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Chemical functional groups of extractives, cellulose and lignin extracted from native *Leucaena leucocephala* bark

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Abstract

Bark from trees is considered a worthless raw material. However, this resource could be economically beneficial if utilized efficiently due to its rich chemical compounds. In this study, an ethanol toluene-soluble extractive, alpha-cellulose and lignin obtained from *Leucaena leucocephala* bark were characterized to determine their chemical functional groups. Based on FTIR spectral analysis, the results indicated that the bands of the functional groups of the extractive from the original bark remain unchanged; however, the absorbance intensity was found to be weaker in the group frequency and fingerprint regions. Removal of extractive, pectin, hemicellulose and lignin from the bark indirectly increased the strong absorbance intensity of cellulose. Broad peaks of OH stretching found in all spectra were assigned to the presence of phenolic OH and aliphatic structures for extractive and aromatic structures of lignin. It was revealed that aromatic functional groups were mainly found in the extractive, while water, carbonyl and ether were the dominant groups in cellulose, and methyl, methylene, carbonyl and carboxyl groups were enriched in lignin.

Graphic abstract

Extended author information available on the last page of the article

Introduction

Generally, barks from trees are regarded as worthless biomass residues and wastes and are mostly discarded, burned or used as fuel in the timber and sawmill industries (Lee and Lan 2006; Niokhor et al. 2009). However, some studies have reported that barks are rich in chemical compounds and could be beneficial to a variety of fields, ranging from the pharmaceutical industry to green polymers and bio-based materials (Pietarinen et al. 2006; Conde et al. 1996; Sen et al. 2010; Valentín et al. 2010). Utilization of these chemical compounds could also contribute to environmental conservation and the economy (Niokhor et al. 2009). All plant biomasses including barks, are rich in cellulose, hemicellulose, and lignin contents (Rutherford et al. 2012; Gonzalez-Vila et al. 2010). Bark, however, is different from other wood parts because it contains high amounts of water, organic soluble extractives, polyphenolics and lignin, as well as inorganic material referred to as ash (Fengel and Wegener 1984; Pereira et al. 2003). Thus, the characterization of chemical compounds and components in bark is vital before exploring their benefits and potential uses.

Infrared spectroscopy is currently one of the most important analytical techniques available to scientists in various fields that analyze almost any type of sample in the form of liquids, solutions, pastes, powders, films, fibers, gases and surfaces (Fan et al. 2012). The advantage of this tool is that it is fast, nondestructive, simple and low in cost (Roggo et al. 2007; Tsuchikawa 2007). In studies investigating lignocellulosic materials, FTIR spectroscopy was used to identify hydrogen bonds, chemical structures and compositions (Fan et al. 2012), certain functional groups or chemical bonds (Yang et al. 2012), phenolic compounds (Ghitescu et al. 2015) and aromatic compounds (Lee and Lan 2006). Apart from that, information obtained from FTIR analysis could also be used to optimize the experimental technique, to evaluate the effect of the operating conditions, to detect the presence of mineral matter and to determine gaseous reactions.

In another study, FTIR was also executed to investigate the differences in cellulose materials, especially in terms of crystallinity and hydrogen bonds of cellulose fibers in bark (Ciolacu et al. 2011). Furthermore, FTIR can examine the formation of inter- and intramolecular hydrogen bonds in cellulose. These properties strongly influence the physical properties of cellulose, including solubility (Kondo et al. 1994), hydroxyl reactivity (Kondo 1997) and crystallinity (Itagaki et al. 1997), and play an important role in the mechanical properties of cellulose (Kondo et al. 1994).

In Malaysia, *Leucaena leucocephala* is a native fast-growing tree often called 'Petai Belalang' by the local population. The genus *Leucaena* belongs to the family *Leguminosae* (flowering plants). This species has been discovered to possess much potential as timber plantation plants of Malaysia (MTIB 2017). Studies have found that the trunks and branches of *L. leucocephala* can be used as raw materials for composite products such as oriented strand boards (OSBs) (Wan Mohd Nazri et al. 2009), particle boards (Marzuki et al. 2011) and fiber boards (Ab. Rahman et al. 2018) This species is also a major raw material in mills for pulp and paper making (Kothiyal et al. 2012). It is known as a multipurpose tree and is widely used in gum production, furniture, construction timber, poles, etc. In fact, this species is now

widely planted to restore land, control erosion and participate in water conservation, reforestation, and land management and has been used as a cover crop and green manure. The density of *L. leucocephala* bark is approximately 690 kg/m^3 (medium to hardwood class timber), which is representative of hardwood species of Malaysia (Ahmad et al. 2011; Babatunde 2008).

In this study, local *L. leucocephala* bark from the trunk was investigated. Bark from this portion normally produces a high percentage of wastes/residues while peeling off from the trunk. Fine barks were screened and mixed before the extraction was conducted. The extracts, cellulose, and lignin were further analyzed using FTIR methods. All peaks of the spectra produced from the analysis were identified, interpreted and compared to resolve their functional group activities.

Materials and methods

Materials

An eleven-year-old *Leucaena leucocephala* tree was harvested from the UMS campus, Kota Kinabalu, Sabah, Malaysia. It was first verified by botanist experts from the Forestry Division, UMS. The trunk was cut from the bottom up to the first branch. The trunk was transferred to a mill for further handling. The bark of the tree was initially peeled manually before being taken to the mill. In this step, the inner and outer bark was not separated. This is because the separation is quite difficult and costly. Furthermore, Xavier et al. (2012) caution that it is important to maintain these fractions to enhance the phenolic content of the skin and improve the properties of produced resins. Then, all the samples were transferred to the Wood Chemistry Laboratory for further processing.

Preparation of samples

The bark was gently washed under tap water to remove dirt before being air-dried in the laboratory at room temperature at 24 °C for 2–3 weeks and protected from direct heat or sunlight. After being dried, the bark was chipped, flaked and pulverized into coarse powder using the laboratory grinder. The powder was then passed through a BS 500-um mesh sieve and retained in a 250-um mesh sieve to obtain 250–500 um particle sizes for extraction.

Extraction of bark

Bark powders were air-dried for several days until they reached the constant weights prior to chemical extraction. In laboratory work, the extractives were obtained by the ethanol toluene solubility method (ASTM D1105 2001), i.e., immersing the ground bark in ethanol-toluene solvent, rotor vaporizing and air-drying, while holocellulose

was obtained by bleaching the bark with four times addition of 1.5% $NaClO_2$ to remove lignin and produce air-dried holocellulose. Subsequently, oven-dried alpha-cellulose was produced by further treating the holocellulose with 17.5% NaOH and filtering the residues before washing them repeatedly with distilled water (ASTM D1103 2001). Then, lignin was obtained after filtering the lignin precipitates that occurred after the extractive-free bark was mixed with concentrated H_2SO_4 and heated with distilled water (ASTM D1106 2001).

Separation of extractives

Approximately 2 g of bark in a thimble (Whatman) was placed in a Soxhlet extraction flask. Extraction was performed with 150 ml of ethanol solution. 427 ml of toluene brought up to 1 L with the addition of ethanol. The solvent was mixed well. Extraction was conducted for 6 h with six siphoning steps. Subsequently, the flask was evaporated and dried in an oven at 103 ± 2 °C for 1 h before being cooled and weighed. Then, the ethanol-toluene soluble bark was ready for FTIR analysis of extractives.

Bleaching of lignin

Approximately 2 g of air-dried extractive-free bark powder was weighed and transferred to a 250-ml tall beaker. The flask was placed in a hot water bath at 70 °C after the addition of 100 ml of distilled water, 1.5 g of sodium chlorite and 5 ml of 10% acetic acid. The content was swirled every 5 min using a glass rod, making sure the flask was closed with a rounded flattened glass and the solution in the flask was just below the level of the water in the bath.

Approximately 5 ml of 10% acetic acid was added after 30 min, followed by the addition of 1.5 g of sodium chlorite for 30 min thereafter. This step was repeated 3 times, followed by a final addition of sodium chlorite. Subsequently, the suspension was cooled in an ice bath prior to filtering into a weighted crucible of porosity 1. Residue (white in color) was finally washed with acetone after being washed with iced distilled water. The residue was transferred to a desiccator after air-drying for a day until it was free of acetone. The sample was ready for cellulose extraction.

Extraction of cellulose

Air-dried holocellulose from the previous step was placed into a 250-ml tall beaker, and 15 ml of 17.5% NaOH was added. The solution was swirled using a magnetic stirrer for 1 min. After the addition of 10 ml of 17.5% NaOH, the solution was stirred for 45 s. Next, the mixture was allowed to stand for 3 min after stirring for 15 s with the addition of up to 10 ml of 17.5% NaOH. After 3 min, another 10 ml of 17.5% NaOH was added and stirred for 2.5 min. This step was repeated 3 more

times (total time: 15 min). The solution was allowed to stand for 30 min (total time: 45 min) before the addition of 100 ml of distilled water and swirling. Subsequently, the solution stood for 30 min (total time: 75 min).

The mixture was filtered into a weighted fritted glass crucible (coarse porosity 3). The beaker and residue were rinsed with 25 ml of 8.3% NaOH solution before being washed with 650 ml of distilled water at 20 °C. Filtration was stopped, and the crucible was filled with 2 N acetic acid for 5 min. Filtration was continued, and the residue was rinsed with distilled water. The residue was oven-dried at 103 ± 2 °C for 24 h before being cooled and weighed. Alpha-cellulose of bark was ready for FTIR analysis.

Lignin extraction

Approximately 1.4 g of air-dried extractive free bark powder was weighed out accurately and transferred to a 50-ml tall beaker. Fifteen milliliters of 72% sulfuric acid was added carefully with a pipette, and the mixture was stirred with a small glass rod (which was left in the beaker). The beaker was placed in a cold water immersion at 20 °C for 2 h with stirring every 10 min. At the end of the period, the mixture was transferred to a 1 L Erlenmeyer flask containing 560 ml of hot distilled water. A condenser reflux was connected to an Erlenmeyer flask, and the sample was boiled on a hot plate for 4 h.

When refluxing was completed, the insoluble lignin was recovered by filtration through the known weight of the crucible (porosity 4). The residue was rinsed with 500 ml of hot water and dried in an oven at 103 ± 2 °C for 24 h before being cooled and weighed. The lignin content was ready for further FTIR analysis.

FTIR analysis of barks

Approximately 500 mg of the fine powder of extractives was used for FTIR analysis to determine functional groups. The sample powder was placed directly on the diamond crystal or light path, and the reflectance spectra were collected. FTIR spectra were recorded on a Perkin-Elmer FTIR (model spectrum 100 series, USA) at ambient temperature, in the wavenumber range of 4000 to 400 cm^{-1} and at the resolution of 4 cm^{-1}. Each spectrum was collected from an average of 4 scans, and the scan speed was 0.2 cm^{-1} s^{-1}. The spectrum was interpreted to determine the functional group of extractives. This analysis was also conducted for cellulose and lignin. The spectrum from the original bark was used as a comparison.

Results and discussion

FTIR analysis of *Leucaena leucocephala* stem bark

In a previous study (Salim et al. 2019), extraction yields of *L. leucocephala* stem bark for extractives, cellulose and lignin were found to be 8.39%, 29.19% and

38.24%, respectively. For chemical characterization, FTIR was used to determine functional groups of the components. The FTIR used infrared light as an energy source and the spectra produced used wavenumbers as units that were directly proportional to energy (a higher wavenumber corresponding to a higher energy); high intensity for FTIR typically means strong bonding, and weak absorption means weaker bonding of the chemical component. High intensity of these contents also means a high chemical content of the *L. leucocephala* extracted components. The spectrum of the original bark was used for comparison before and after extraction.

Functional group of the main chemical components of bark

The IR absorption spectra of the bark components (extractives, cellulose and lignin) and the original bark, recorded within the range of 4000–500 cm^{-1} are shown in Fig. 1.

The spectrum of the extractives demonstrates the highest peak with a sharp and broad shape followed by the spectra of lignin and cellulose, which mostly appear as small and tiny peaks. The original bark shows similarly shaped peaks with lignin and cellulose but at lower intensity. However, several peaks of the bark overlapped with the extractives. The majority of the peaks were sharp in the fingerprint region (1800–500 cm^{-1}), while in the group frequency region (1800–4000 cm^{-1}), common peaks were broader than the others. The intensity of the group frequency and fingerprint regions recorded in the range of 40.55 to 116.65 (a.u) shows that cellulose and lignin had stronger absorption bands and dominated the highest intensity
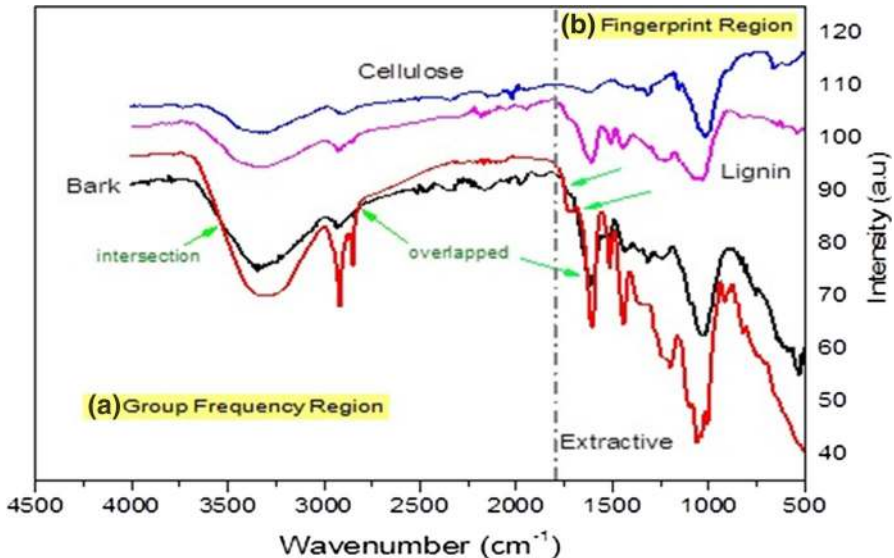

Fig. 1 FTIR spectra of chemical components of *L. leucocephala* bark **a** Group frequency region, **b** fingerprint region

range at 100.24 to 116.65 (a.u) and at 92.14 to 107.40 (a.u), respectively. Moreover, extractives and the original bark had the lowest intensity ranges of 40.55–74.82 and 55.03–93.70 a.u, respectively.

Compared to the original bark, extractives showed broader peaks and intersected at 3538 cm^{-1} and 2871 cm^{-1} in the group frequency region. The peak of extractives also were sharp at 2918 cm^{-1} and, at the same time, showed a new sharp peak at 2850 cm^{-1} (Fig. 1). The appearance of these two sharp peaks revealed the formation of a new bond of the extractives.

In the fingerprint region, the overlapping peak between the original bark and extractives at 1753–1530 cm^{-1} indicates that the extractives have a similar composition after separation from the bark. The peaks are consistent with the same functionalities in the tannin of *L. leucocephala* bark in this study. However, an extraction reduces the absorbance intensity of extractives, even though bands of the functional groups of the extractives remain unchanged (Eberhardt et al. 2007) due to an opening of the cyclic ether structure of polyflavonoids (Soto et al. 2005).

Holocellulose (hemicellulose and alpha-cellulose) was obtained by bleaching the extractive-free bark using sodium chlorite to remove lignin. Hemicellulose is the most unstable component and easily decomposes in sodium hydroxide (NaOH) solution. Thus, when holocellulose is treated with 17.5% NaOH, alpha-cellulose or cellulose is obtained. These processes contributed to the disappearance of three peaks of lignin in the cellulose spectrum, as illustrated in Fig. 1. The absence of lignin caused OH peaks to appear in the cellulose spectrum, indicating that hemicellulose and lignin were removed extensively during chemical treatment (Li et al. 2009). Removal of extractives, pectin, hemicellulose and lignin from the original bark contributed indirectly to the increase in the intensity absorbance of cellulose, as shown in Fig. 1.

Lignin extraction involves the breakdown of cellulose structures by sulfuric acid. This concentrated acid is able to absorb water so strongly that it can breakdown cellulose into carbon and water and subsequently separate lignin as a precipitate.
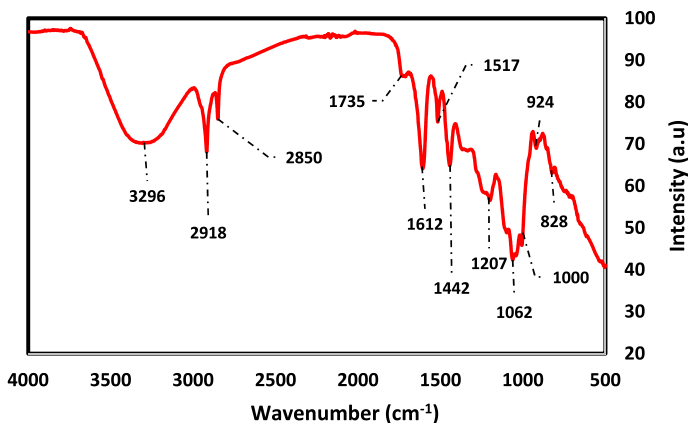

Fig. 2 FTIR spectra of extractives of *L. leucocephala* bark

Figure 1 shows the absorbance intensity of lignin, which is lower than that of cellulose. Lignin intensity, however, was found to increase after the removal of extractives, pectin and hemicellulose (Ciolacu et al. 2011).

Extractives of bark

Figure 2 shows the functional group of FTIR spectra of the extractives of *Leucaena leucocephala* bark.

The broad and strong band at 3296 cm^{-1} was from the hydroxyl groups due to moisture in the bark. It is assigned as an OH stretch vibration in phenolic and aliphatic structures. This finding is almost similar to findings by previous researchers, who found that the band at 3300 cm^{-1} represents the stretching vibration of hydrogen bonded –OH groups in phenolic and aliphatic structures (Caron 2010; Williams and Fleming 1989). Feng et al. (2016) also found the broad spectra of OH stretching at the wavelengths of 3600–3200 cm^{-1}, which may be attributed to the presence of phenolic OH.

The two sharp and strong absorbed peaks that appeared at 2918 cm^{-1} (symmetric) and asymmetrically at 2850 cm^{-1} in Fig. 2 are attributed to the CH stretching vibration in the aromatic methoxyl group and methyl and methylene groups. Dirckx et al. (1992) also indicate that a similar value of the signal at 2850 cm^{-1} raised the C–H stretching vibration in the aromatic methoxyl groups of lignin and methyl (–CH$_3$) and methylene (–CH$_2$) groups of side chains. This is similar to a study by Ferreira et al. (2013), who found that major peaks at 2920 and 2851 cm^{-1} are mainly attributed to the aliphatic chains of suberin, accounting for asymmetric and symmetric C–H stretching vibrations, respectively. Zhao et al. (2013) also imply that the main structures of the resins from bark changed to poly-aromatic structures during thermal degradation. The intensities of the peaks at 2914 and 2848 cm^{-1} were due to the aliphatic CH$_2$ asymmetric stretch and aliphatic CH$_2$ symmetric stretch, respectively. Lee and Lan (2006) also confirm that FTIR analysis showed that an aromatic compound is the main component in bark extracts.

The shoulder peak at 1735 cm^{-1} in Fig. 2 is assigned to the carbonyl group, C=O. Previous researchers found that the presence of C=O stretching vibration at 1735 cm^{-1} corresponds essentially to the ester groups in suberin (Ferreira et al. 2013) and the carbonyl group (Eberhardt et al. 2007). Sinha and Rout (2009) also confirm the absorption bands at 1730 cm^{-1} assigned to C–O stretching in the carboxyl and ketone groups. The peak centered at 1736 cm^{-1} is attributed to the acetyl and uronic ester groups of hemicellulose and ρ-coumaric acids of lignin and/or hemicellulose (Li et al. 2009). Moreover, Soto et al. (2005) suggest that the peaks between 1400 and 2000 cm^{-1} show the aromatic nature of the compound in the extract.

C=C stretching in the aromatic ring appeared at 1612 cm^{-1} and 1442 cm^{-1} (Cheng et al. 2011; Feng et al. 2014). Figure 2 also shows that the intensities of the absorption bands at 1612, 1517 and 1442 cm^{-1} were due to the aromatic stretching vibration. At 1442 cm^{-1}, OH in-plane deformation absorption bands were weaker. The intensity of the bands is attributed to the carbohydrates that have diminished

significantly in all extract spectra. This indicates that few carbohydrates have been extracted under the experimental conditions.

The presence of C–O–C stretch was detected at 1207 cm^{-1} (Dirckx et al. 1992; Schwanninger et al. 2004) as shown in Fig. 2. Moreover, the lower intensity of the peak was absorbed at 1062 cm^{-1} corresponding to –CO stretch vibration. Li et al. (2009) also confirm that the peak at 1061 cm^{-1} is associated with the C–O stretching that appeared in all of the spectra. The cyclic nature of the ether was reflected by the signal located at 1030 cm^{-1}, which was produced by the aliphatic C–O stretching. However, Liu et al. (2010) declared that the region between 1000 and 1150 cm^{-1} corresponded to the strength vibration of C–OH side groups and the C–O–C gly-cosidic bond vibration. Figure 2 shows the FTIR spectra of extractives of *L. leuco-cephala* bark. The band intensity detected at 1000 cm^{-1} is attributed to C–OH side groups (Feng et al. 2016), while the peak at 924 cm^{-1} region is assigned to the C-H bending vibration (Liu et al. 2011a). Absorbance at 828 cm^{-1} is attributed to the –CH bend in aromatics (Feng et al. 2016). The absorption peaks located at approximately 828 cm^{-1} are also attributed to –C–N stretching vibrations of the amine groups (Shameli et al. 2012).

Cellulose of bark

Figure 3 shows a large and broader absorption band detected in the range of 3000–3750 cm^{-1} assigned to the stretching vibration of O–H groups.

This finding is supported by Sathishkumar et al. (2013), who found that the region at approximately 3340 cm^{-1} is attributed to hydroxyl group (OH) activities. Sara-vanakumar et al. (2013) also showed that the peak that appeared at 3342 cm^{-1} was due to the OH stretching of alpha-cellulose. The broad band in the 3600–3100 cm^{-1} region, which is due to the OH stretching vibration, gives considerable information concerning the hydrogen bands, which can be correlated with the scission of the intra- and inter-molecular hydrogen bonds (Ciolacu et al. 2011).
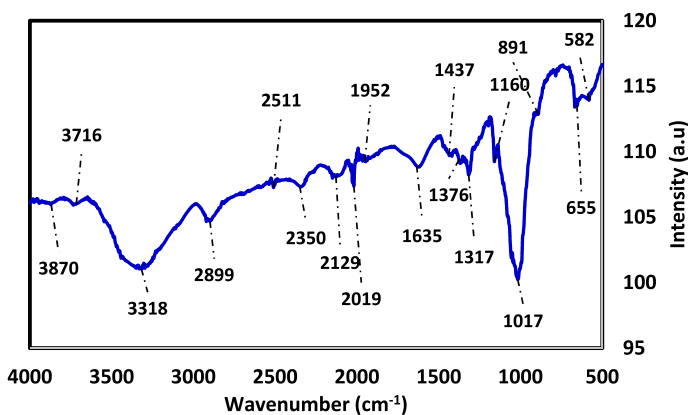

Fig. 3 FTIR spectra of cellulose of *L. leucocephala* bark

The peak at 2899 cm^{-1} is attributed to the C–H stretching vibration (Fig. 3). Ciolacu et al. (2011) found that the presence of amorphous cellulose samples can be further confirmed by the shift of the band from 2900 cm^{-1}, which corresponded to the C–H stretching vibration. The peak at 2848 cm^{-1} is associated with the CH_2 groups of cellulose and hemicellulose (Fiore et al. 2014; Indran et al. 2014).

In Fig. 3, the peaks appearing at 2350 cm^{-1} are due to the presence of carbon dioxide, while the peaks at 2129 and 2019 cm^{-1} are assigned to the C=C stretch vibration. The appearance of these three peaks might possibly be due to the delignification of bark during cellulose extraction. Li et al. (2009) believe that the peak was predominantly contributed by the aromatic C=C stretching of the aromatic ring in the lignin since this peak disappeared in the other three spectra due to the removal of lignin. The band at the 1635 cm^{-1} region is attributed to the adsorbed water as reported by Liu et al. (2011a) for mulberry bark. The appearance of a tiny shoulder at 1437 cm^{-1} and the peak at 1376 cm^{-1} in Fig. 3 indicates the characteristic peaks of aromatic skeletal vibration (Liu et al. 2011a; Sun et al. 2005) and methoxyl vibration (Moran et al. 2008), due to the presence of small amounts of lignin. In addition, the FTIR absorption band at 1430 cm^{-1} was related to the symmetric CH_2 bending vibration (Ciolacu et al. 2011). This band is also known as the "crystallinity band," and a decrease in its intensity reflects a reduction in the degree of crystallinity of the samples (Ciolacu et al. 2011). Subramanium et al. (2005) found that the peak at approximately 1427 cm^{-1} and 1371 cm^{-1} corresponds to CH_2 and CH groups of cellulose (Belouadah et al. 2015). The tiny peak found at 1317 cm^{-1} as shown in Fig. 3, is attributed to the presence of the small amount of C–O stretching vibration of the syringyl ring units (Ghitescu et al. 2015; Shi et al. 2011; Faix 1992), while the peak at 1158 cm^{-1} signifies the C–O–C groups of cellulose and hemicellulose (Seki et al. 2013; Belouadah et al. 2015). The sharp tiny peak located at 1160 cm^{-1} is linked to the C–O–C asymmetrical stretching.

Liu et al. (2010) state that the region between 1000 and 1150 cm^{-1} corresponds to the strength vibration and the C–O–C glycosidic bond vibration, and this is almost similar to the peak at 1160 cm^{-1} as evidenced in Fig. 3. The broad sharp peak absorbed at the 1017 cm^{-1} region as shown in Fig. 3 is attributed to the C–O stretching vibration from the cellulose, which increased the peak intensity after the removal of pectin, hemicellulose and lignin (Liu et al. 2011b). Ciolacu et al. (2011) and Li et al. (2009) confirm that 897 cm^{-1} is associated with the C-H rocking vibrations of cellulose, which appeared in all of the spectra. The features of the characteristic region (1700–850 cm^{-1}) are due to the constituents of alpha-cellulose, lignin and hemicellulose (Khan et al. 2005; Miao et al. 2016). The band at 891 cm^{-1}, shown in Fig. 3 corresponds to the C-H rocking vibrations from the cellulose. However, the presence of a peak at approximately 898 cm^{-1} is associated with the C–O groups, which are related to the β-glycosidic linkages (Reddy et al. 2014; Seki et al. 2013). The little peaks at approximately 662 cm^{-1} and 588 cm^{-1} correspond to the C–OH bending (Fan et al. 2012; De Rosa et al. 2010). These similar peaks are also found at 655 and 582 cm^{-1}, as illustrated in Fig. 3.
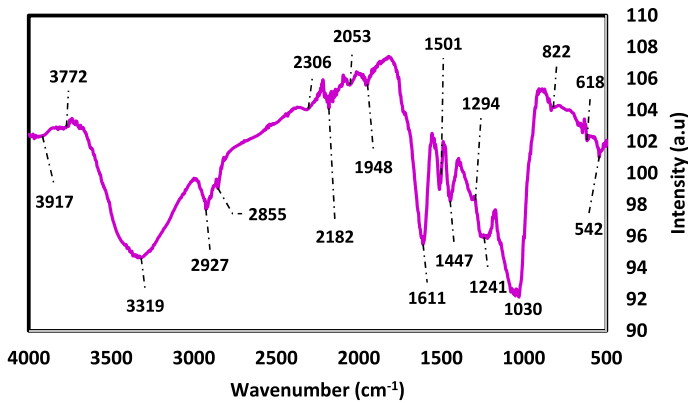
Fig. 4 FTIR spectra of lignin of *L. leucocephala* bark

Lignin of bark

Figure 4 shows the FTIR spectrum of the lignin *L. leucocephala* bark. The band at 3319 cm^{-1} is attributed to the OH groups in lignin. Guo et al. (2008) found that the broad band at 3412 cm^{-1} was dominated by the stretching vibrations of aromatic and aliphatic OH groups.

Peaks appearing at 2927 and 2855 cm^{-1} could possibly correspond to the C–H stretching in the lignin molecules. Sahoo et al. (2011) found this chemical group at 2919 and 2849 cm^{-1}, and Guo et al. (2008) found that the peaks at 2925 and 2849 cm^{-1} predominantly arose from the C–H stretching in methyl of aromatic and methylene groups of side chains and aromatic methoxyl groups. Similar findings by El Mansouri and Salvado (2007) and Gosselink et al. (2004) indicated that C–H stretch in methyl and methylene groups is present in regions between 3000 and 2800 cm^{-1}.

The presence of three small peaks at 2182, 2053 and 1948 cm^{-1}, as shown in Fig. 4, was attributed to the appearance of NCO from isocyanate groups. Schaber et al. (2004) found this functional group peak at 2167 cm^{-1}. The strong and sharp peak at 1611 cm^{-1} was attributed to the aldehyde group of lignin. Wahab et al. (2010) also confirmed this group at 1616 cm^{-1}. According to Gosselink et al. (2004), aromatic skeletal vibration bands were observed for all lignins at 1600 and 1500 cm^{-1} wavenumber ranges. Thus, the medium and sharp peaks found at 1501 cm^{-1} in Fig. 4 could be attributed to CH deformation (methyl and methylene), while the peak at 1447 cm^{-1} band could correspond to the aromatic skeletal vibration combined with C-H in plane deformation. The peaks at 1460, 1510 and 1600 cm^{-1} in the FTIR spectra are assigned to C–H deformations (asymmetric in methyl, methylene and methoxyl group), the aromatic skeletal vibrations coupled with C–H in plane deformations, and the aromatic skeletal vibrations, respectively (Tian et al. 2010).

In Fig. 4, lignin structures at 1294 and 1241 cm^{-1} wavenumber ranges are possibly due to the C=O stretching of syringyl and guaiacyl ring, respectively. Shi et al. (2011) confirmed this compound at 1280 and 1120 cm^{-1}, and Guo et al. (2008) affirmed this absorption at 1329 cm^{-1} (syringyl) and 1217 cm^{-1} (guaiacyl). The peak at 1030 cm^{-1} corresponded to the guaiacyl type units (Fig. 4). However, Kosikova and Slavikova (2004) found that the C–O bands at 1097 and 1035 cm^{-1} were connected to the secondary and primary alcohols, respectively. They do not disappear because they fall together with the C–O stretch from aliphatic ethers and the aromatic guaiacyl type C–H, respectively, both of which were unaffected by the butyration reaction.

The small peak at 822 cm^{-1} is attributed to the C–H stretch vibration, and two peaks appeared at 618 and 542 cm^{-1} band corresponding to the C–H bend, as shown in Fig. 4. Dirckx et al. (1992) and Schwanninger et al. (2004) also found that the aromatic –CH stretch vibration was detected for all wavenumbers less than 900 cm^{-1}.

Main chemical functional groups of *Leucaena leucocephala* stem bark

Based on the results and discussion, the functional groups of extractives, cellulose and lignin of *Leucaena leucocephala* stem bark are summarized in Table 1.

Aromatic compounds were the main functional groups (Table 1). They appear sharply at 1612, 1517 and 1442 cm^{-1} band, followed by methyl and methylene at 2918 and 2850 cm^{-1} wavenumber range, while a strong, broad and wide peak in the 3296 cm^{-1} region is dedicated to the hydroxyl group. The rest of the compounds, which existed as small peaks (carbonyl and ether), tiny (alkene) and very tiny shapes (alkane), appeared weak and less functional in the bark extractives. According to Feng et al. (2016), IR spectra of all the bark are similar with respect to the IR absorbance of some typical functional groups, i.e., (a) aromatics, (b) methylene (–CH$_2$-), (c) C–O–C and (d) Ar–CH.

Table 1 also lists the carbonyl and hydroxyl groups as the main functional groups of cellulose. Carbonyl and hydroxyl possess the highest intensity and sharp peak at 1017 cm^{-1} and broad and wide peak at 3318 cm^{-1}, respectively. H–O–H deformation of absorbed water was observed at 1635 cm^{-1}, while a methyl group existed in the 2899 cm^{-1} region. The peaks were of sharp shape and medium width. Carbon dioxide, alkyne, alkane, carbonyl and carboxyl groups, on the other hand, were present as small and tiny peaks. Apart from that, ethers, methyl and carboxyl existed as very tiny peaks and with less functionality. The formation of inter- and intra-molecular hydrogen bonds in the cellulose not only has a strong influence on the physical properties of cellulose, including solubility (Kondo et al. 1994; Kondo 1997), hydroxyl reactivity (Kondo 1997) and crystallinity (Itagaki et al. 1997), but also plays an important role in the mechanical properties of the cellulose (Kondo et al. 1994).

For lignin, carbonyl (1611 cm^{-1}), carboxyl (1030 cm^{-1}) and hydroxyl (3319 cm^{-1}) were the main functional groups, where the peaks clearly appeared with broad, sharp and wide shapes (Table 1). The majority of the chemical function

Table 1 Summary of the peak location and shape of the IR bands of the main chemical functional groups of *L. leucocephala* stem bark obtained from FTIR analysis

Component	Peak wavenumber (cm^{-1})	Functional group		Peak shape/size
Extractive	3296	OH	Phenolic and aliphatic structures	Broad, width
	2918	C–H	Methylene group	Broad, sharp
	2850	C–H	Methyl, methylene and methoxyl groups	Medium, sharp
	1735	C–O	Carbonyl	Shoulder
	1612	C=C	Aromatic	Broad, sharp
	1517	C=C	Aromatic	Medium, sharp
	1442	C=C	Aromatic	Broad, sharp
	1207	C–O–C	Ether	Small
	1062	C–O	Carbonyl	Small
	1000	C–OH	Carboxyl	Very tiny
	924	C–H	Alkene	Tiny
	828	C–H	Alkane	Very tiny
Cellulose	3318	OH	Hydroxyl group for alpha-cellulose	Broad
	2899	C–H	Methyl group	Medium
	2350	–COO	Carbon dioxide	Small
	2129	C=C	Alkyne	Small
	2019	C=C	Alkene	Tiny sharp
	1635	H–O–H	Adsorbed water	Medium
	1437	CH$_2$	Alkane	Tiny
	1376	C–H	Alkane	Tiny
	1317	C–O	Carbonyl	Tiny sharp
	1160	C–O–C	Ethers	Very tiny sharp
	1017	C–O	Carbonyl	High, sharp
	891	C–H	Methyl	Very tiny shoulder
	655	C–OH	Carboxyl	Asymmetric tiny shoulder
	582	C–OH	Carboxyl	Very tiny

Table 1 (continued)

Component	Peak wavenumber (cm^{-1})	Functional group		Peak shape/size
Lignin	3319	OH	Aromatic and aliphatic OH groups	Broad, width
	2927	C–H	Methyl, methylene and methoxyl groups	Medium
	2855	C–H	Methyl, methylene and methoxyl groups	Very tiny
	2182	NCO	Isocyanate groups	Small sharp
	2053	NCO	Isocyanate groups	Small sharp
	1948	NCO	Isocyanates groups	Small sharp
	1611	C=O	Aldehyde groups	Broad, sharp
	1501	C–H	C-H deformation (methyl and methylene)	Medium, sharp
	1447	C–H	Aromatic skeletal vibration combined with C-H in plane deformation	Medium, sharp
	1294	C–O	C-O stretching of syringyl ring	Very tiny shoulder
	1241	C–O	C-O stretching of guaiacyl ring	Medium
	1030	C–OH	C–OH stretch vibration in Guaiacyl units	Broad
	822	C–H	C-H rock vibration	Very tiny
	618	C–H	Alkyne	Very tiny
	542	C–H	Alkanes	Tiny sharp

of lignin spectra is attributed to the methyl, methylene and methoxyl groups (2927, 2855, 1501 and 1447 cm^{-1}), which existed in medium sharp peaks. Isocyanate and aromatic compounds were also detected in lignin as small, sharp and very tiny peaks. According to Gosselink et al. (2004), the hydroxyl, methoxyl, carbonyl, and carboxyl groups are the most important chemical functional groups in lignin, and they can be used for its identification (Shamsuri and Abdullah, 2010). However, lignin should be differentiated according to the hydroxyl groups and amine contents (Sahoo et al. 2011).

Conclusion

Leucaena leucocephala barks were characterized to determine their chemical functional groups. The extractives, cellulose and lignin of *L. leucocephala* bark were extracted through separation methods before the functional groups were determined. The research results found that the separation of the extractives from

the original bark did not change the bands of the functional groups; however, the absorbance intensity weakened in the group frequency and fingerprint regions. Removal of extractives, pectin, hemicellulose and lignin from the original bark contributed indirectly to the increase in the intensity absorbance of cellulose. Broad peaks of OH stretching were found for all the spectra, which are attributed to the presence of phenolic OH and aliphatic structures of extractives and the aromatic structures of lignin. This study also contributed to the abundant information concerning hydrogen bonds, which were correlated with the scission of the intra- and inter-molecular hydrogen bonds of cellulose. Overall, the results from this study indicate that an aromatic compound is the main chemical functional group of extractives, while water, carbonyl and ether are the main components of cellulose. In addition, it was found that methyl, methylene, carbonyl and carboxyl groups are the main chemical functional groups of lignin in *L. leucocephala* stem bark.

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