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EXTRACTION OF TANNIC ACID FROM KENAF BAST FIBRE USING ULTRASONIC ASSISTED EXTRACTION

Tannic acid or tannin, type of phenolic compound contains in kenaf bast fibre. Conventional extraction has certain limitations in terms of time, energy, and solvent consumption. Ultrasound assisted extraction (UAE) can extract bioactive components in shorter time, low temperature, with lesser energy and solvent requirement. UAE as alternative extraction technique is better equipped to retain the functionality of the bioactive compounds. In this study, the conditions for ultrasound assisted extraction (UAE) of tannic acid from kenaf bast fibre by assessing the effect of sonication time and different duty cycles were optimized. The use of ultrasound to extract tannic acid from kenaf bast fiber was evaluated. Ultrasound-assisted extraction (UAE) was carried out using ethanol as solvent to intensify the extraction efficacy. Phytochemical screening was conducted to identify the presence of tannic acid in extracts. The extracts then were analyzed using High-Performance Liquid Chromatography (HPLC) and Scanning Electron Microscopy (SEM). It was found that 0.2429 mg/mL of tannic acid was obtained under the extraction conditions of extraction temperature of 40°C, sonication time of 20 minutes and duty cycle of 50%. From SEM analysis, it was found that the raw sample demonstrated rough surface and no porous but kenaf bast fibre display smoother surface with less impurities and few pores appeared after the extraction process using UAE. These results indicate that ultrasound-assisted extraction is an efficient method for extracting tannic acid from kenaf bast fibre with the advantages of lower extraction time and higher extraction yield.

Keywords: Ultrasonic Assisted Extraction (UAE); Sonication Time; Duty Cycle; Extraction Process; Kenaf Bast Fibre

1. Introduction

Kenaf is cultivated in many countries in the world like Africa, Vietnam, India, Thailand, Australia, Indonesia, Malaysia, southern Europe and China [1]. The development of agricultural practices for sustainable production holds high potential in multiple industrial areas such as paper, biofuels, automobile parts, construction and packaging materials, animal feed and environmental cleaners [2]. Generally, kenaf fibre is polymeric composite that contain hemicellulose, cellulose, lignin, pectin and other extractives. The fibre products exhibited responsible for most of physical and chemical properties [3]. Kenaf have shown as fascinating sources of possible bioactive molecules, likes phenolic compound, cardioprotective, triterpene derivatives, phytosterol, antioxidant, antihypertensive and antiproliferative activities [4].

In present years, natural plant product has been broadly used in food, medicine, cosmetic, tanning, metallurgical and others industry in tannic acid. Polyphenols have the innumerable phenolic hydroxyl group likes tannic acid where the structure can give outstanding chemical and physical properties. Also, tannic acid can interconnect with alkaloids, metal proteins, pharmacological and carry out some ecological and physiological effects with extraordinary pharmacological biological activities [5]. Moreover, tannins are broadly delivered in numerous plants. The purpose to preserve the plant tissues that consider as defensive molecules from herbivorous beats due to the astringent taste. It has been revealed that some natural tannins and associated compound have several biological activities such as antioxidant, hypolipidemic, hypoglycaemic, antioxidant, antibacterial activities and antitumor [6]. Discovery of bioactive compound properties with increasing the properties of materials for biomedical appearance known as organic polymer additive [7]. In terms of the research tannin related activities has through a great improvement, especially in green feedstock for material in various fields. Nonetheless, for the valorizations, the extraction process remains as main blockage because of their heterogenous nature [8].

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In the current review, extraction techniques were conducted based on the works from the past 20 years as an inclusive study of main types of tannins. Recently, an increasing demand for the alternative extraction technique that enabling process automation, shortening extraction times, and reducing consumption of organic solvent. In this present study, the extraction of phenolic compounds, tannic acid from kenaf bast fibre was performed using ultrasonic-assisted extraction (UAE). Scanning Electron Microscopy (SEM) was used to study the surface morphology of kenaf bast fibres for the comparison of raw sample and kenaf bast fibre after extraction. High-Performance Liquid Chromatography (HPLC) analysis was used to determine the extraction yield of tannic acid. Several parameters include sonication time and duty cycle were studied to determine the optimum conditions for UAE method.

2. Methodology

Materials

Materials that used are kenaf bast fibre, components that derived from the kenaf stalk. Stalks of kenaf were harvested from kenaf plantation in Bukit Bunga, Kelantan, Malaysia. Kenaf tree that already achieved the maturity of 3 to 4 months and suitable for harvesting was chosen.

Chemicals

The chemicals that used are ethanol, distilled water, deionized water, acetic acid (HPLC grade), methanol (HPLC grade), ferric chloride and standard of tannic acid.

Preparation of kenaf fibre sample

Stalks of kenaf that were harvested were decorticated using kenaf decorticator to separate kenaf bast fibre and kenaf core. 1.2 kg of green kenaf bast fibre was taken as sample. All sample taken from middle part of kenaf bast fibre that was cut into 3 to 5 cm. Then, samples were dried at 45°C in a vacuum oven for 30 h. The dried kenaf bast fibre were further pulverized into powder using a laboratory grinder. This kenaf bast fibre powder was passed through a standard sieve (355-µm mesh) and was kept in a water and airtight high-density polyethylene (HDPE) pouch at −18°C till further experimentation.

Extraction process using Ultrasound Assisted Extraction (UAE)

The ratio of solvent to sample used are 1:40 which refer to 5 grams of sample dissolved in 200 mL of ethanol. Manipulated parameters that were used in UAE are sonication time and duty cycle. The sample was undergone ultrasonic extraction of 50% duty cycle and 40°C temperature in different sonication time which are 20, 40, 60 minutes. TABLE 1 shows the parameters of extraction process that was designed for the effect of sonication time. The sample was undergone ultrasonic extraction using the optimum value of sonication time that was obtained from previous study for the effect of sonication time and 40°C temperature with different of duty cycles which are 10%, 25% and 50%. The effect of duty cycle parameters of UAE is shown in TABLE 2.

TABLE 1

Parameters used for the effect of sonication time

Constant parameters	Duty cycle	50%
	Temperature	40°C
Manipulated parameters	Sonication time (minutes)	20, 40, 60 min

TABLE 2

Parameters used for the effect of duty cycle

Constant parameters	Sonication time	Was obtained from previous study for the effect of sonication time
	Temperature	40°C
Manipulated parameters	Duty cycle	10%, 25%, 50%

After the extraction process, the samples were transferred into 50 mL of centrifuge tube for centrifugation. Then, the samples were centrifuged for 15 minutes at 4000 rpm. The samples then were filtered using filter paper to remove any dirt. After that, the sample was transferred again into the 50 mL centrifuge tube and was kept in the chiller under temperature of 5°C.

Phytochemical screening of tannic acid

2 mL of each sample was heated for 2 minutes. The test tube was allowed to cool. 3 to 5 drops of ferric chloride solution were added to each of sample extracts. Lastly, orange colour was observed for the presence of tannic acid in the extracts.

High-Performance Liquid Chromatography (HPLC) analysis

An Agilent 1100 HPLC system Agilent technologies operated by Windows NT based MetaChem C18 software used. 1 mL of extracts was filtered using 0.45 µm filter pore size membrane filter into HPLC vial before HPLC analysis. Tannic acid was used as standard calibration curve. The flow rate used is 1 mL/min with the injection volume of 5 µL. Then, the running time for the analysis is 8 minutes with the wavelength of 270 nm. All the parameters and conditions were tabulated in TABLE 3 for HPLC analysis of extracts.

TABLE 3

Parameters for HPLC analysis

Parameters	HPLC conditions
Column size	C18 (250 mm × 4.6 mm × 5 µm)
Mobile phase: Solvent A Solvent B	Methanol (HPLC grade) Acetic acid and deionized water
Flow rate	1.0 mL/min
Injection volume	5 µL
Running time	8 minutes
Wavelength	270 nm

Scanning Electron Microscopy (SEM) analysis on kenaf bast fibre sample

Kenaf bast fibre was characterized using SEM for analysis of surface morphology. Two samples were analyzed includes raw sample of kenaf bast fibre and sample from the optimum conditions obtained from UAE. The dried samples were tested using a Jeol-JSM-IT200 scanning electron microscope. The SEM was operated in high vacuum at an accelerating voltage of 10 kV. The fibre was examined up to 200 mm (7.87 in) in diameter and 80 mm (3.14 in). The magnifications that being used are at 500× and 1000×.

3. Results and discussion

Phytochemical screening of tannic acid

Phytochemical screening of tannic acid for the extracted sample was performed qualitatively. TABLE 4 indicates the results of phytochemical screening for the presence of tannic acid in extracts. A total of 12 extracted samples were tested for this phytochemical screening. All the samples were extracted using ethanol as solvent. The colour of the kenaf bast fibres before extract is light green and immediately changed to orange after a few drops of ferric chloride. According to TABLE 4, positive results on tannic acid were observed. Based on previous study [9,10], it was found that a total of 13 phytochemical compounds in kenaf leaves, including phenol, flavonoid and tannin in the leaf extract. TABLE 4 shows that tannic acid in samples ST 40.1, ST 40.2, SC 10.1 and SC 10.2 are mildly present compared to other samples which indicates highly present. Each sample was tested in duplicate. The differences in total tannin content might be due to the changes in difference parameter during the extraction. Fig. 1 shows phytochemical screening of tannic acid for the effect of sonication time and effect of duty cycle.

TABLE 4

Qualitative analysis of phytochemical in extracts

Parameters		Sample ID	Absent/ present
Sonication Time	20 minutes	ST 20.1	+++
		ST 20. 2	+++
	40 minutes	ST 40. 1	++
		ST 40. 2	++
	60 minutes	ST 60. 1	+++
		ST 60. 2	+++
Duty cycle	10%	SC 10. 1	++
		SC 10. 2	++
	25%	SC 25. 1	+++
		SC 25. 2	+++
	50%	SC 50. 1	+++
		SC 50. 2	+++

++ mildly present; +++ highly present

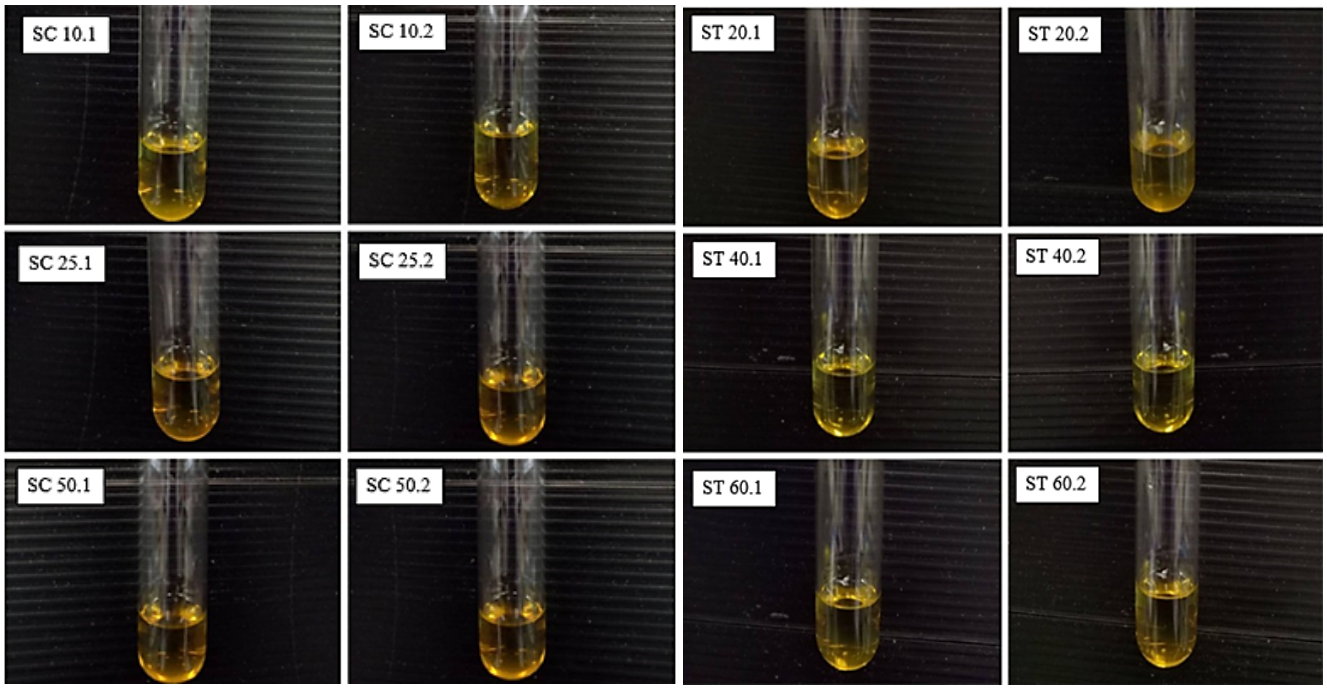

Fig. 1. Phytochemical screening of tannic acid for effect of sonication time (sample SC 10.1, SC 10.2, SC 25.1, SC 25.2, SC 50.1, SC 50.2) and effect of duty time (sample ST 20.1, ST 20.2, ST 40.1, ST 40.2, ST 60.1, SC 60.2)

Effect of sonication time towards extraction yield of tannic acid

The effect of sonication time of UAE to the concentration of tannic acid from kenaf bast fibre is shown in Fig. 2. Sample with 20 minutes sonication time labelled as ST 20 resulted extraction yield of 0.2429 mg/mL. ST 20 shows the highest concentration of tannic acid compared to sample ST 40 and ST 60. In addition, 40 minutes of sonication time namely as ST 40 obtained extraction yield of 0.0521 mg/mL which is higher compared to ST 60 that obtained extraction yield of 0.0390 mg/mL for 60 minutes of sonication time.

Similar trend was also observed for the extraction of phenolic antioxidants from kenaf seeds extracted by pulsed ultrasonic-assisted extraction by Tan et al. in 2014 [11]. In the previous study, 15 minutes was found to be the optimum extraction time and after 15 minutes, the extraction yield decrease. The total phenolic content (TPC) and total flavonoid content (TFC) for kenaf seeds extracted with 100% ethanol decreased as the extraction time increased. This trend can be explained by the potential extraction time increase by the loss of antioxidants following heat or oxygen exposure [11,12]. This finding is in agreement with the extraction of total phenolic and flavonoid compound from Aloe *barbadensis* Miller [13] and the extraction of gallic acid from should be in italic for Chromolaena odorata [14]. Similar results reported by Daghaghelea et at. (2021) [15] for the extraction of antioxidant compounds from *Moringa Oleifera* leaves stated that the extraction yields first followed a rising trend and then a falling trend after the extraction time of 15 minutes.

According to the previous study, the extraction occurs in a rapid period, appoint that the extraction cannot be more than 30 minutes for tannic acid [10]. At this rate, that up to 90% of the recovery of the total content of the phenolic compounds can be achieved thus indicating a considerably rapid extraction rate [16]. Other study examined that more than 40 minutes of sonication time with higher energy levels, more than 20 kHz can affect on phytochemical as the decrease of diffusion area and increase diffusion distance cause decrease of yield of phenolic compound and flavonoid content [17]. The preferred sonication time was 20 min, corresponding to the maximum extraction yield. This duration was used as a constant parameter for further experiments.

Effect of duty cycle towards extraction yield of tannic aAcid

Fig. 3 shows the effect of duty cycle to the concentration of tannic acid from kenaf bast fibre. At 10% of duty cycle, SC 10 recorded extraction yield of 0.0372 mg/mL. According to Fig. 3, SC 10 is higher than SC 25 which represents 25% of duty cycle. The concentration of tannic acid in SC 25 is 0.0263 mg/mL. 50% of duty cycle that labelled as SC 50 in Fig. 3 shows the highest value of 0.2429 mg/mL compared to others.

In this study, the solid to liquid ratio that was used is 1:40 suggesting that it is ideal to supply the value of solvent needed to enter the cells by enhancing the permeation of the phenolic compounds. It was found that the optimum operating conditions for UAE are sonication time of 20 minutes and duty cycle performed at 50% with the sample labelled as SC 50.

Similar finding was observed by Torres et al. [18] when comparing anthocyanin and tannin extraction yields using wave amplitudes of 20% and 50%, which suggests that a better ultrasonic duty cycle induces a greater value of cavities also increasing the extraction process. Unnecessary increase on timing can cause of excessive heating and excessive energy consumption. The previous study showed that hydro-alcoholic mixtures, especially ethanol, are the solvent systems that most suitable for extraction due to the different polarities of the phenolic compounds and the acceptability of this system for human consumption [18]. Xu et al. [19] reported that pectin yield from grapefruit peel first increased with increase in duty cycle and then decreased after reaching a peak of 50% duty cycle which is in good agreement with the findings of this study. At high duty cycle of 50-70%, the cavitation effect decreases due to saturation effect and inter-bubble collision as discussed in effect of power.

Duty cycles indicate the availability of more tannic acid can be extracted from kenaf bast fibre. This might be due to the duty cycle enhance the mass transfer of tannic acid from the plant to the solvent for better penetration. Moreover, the disruption of

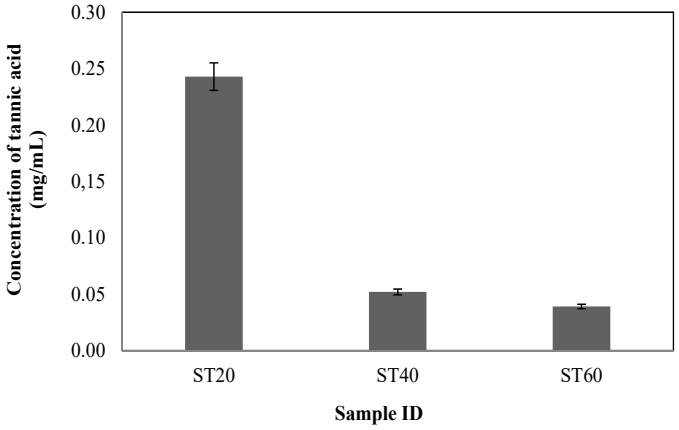

Fig. 2. Effect of sonication time towards concentration of tannic acid

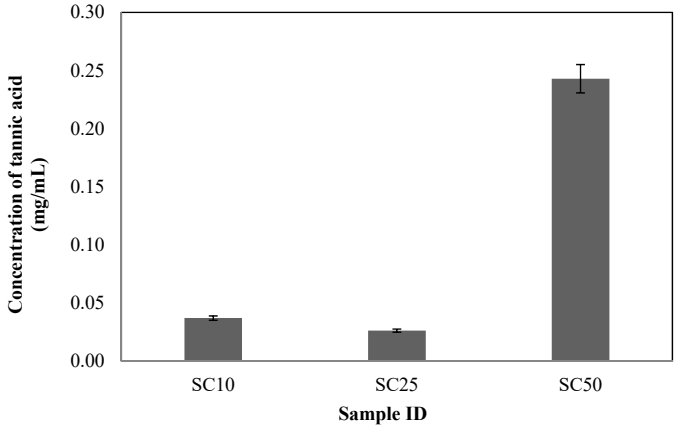

Fig. 3. Effect of duty cycle towards concentration of tannic acid

matrix increases the cell wall permeability, thereby enhancing the extraction of bioactive compounds towards extract [20]. Ultrasound leads to the localized damage to the plant tissues as erosion that may also be attributed to the implosion of the cavitation bubbles on the surface of plant tissues. The eroded part facilitates the contact of solvent, increasing the extraction yield. Higher temperature would decrease the extraction rate due to the lessen cavitation intensity that obtain lower surface tension and increase vapor pressure in cavitation bubbles. Larger volume of solvent used for the extraction process aid to accelerate or speed up the diffusion process [21].

SEM analysis for kenaf bast fibre sample

SEM analysis was performed in this study to analyze the surface morphology of kenaf bast fibre as well as to gain greater insight into the surface structure. Two samples from raw kenaf bast fibre (a) and sample from the optimum conditions obtained from UAE, kenaf bast fibre sample after extraction (b) was examined under magnification of 500× and 1000× as shown in Fig. 4. Sample (a1) shows that raw kenaf bast fibre still contains other impurities and the surface was smooth. From the other side can be seen clearly on magnification of 1000× for sample (a2). After extraction, most of the impurities was clearly removed using ethanol, the surface of the kenaf bast fibre tend to be smoother in sample (b1) under 500× magnification and clearly shown under 1000× magnification labelled as (b2).

Apart from that, there is no pore obtained from the raw kenaf bast fibre in sample (a1) and sample (a2). A few pores present after the extraction process on sample (b1) as can be seen clearly on sample (b2) under larger magnification. The results emphasize on the pore size that have clearly observed for UAE to show the cavitation effect created by the ultrasound. Hence, the solvent could more easily penetrate the cell to enhance the extraction efficiency [22]. Moreover, vibrations certainly existed for bubbles inside newly created pores. The oscillations and slump of these bubbles created an internal pressure that led to an expansion of the structure and open pores [23-26].

4. Conclusion

In conclusion, extraction of tannic acid from kenaf bast fibre was successfully carried out using ultrasound assisted extraction (UAE). The phytochemical screening on kenaf bast fibre extracts indicated the presence of tannic acid. Lower sonication time of 20 minutes and higher duty cycle at 50% was found as the optimum

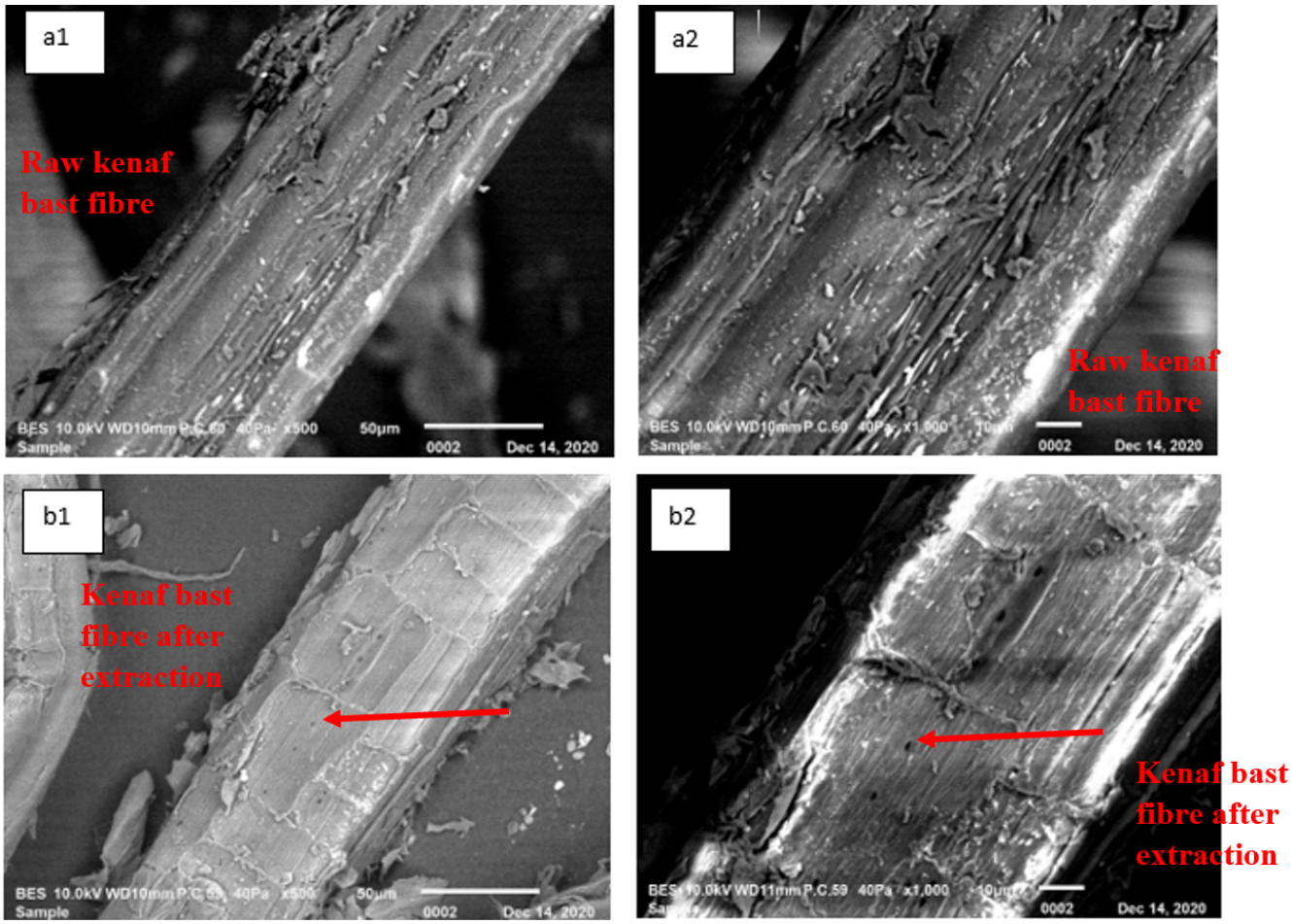

Fig. 4. SEM images of (a1) raw kenaf bast fibre, 500× magnification, (a2) raw kenaf bast fibre, 1000× magnification, (b1) kenaf bast fibre sample after extraction, 500× magnification, (b2) kenaf bast fibre sample after extraction, 1000× magnification

parameters that obtained the highest tannic acid concentration of 0.2429 mg/mL. Study on SEM indicates the changes before and after the extraction process. Almost all the impurities were completely removed after the extraction process. Also, some of the pore derived after the extraction process. Thus, it can conclude that ultrasound assisted extraction of kenaf bast fibre is an effective method to extract with the benefits of lower time and solvent requirement and higher extraction yields.

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