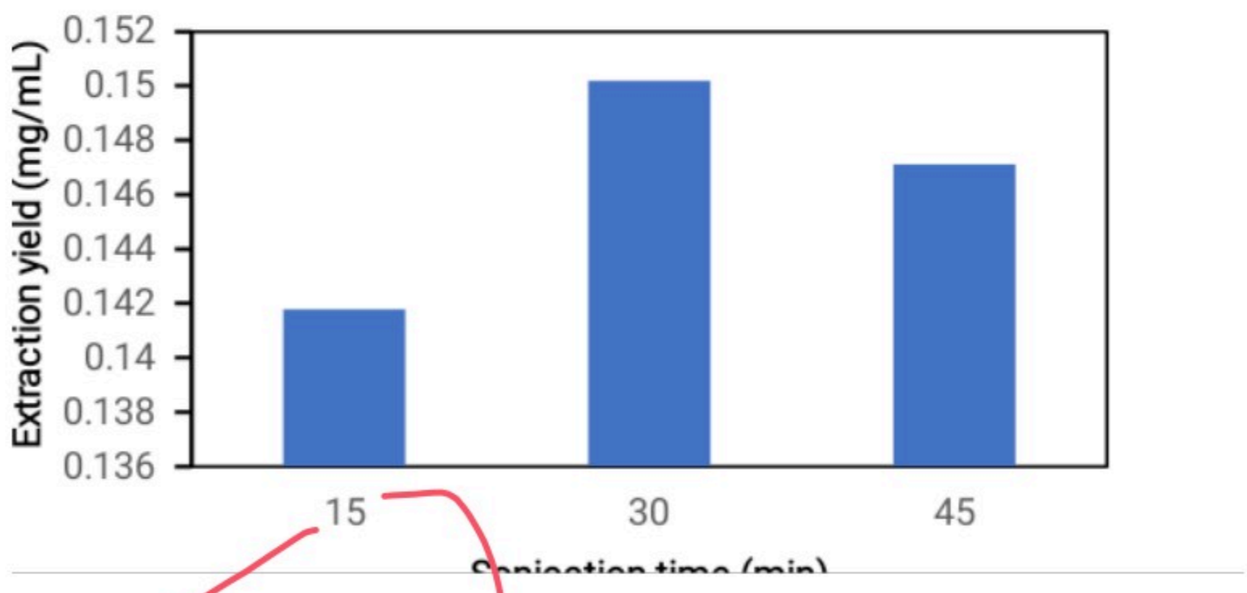

FIGURE 2. Effect of sonication time using UAE.

HPLC analysis of ultrasonic -assisted extraction for the effect of duty cycle

The effect of the duty cycle on the extraction efficiency was conducted by fixing sonication time of 30 minutes, temperature at 50℃ and the solid to liquid ratio at 1:20 while varying the duty cycle from 25 to

75% as presented in Fig. 3. Based on the figure, 25% duty cycle was determined as the optimum duty cycle in extracting tannic acid in *Brucea javanica* sp. It is shown that 50% and 75% duty cycle did not have a pronounced advantage over the 25% duty cycle as the ultrasonic irradiation that was sufficient to affect extraction process. Overall, 25% duty cycle was suitable for enhance the extraction to replace continuous ultrasonic irradiation by reduce the energy consumption and there was efficient cavitation effect to break down the cell wall structure and accelerates diffusion through membrane without damaging the component of the cell [5].

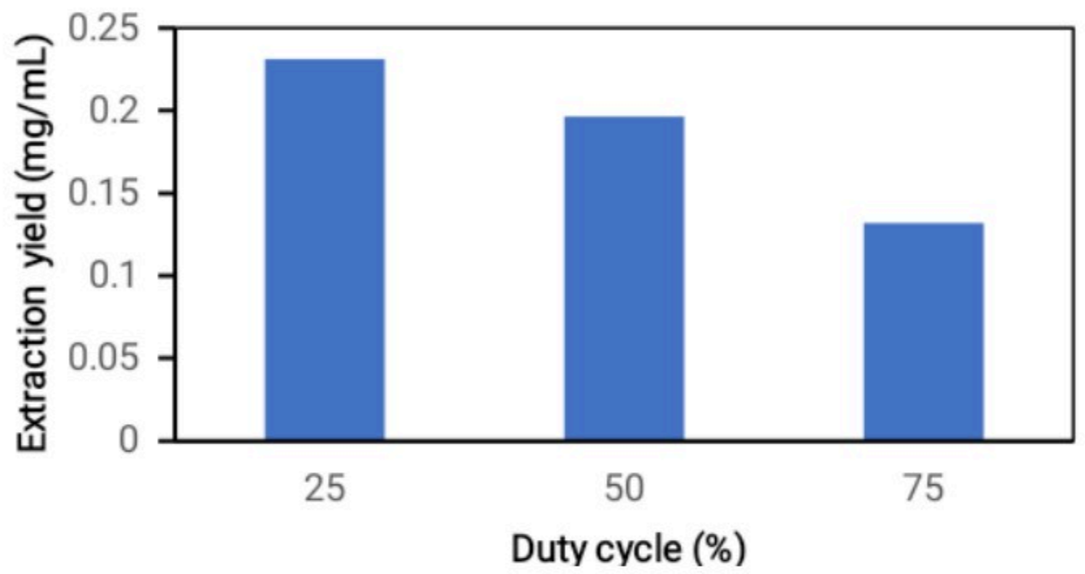

FIGURE 3. Effect of duty cycle using UAE.

Fourier Transform Infrared Spectroscopy (FTIR) analysis

In this study, the pure powder of *Brucea javanica* sp and the extracts were analysed using FTIR for the identifation of functional group. Fig. 4 shows the FTIR spectra of tannin acid from *Brucea javanica* sp plant extract. For untreated sample, the wide and strong appearance of absorption band observed at 3288 cm^{-1} in the region absorption from 3600-3200 cm^{-1} which showing the stretch vibration of the hydroxyl group (O-H) H-bonded broad. There were small sharp peaks near 2922 cm^{-1} and 2852 cm^{-1}, due to the alkane medium (C-H) is observed. The sharp peak around 3000 and 2850 cm^{-1} associated symmetric and antisymmetric stretching with –C-H vibrations of the corresponding CH_2 and CH_3 classes. A weak signal at 1635 cm^{-1} was observed. This band is related to carbonyl groups due to the C-C aromatic compound. The peak at 1034 cm^{-1} was observed, this bands were related to CO-O-CO bridges. FTIR analysis of tannic acid spectra in conventional and UAE extracts show identical peak patterns. The wide bonds in the 3400-3200 cm^{-1} range are correlated with the –OH stretching of the phenolic group. Peaks were observed at 3327 cm^{-1} in conventional extract and 3319 cm^{-1} in UAE extract. The aromatic C-C stretching vibrations can be assigned to peaks around 1635 cm^{-1}, both in the conventional and UAE extract range. Compared with the FTIR spectrum of untreated sample, the spectra of conventional and UAE extract have undergone changes. The peak between 3000 and 2700 cm^{-1} gradually became broader after undergoes extraction for both extraction methods. It could be because the existence of intense hydrogen bond. The peaks at 1034 cm^{-1} associated to the CO-O-CO bridges gradually became combined and broadened. This transition may be due to environmental changes to untreated sample as the vibration of the C=O groups was influenced by the formation of bridges -CH_2-.

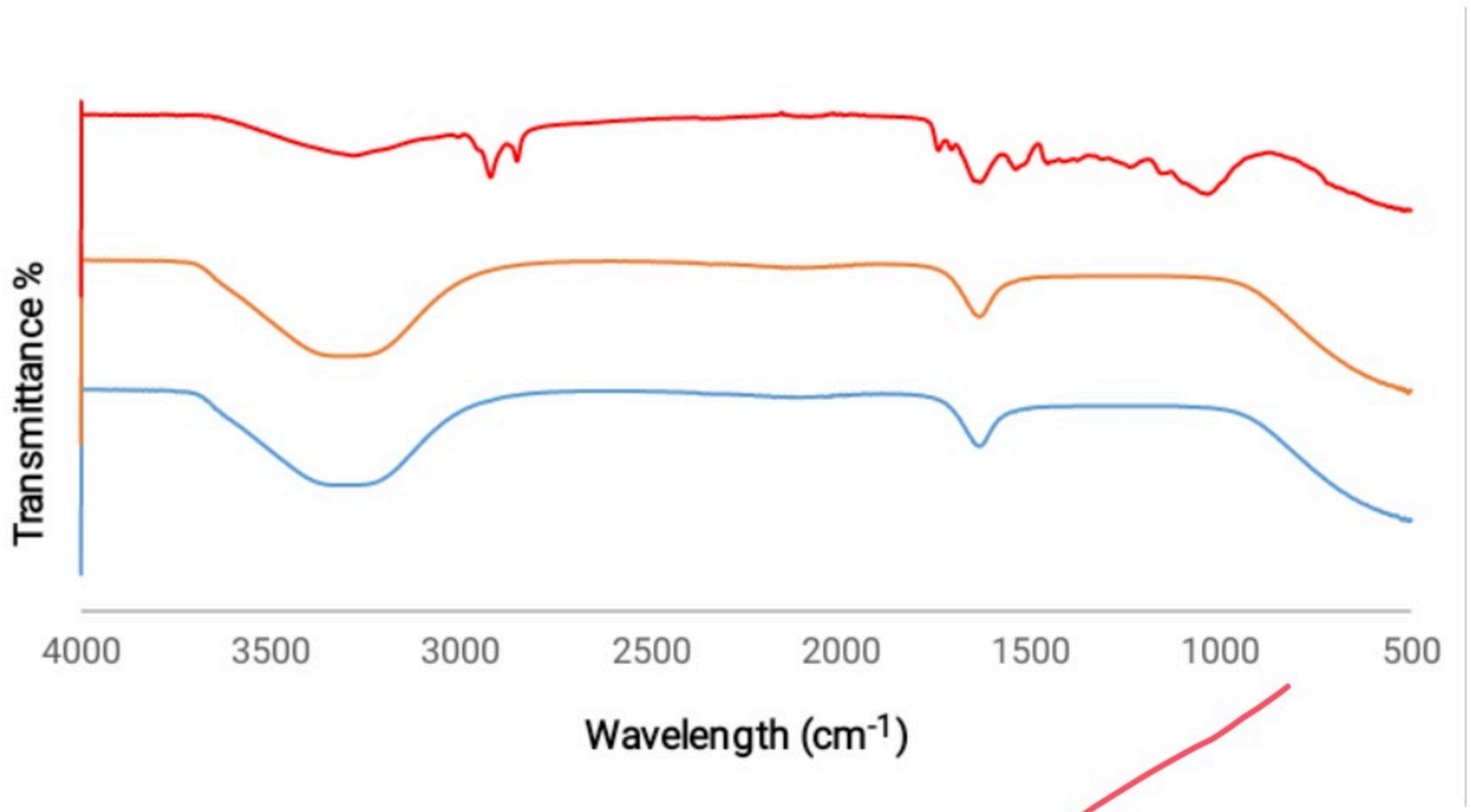

FIGURE 4. FTIR spectra of *Brucea javanica* sp plat extract; (a) untreated sample; (b) conventional extract; (c) UAE extraction

CONCLUSION

The comparison for the extraction yield between conventional extraction and ultrasonic-assisted extraction (UAE) were determined by obtaining the optimum parameters for both methods. The highest concentration of tannic acid obtained from the conventional extraction method with the optimum conditions of temperature 50℃ and 6-hours extraction time was 0.1527mg/mL. The highest concentration of tannic acid obtained from the ultrasonic-assisted extraction with optimum conditions at 30 minutes of sonication time and 25% duty cycle was 0.2313 mg/mL. Based on the results obtained, it was found that UAE extraction method gave more advantages performance as alternative extraction with green technology with higher extraction yield.

ACKNOWLEDGMENTS

The authors would like to acknowledge the financial support received in the form of grants from the Ministry of Higher Education Malaysia through Fundamental Research Grant Scheme (FRGS), R/FRGS/A1300/01155A/003/2018/00559.

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