Characterization of Powdered Rhizophora Mucronata Bark Tannins Extractives using Different Drying Methods

Ahmad Hafizan Muhammad Muhayyidin, Noor Fitrah Abu Bakar, Nurul Aimi Ghazali, Arina Sauki, Wan Asma Ibrahim

Abstract*: The effect of different drying methods using spray dryer and rotary evaporator towards the physico-chemical properties and thermal stability of powdered tannin extractives from Rhizophora Mucronata bark was investigated. Prior to spray drying at 130°C and rotary evaporating at 80°C, tannin was extracted using water-based boiling extraction at temperature ranging from 80 to 90°C. Powdered tannin extractives obtained by spray dryer decomposed at higher temperature (at 270°C) than those using rotary evaporator (at 210°C). The powdered tannin extractives from spray dryer was higher in thermal stability due to the high crystallinity peak appeared from X-ray Powder Diffraction (XRD) analysis. Condensed and hydrolysable tannins were also quantified using Reverse-phase High Performance Liquid Chromatography (RP-HPLC) for both methods. Powdered tannins extractives using spray dryer contained 27.8% condensed tannins and 0.001% hydrolysable tannins, in which the condensed tannins are slightly higher in concentration than those formed using rotary evaporator which was 26.5%. The findings revealed that the used of spray dryer is more beneficial to obtain a stronger thermal stability and a higher concentration of powdered Rhizophora Mucronata bark tannins extractives.

***Keywords* : Condensed tannin, Hydrolysable tannin, Rhizophora Mucronata, Rotary evaporator, Spray dryer.**

I. INTRODUCTION

Tannin is derived from the French "tanin" and is used for a range of natural polyphenols because of its non-toxic, biodegradable and naturally existing [1], [2]. Tannin in vascular plants occurs as two types; condensed and hydrolysable tannins [3], [4]. Condensed tannin which is also known as proanthocyanidins are oligomers and polymers of flavan-3-ols units that are frequently linked either via C4-C6 or C4-C8 bonds [5], [6]. The most common condensed tannins are procyanidins comprising of catechin, epicatechin and/or their gallic acid esters, and prodelphinidins comprising of gallocatechin, epigallocatechin and/or their galloylated derivatives [7].

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Tannin is the main element in mangrove species, as high as 20% dry weight and the fourth most abundant compound in vascular plant tissue [8]. The commonly found mangrove species are *Rhizophora stylosa*, *Rhizophora mangle*, *Rhizophora apiculata* and *Rhizophora Mucronata* [9], [10]. Tannin is frequently obtained or extracted from red mangle (*Rhizophora Mangle*); quebracho (*Scinopsis Balansae*); mimosa (*Acacia Meamsii* and *Acacia Dealbata*); valonea (*Quercus Macrolepis*); and chestnut (*Castanea Dentata*) [11], [12]. *Rhizophora* species in eastern and southeast Asia are known as therapeutic plants [13]. Useful tannic compounds are normally obtained in the gall, roots, barks or in the leaves of the mangrove species [14], [15]. According to Basak, et al. [16], tannin extracted from the leaves of mangroves has a significant range of extraction percentage values from 8.39 to 44.27%. Meanwhile, 54 to 80% of tannin extracted from the barks of mangroves which is higher compared to if extracted from the leaves [17], [18]. In this invent, tannin was extracted from the bark of *Rhizophora Mucronata* species of mangrove. This species is one of the major species in Malaysia that offered a great possibility as a source of tannin [19], [20], [21]. On the other hand, the bark is typically thrown away as a waste product because it disrupts the carbonization process due to its high moisture content [22]. Thus, the wasted bark can be used as a potential source of tannin, which can be retrieved by extraction process.

Commonly, tannins extracted using conventional Soxhlet and its effectiveness mainly depends on the selectivity of the solvent such as water and organic solvents [19], [20], [21]. However, a past research shows that extraction by boiling at larger scale such as in food industry could be operated in order to obtain crude extractives [23]. After extraction, liquid extract or extractant that contains of high volume of water may degrade the bioactive compounds and lead to bacterial growth due to humidity, light, temperature and presence of oxygen [24]. Nevertheless, this can be avoided by evaporating the amount of solvent (water) using spray drying and rotary evaporating [25], [26], [27]. Drying process is used to ensure the products microbiological stability, reduce and avoid any chemical and biological degradation risks, lower the cost of storage and transportation, and lastly attain a product with specific characterization and properties [26]. Tannin was used as a deflocculant in drilling fluid to reduce the plastic viscosity and yield point value [28].

Thus, this study investigates the effect of using different drying techniques for transformation of water-based tannin extractant into powder. The physical and chemical properties of the powdered tannin from *Rhizophora Mucronata* using spray dryer and rotary evaporator were characterized. The properties of the powders are expected to be varied because of the different drying process and heating conditions.

II. MATERIALS AND METHODS

A. Extraction of Tannin by Boiling Prior To Drying Processes

150 g of unsieved milled bark sample was put in a porous sealed bag and placed in a beaker with distilled water. Distilled water was heated at temperature ranging from 80 to 90 °C and stirred for six hours using hot plate stirrer. Using bark/solvent ratio of 1:15, the volume of solvent was changed to 1500 mL. Distilled water was continuously added to maintain a constant volume of the solvent [21]. After boiling, the porous sealed bag was removed and the extractant was evaporated using spray dryer and vacuum rotary evaporator.

B. Formation of Tannin Powder Using Spray Dryer

The extractant was evaporated using a LabPlant SD-Basic spray dryer with the inlet temperature set at 130 °C. The compressor was switched on and the valve was opened at a pressure of 1 bar. The pump rate was set at 3 revolutions per minute (rpm). The extractant was sprayed and dispersed with a pressure nozzle. Throughout the drying process, the outlet temperature was in range of 75 – 80 °C. In the same direction of the hot air flow, the extractant was sprayed into a drying chamber. The chamber was designed such that the air flow rate provides a droplet resistance time in the chamber, so that the desired droplet moisture removal is completed. The amount of the powder collected in cyclone was packed and weighed.

C. Formation of Tannin Powder Using Vacuum Rotary Evaporator

Eyela vacuum rotary evaporator N-1200BS series was used. The extractant was loaded into round bottom flask and attached to the condenser. The temperature of the water bath was set at 80 °C and the round bottom flask was lowered into the water bath. The dial on the rotary evaporator was set at 6 for the spin speed. Then the chiller was turned on at temperature of 15 °C. The release valve at the top of the condenser was closed and the vacuum was turned on. Evaporation process was completed when all the extractants were stuck on the wall of the round bottom flask. The round bottom flask was placed in an oven at temperature 105 °C overnight. The extractives were obtained by scrapping the wall of the round bottom flask and was packed in an airtight container.

D. Characterization of Spray Dried and Rotary Evaporated Tannin Powder

Field Emission Scanning Electron Microscope (FESEM). The microstructure of the surface of the tannin powder was analysed for both samples using a field emission scanning electron microscope (FESEM) (Hitachi SU8220). Analyses of the sample surfaces were performed under vacuum, using a 10-kV acceleration voltage.

Fourier-transform infrared spectroscopy (FTIR) spectra analysis. FTIR was used to study the functional groups of the tannin powder. The analysis was performed using a Perkin Elmer Spectrum One instrument. 0.2 mg of tannin powder were deposited and scanned at wavelength of infrared was 4000–500 cm^{-1}.

Reverse-phase High Performance Liquid Chromatography (HPLC). A chromatograph, composed of an LC 410 pump and autosampler LC ISS 200 410 of Perkin–Elmer (Norwalk, USA), joined to a diode array detector 1100 of Hewlett-Packard (Palo Alto, USA) was used and set at 280 nm. For both analyses, the flow rate was set at 1ml/min, oven temperature 25 °C and injection volume at 20 µl. The column used was a Hewlett-Packard C18 Nucleosil 5 µm (200mm by 4mm i.d.). Solvents employed for elution were: A-methanol/H_3PO_4 (999:1); and B-H_2O/H_3PO_4 (999:1). The percentage gradient profile was as follows: 80B+20A in 0–40 min to 100A in 5 min. Polyphenols were identified by comparing retention times with those of pure standards and by spiking the samples with standard solutions.

Thermogravimetric analysis (TGA). Thermal decomposition was performed using a Mettler Toledo (TGA851/LF/1600) to study the thermal stability of the tannin powder. The tannin powder was weighed and heated from room temperature to 700 °C at a constant heating rate of 20 °C/min. The measurements were conducted under nitrogen gas purge of 30ml/min.

X-ray Powder Diffraction (XRD). The morphology of the particles was analysed using Rigaku (Tokyo, Japan) with Cu target and Ni filter. The data were collected in scanning angle of 3 to 90° and scanning speed of 1°/min. The accelerating voltage and the applied current were 40 kV and 40 mA, respectively.

III. RESULTS AND DISCUSSION

A. Field Emission Scanning Electron Microscope (FESEM)

The effects of spray dry and vacuum rotary evaporator on the physical structure of the powdered tannin from *Rhizophora Mucronata* were evaluated through FESEM. Fig. 1 shows different morphology of the produced particles using both methods. Powdered tannin using spray dry as shown in Fig. 1(a) was large but showed a shrunken surface due to rapid and high heating temperature during atomization of the droplets at 130 °C. On the other hand, powdered tannin produced using rotary evaporator consist of small and round shape particles with almost uniform size distribution as shown in Fig. 1(b), due to lower heating temperature than the spray drying technique i.e. approximately 80 °C. Besides, the finer size powder had passed through the exhaust due to ineffective separation at the cyclone [29].

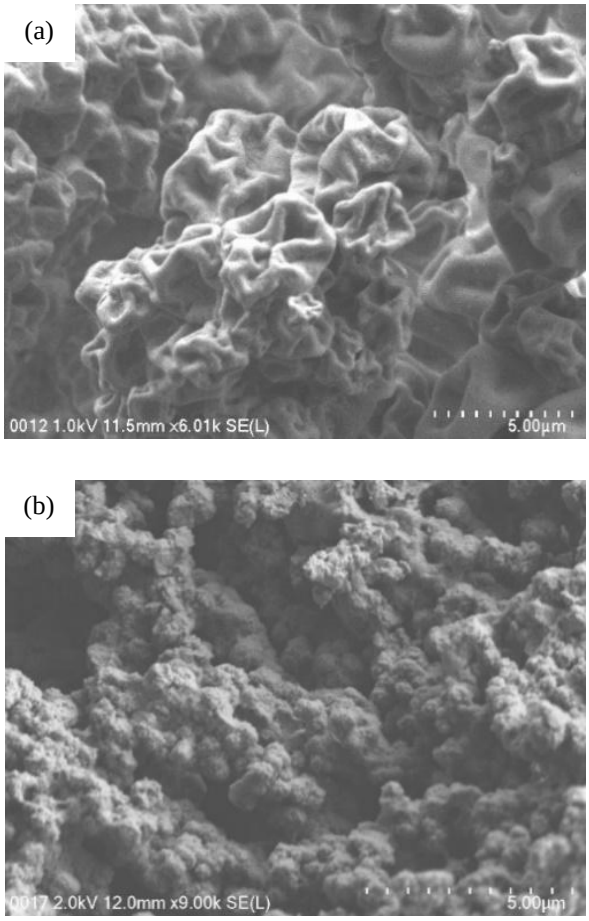

Fig. 1.Field Emission Scanning Electron Microscope (FESEM) images of powdered tannin by using (a) spray dried and (b) rotary evaporated methods.

B. Fourier-Transform Infrared Spectroscopy (FTIR) Spectra Analysis

The Fourier transform infrared spectroscopy (FTIR) analysis showed the presence of components of carbonyl and hydroxyl, which are the functional group of the powdered tannin.

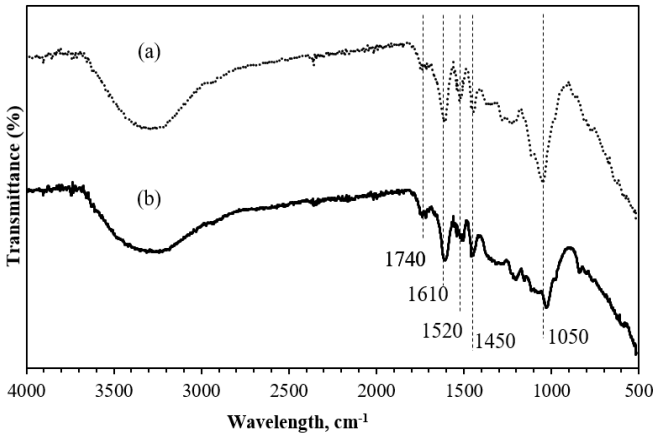

Fig. 2.Fourier-transform infrared spectroscopy (FTIR) spectra for powdered tannin sample using (a) spray dried (b) rotary evaporated methods.

Fig. 2 shows the spectra of powdered tannin from both drying techniques and it exhibited that the broad band absorption around region 3100 to 3500 cm^{-1} centred at 3243 and 3273 cm^{-1}. These bands exist due to the ample variety of hydrogen bonding between OH from presence of phenolic group (OH) stretching vibration [30]. A signal at 1740 cm^{-1} from both spectra is assigned to carbonyl groups of tannin which indicates the presence of catechin and epicatechin unit [31].

Most of the condensed tannins consisting in plant tissues are procyanidins, which are derived from catechin or epicatechin and may contain gallic acid esters [32]. Hence, single peak at 1520 cm^{-1} indicated that the tannins are predominantly consist of procyanidins [33].

The frequency peak at 1610 cm^{-1} of the carbon-carbon stretch shows the presence of C4-C8 interflavonoid linkages [34] and the phenolic compounds in the tannin were indicated with the occurrence of aromatic ring vibrations [35]. Moreover, various peaks are found in the region of 1400 to 1500 cm^{-1} and 1100 to 1300 cm^{-1} which correspond to the deformation vibration C-C bonds in the phenolic groups and aromatic C-H in-plane bending vibration bonds [36].

C. Reverse-Phase High Performance Liquid Chromatography (RP-HPLC)

The concentration and type of polyphenols present in the powdered tannin can be quantified and determined by RP-HPLC. Flavanol, (catechin) which is condensed tannin, and phenolic acid (gallic acid), which is hydrolysable tannin, were the two major classes of polyphenols that were analysed. Two main peaks were identified from the chromatogram in Fig. 3. Gallic acid corresponded at retention time of 4 minutes (min) for the first peak which has been compared with gallic acid standard [37]. The concentration of hydrolysable tannin in both powders were only 0.001%.

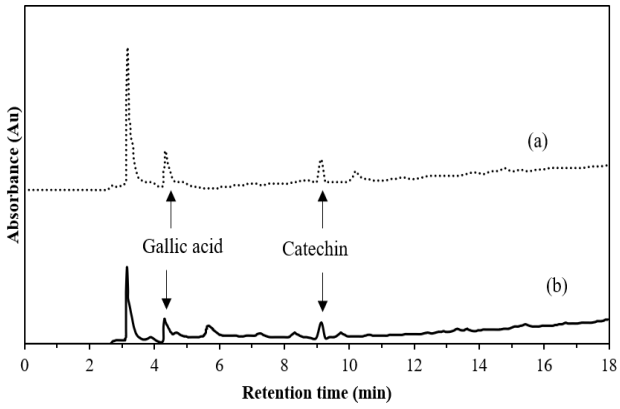

Fig. 3.Reverse-phase High Performance Liquid Chromatography (RP-HPLC) for powdered tannin by using (a) spray dried (b) rotary evaporated methods.

For the condensed tannin analysis of the powdered tannin, the samples were spiked with standard catechin solution and detected at the peak corresponded at retention time of 9.2 and 9.1 min for spray dried tannin and rotary evaporated tannin powder. The concentration of catechin measured for both spray dried and rotary evaporated tannin powder were 27.8% and 26.5% respectively. The concentration in spray dried was slightly higher than rotary evaporated due to some degradation of catechin during overnight exposure at 105°C in the oven during completion of drying process from rotary evaporated for producing the tannin powders [38].

D. Thermogravimetric Analysis (TGA) of the *Rhizophora Mucronata* Tannin Powder

Fig. 4 and 5, which show the dependence of the mass loss of the samples expressed as a percentage of the initial mass and temperature. In Fig. 4(a), for spray dried powder, first mass loss at 114°C was higher than the tannin powder from rotary evaporator which is 10%. This indicates that the spray dried powder contains more moisture than the other one. The major mass loss peaked at 270 °C [39], which the degradation of tannin occurred. The degradation accounts for 35% mass loss and leaving behind 55% char residue at 700 °C.

While for the rotary evaporated powder showed in Fig. 4(b), the first mass loss focused at 71 °C and about 4% mass loss which corresponds to the loss of water and absorbed moisture [40]. The second mass loss which corresponds to tannin decomposition of the powder started at 210 °C. After 700 °C, the carbonized residue remained was 51%. The results from this analysis can be compared to Pantoja-Castro and González-Rodríguez [41] which is on par with their findings. Moreover, Li, et al. [35] found that the tannin used in their experiment degraded at 225 °C and stated the thermal decomposition of tannin occurred at the range temperature of 150-350 °C.

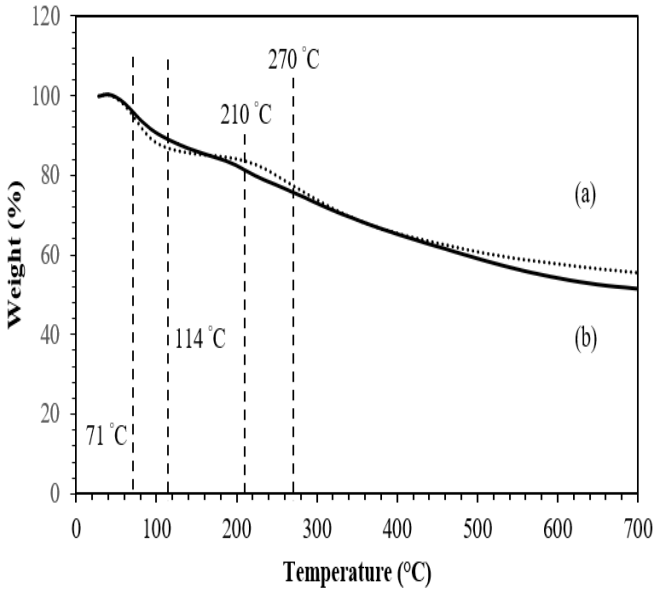

Fig. 4. Thermogravimetric analysis (TGA) Graph for powdered tannin using (a) spray dried and (b) rotary evaporated methods.

It was also evident from the Differential Thermogravimetry (DTG) curve in Fig. 5 that the degradation process under nitrogen occurred in two distinct steps. The first step indicates the loss of water and absorbed moisture and the second step is when the tannin starts to degrade. Tannin extractives from *Rhizophora Mucronata* were thermally stable than Aleppo pine tannin which decomposed at 179 °C and commercial tannins such as mimosa tannin, quebracho tannin and maritime pine tannin which decomposed at 146 °C, 145 °C and 130 °C respectively as reported by Saad, et al. [42].

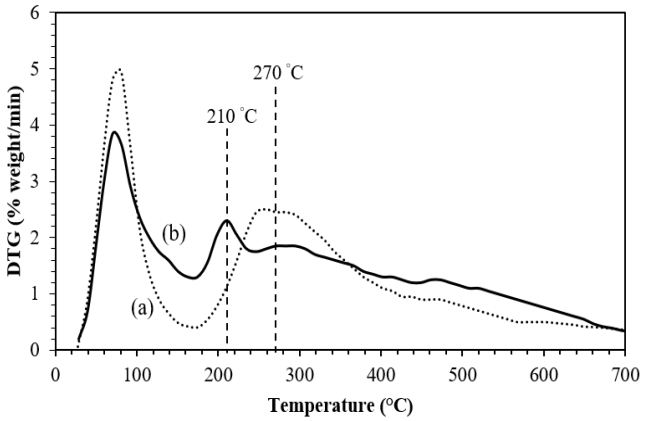

Fig. 5.Differential Thermogravimetry (DTG) curves for powdered tannin sample using (a) spray dried and (b) rotary evaporated methods.

E. X-ray Powder Diffraction (XRD)

The XRD patterns for both spray dried and rotary evaporated tannin powders are shown in Fig. 6. The tannin powder proved to have an amorphous structure as their diffraction peaks emerge in the 2θ = 15° - 30° range [43], [44]. The peaks that appear at around 2θ = 32°, 45° and 56° were comparable to the peaks of crystalline carbonaceous structure such as graphite. This affirms that as the tannin extractives and the small peaks are consistent with impurities naturally present in tannin [45].

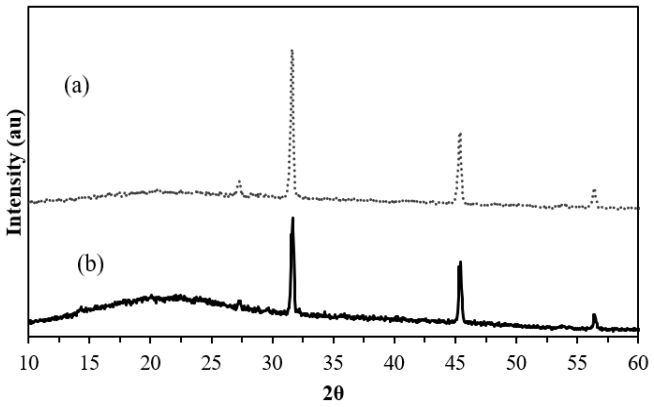

Fig. 6.X-ray Powder Diffraction (XRD) peaks for powdered tannin using (a) spray dried (b) rotary evaporated methods.

The intensity of crystalline peak at 2θ = 32° for spray dried tannin powder is higher than those extracted by rotary evaporated method. This difference indicates that tannin extractives from spray dried have more structured and stable compound compare to those extracted by rotary evaporated [46]. This statement is supported by the TGA (Fig 5) since tannin powder of spray dried has higher thermal decomposition at 270 °C and 210 °C for rotary evaporated.

IV. CONCLUSION

In this study, the formation of tannin powder was demonstrated by using two instruments; spray dryer and vacuum rotary evaporator respectively. The vacuum rotary evaporator produced a higher percentage of powdered tannin extractive than spray dryer due to the loss of the powder at the walls in the heating chamber of the spray dryer. The difference of heating temperature affected the morphology of powdered extractives based on FESEM analysis. The FTIR results verified that the condensed and hydrolysable tannin still existed in the powdered tannin. RP-HPLC confirmed the presence of condensed tannin in both powders with concentration of 27.8% and 26.5% for spray dried and rotary evaporated respectively, while 0.001% for hydrolysable tannin in both drying methods. Thermogravimetric analysis shows that thermal decomposition for both powdered tannin occurred in range of 150°C - 350°C. Furthermore, XRD analysis affirmed that both powdered tannins were amorphous structure but the spray dried tannin powder was higher in crystallinity than the rotary evaporated. This study suggests that spray drying technique is more suitable to be used for the extraction process as it will improve the thermal stability of the powdered tannin.

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