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Isolation and Physicochemical Characterization of Lignin from *Chromolaena Odorata* and *Tithonia Diversifolia*

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ABSTRACT: This study dealt with isolation of lignin from soft woods namely; *Chromolaena odorata* (Siam Weed) and *Tithonia diversifolia* (Mexican Sunflower) using alkali pretreatment method. The raw samples were characterized by some physico-chemical parameters before extraction. *Chromolaena odorata* gave higher lignin yield and lesser ash content of 15.12 % and 4.22 % respectively compared to *Tithonia diversifolia* with 7.40 % and 9.56 % respectively. The isolated lignins were characterized by some physico-chemical parameters and spectroscopy methods like Futran Transform Infrared Spectroscopy (FT-IR) and Scanning Electron Microscopy (SEM). Lignin from *Chromolaena odorata* was found to be more pure than the one from *Tithonia diversifolia* as evident from the values of ash content (4.22%) and klason lignin (33.65 %) for *Chromolaena odorata* while ash content (9.56 %) and klason lignin (21.96 %) were obtained from *Tithonia diversifolia*. The FT-IR spectra of both lignins revealed the presence of syringyl and guaiacyl units. © JASEM

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KEYWORDS: *Chromolaena odorata, Tithonia diversifolia*, Lignin, FT-IR, Physico-chemical parameters.

Introduction

Lignin is a renewable material obtained in huge quantities as a by-product of the pulp industry. It has mainly been used as a fuel and only a small amount has been isolated and commercialized. Nevertheless, worldwide, amount of lignin has been estimated at 2% and corresponds to 1 million tons per year (Ammar *et al.*, 2014). This macromolecule is complex and it is widely accepted that the biosynthesis of lignin stems from the polymerization of three types of phenylpropane units, normally referred to as monolignols (Lipersguer *et al.*, 2009). These units are coniferyl, sinapyl and p-coumaryl alcohols. The three structures are depicted in Figure 1.

The amount and composition of lignin vary among taxa cell types and individual cell wall layers. Softwood lignin consists almost exclusively of G-type lignin, hardwood lignin consists of G and S-type units (H units being minor components), while gramineae has all the three (Kang et al., 2012). Additionally, based on its interesting functionalities and properties, lignin offers a perspective for higher value-added applications. The interest in developing lignin-based applications is nowadays driven by three important factors, namely (i) the availability of new lignin sources, such as, sulfur-free, organosolv, steam explosion lignins and so on. (ii) the growing interest in the bio-refinery concept, where lignin valorization is becoming relevant, because it is the only bio-sourced molecule containing aromatic moieties, and (iii) the approach of sustainable chemistry, where green processes and bio-based products are in focus (Ammar *et al.*, 2014).

However, it has long been recognized for its negative impact on forage quality, paper-making and cellulosic biofuel production. Therefore, elucidating the lignin structure can play an important guiding role on efforts to remove lignin fractions from lignocellulosic materials. Moreover, effectively overcoming the recalcitrance is an important and urgent research priority for the development and implementation of the lignocellulosic biorefinery concept (Meng, *et. al.*, 2012).

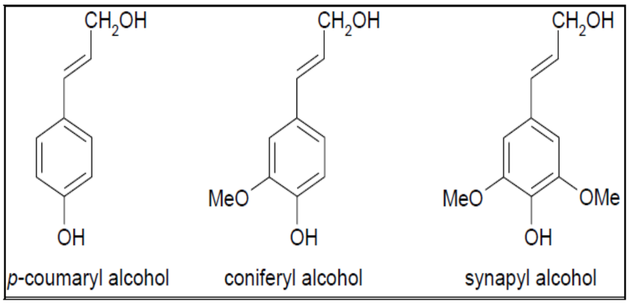


Fig 1: The major building blocks of lignin (Dence and Lin, 1992)

Many works on isolation of lignin from different plants and/or agricultural wastes are available in literature (Ammar *et al.*, 2014; Rencoret *et al.*, 2011; Mohammad et al, 2006).

Chromolaena odorata commonly known as Siam weed is a tropical species of flowering shrub in the

Corresponding author Email: f.o.nwosu@gmail.com, nwooyef@yahoo.com sunflower family, Asteraceae. It is an invasive deep-rooted shrub recorded as part of the 100 worst invasive species in the world (Iondoh *et al.*, 2013). (Baxter (1995) has noted that the plant spreads widely throughout the tropics and has been declared as a noxious weed due to the difficulty in controlling it by both curative and preventive measures. *Tithonia diversifolia* commonly known as Mexican sunflower was probably introduced into West Africa as an ornamental plant, a member of the family Asteraceae. It is an annual, aggressive weed growing to a height of about 2.5 m and adaptable to most soils. It had been observed to be widely spread in Nigeria where it is found growing on abandoned/waste lands, along major roads and waterways and on cultivated farmlands (Olabode *et al*., 2007).

This study is aimed at isolating and examination of physico-chemical characterization of lignin from both *Chromolaena odorata* and *Tithonia diversifolia* stems.

MATERIALS AND METHOD

Materials: The *C. odorata* and *T. diversifolia* used in this work were collected from along road side towards the gate of university of Ilorin, Nigeria. The leaves were detached and the stems were washed, rinsed with distilled water, in order to eliminate sand and contamination, cut into small pieces, then air dried under laboratory conditions of temperature of 30 °C. The obtained materials were milled and sieved to particle sizes in the range of 0.5-1.0 mm. The powdered sample was kept in air tight container prior to extraction.

Extraction of lignin: The extraction of lignin from *C. odorata* and *T. diversifolia* was carried out by alkali pretreatment method using 5M NaOH solution in a round bottom flask using a Solid to liquid ratio of 1: 10. The flask was equipped with a condenser and placed on heating mantle set at 100 °C for 7 hours. The mixture was filtered and the filtrate (black liquor) precipitated with 50 % H_2SO_4 solution and made up to pH 2 with the aid of addition of either 2 M of NaOH or H_2SO_4. The lignin was isolated through vacuum filtration and washed with acidified water (pH=2) several times. The lignin cake was then socked dry under vacuum and finally dried in an oven at 50 °C for 8 hours (Khan and Ashraf, 2006).

Characterization of extracted lignin: The physico-chemical parameters such as pH, conductivity, bulk density, moisture content, ash content were determined using standard methods as described in literature respectively (Khan and Ashraf, 2006; Ahmenda *et al*. 2000; Yoshiguki and Yukata, 2003;Toledano et al.,2012).

Determination of acid insoluble and acid soluble lignin: Acid insoluble lignin (AIL) which is also known as klason lignin was determined by subjecting lignin to an acid hydrolysis process. The acidic hydrolysis was carried out by adding 3.75 ml of sulphuric acid (72%) to 0.375 g of lignin. The mixture was left for 1 hour at 30 ◦C. Then it was diluted with 36.25 ml of deionized water and heated at 100 ◦C for 3 hours. The solution was cooled for 15 minutes and then filtered under vacuum. The remaining solid is the acid insoluble lignin (Toledano *et al*., 2012).

The acid-insoluble lignin was calculated as follows:

$$AIL, \% = \left[\frac{B-A}{C}\right] X\ 100$$

Where A is the weight of filter paper (g), B is the weight of filter paper plus dried lignin residue (g) and C initial weight of the lignin sample (g). Acid soluble lignin (ASL) was determined by spectrophotometry (UV absorption at 205 nm). The filtrate above was used. The filtrate was diluted with 1M H_2SO_4 until the absorbance is between 0.1 to 0.8 cm^{-1} (Vishtal and Kraslaw, 2011). The acid-soluble lignin was calculated as follows:

$$ASL, \% = \left[\frac{A\ X\ B\ X\ C}{D\ XE}\right]$$

Where A is the absorbance at 205 nm, B is dilution factor, C is filtrate volume (L), D is extinction coefficient of lignin (110 $g.L^{-1}.cm^{-1}$) and E is the initial lignin weight (g).

FTIR and SEM analysis: FT-IR spectroscopy (Shimadzu 8400 FTIR spectrometer) was used to collect the IR spectra of the dried lignin sample from *C. odorata* and *T. diversifolia*. Scanning Electron Microscope (FEI NOVA NANOSEM 230), which was equipped with an energy dispersive X-ray microanalysis (EDX) system (FEI, Eindhoven, Holland) was used to study the morphology and chemical analysis of the lignin sample.

RESULTS AND DISCUSSION

Lignin extraction efficiency: The yield of *C. odorata* and *T. diversifolia* lignins on dry basis is presented in Table 1. CO has higher lignin yield of 15.12 % than TD with 7.40 %. Lignin content of 6.5 % has been reported for *T. diversifolia* lignins (Olabode *et al*., 2007). The wide difference in the yield can be attributed to the difference in intrinsic properties of raw materials since the same extraction method was used. Moreover, the geographical location and environmental effect might affect the amount of lignin contents in each of the plant stems studied.

Physico-chemical properties of isolated lignin: The physico-chemical properties of isolated lignin from *C. odorata* and *T. diversifolia* are also presented in Table 1.The pH of the *C. odorata* lignin and that of *T. diversifolia* lignin are acidic with the values of 4.57 and 4.28 respectively, which can be attributed to the fact that lignin is a polyphenolic compound which contains acidic OH groups.

The conductivity value for *T. diversifolia* lignin is higher than that of *C. odorata* lignin which is 639 and 419 µS/cm respectively. This is in agreement with their ash content. The ash content of *T. diversifolia* lignin is higher than that of *C. odorata* lignin which has been found to be 9.56 and 4.22 % respectively. This indicates that *C. odorata* lignin has higher degree of purity than *T. diversifolia* lignin. This is a requirement in some applications of lignin such as production of phenolic resins, animal nutrition and dispersants where lignin with low ash contents are utilized (Vishtal and Kraslawski, 2011).

Moisture content of a sample is a measure of the amount of water present in the sample. It implies that sample with low moisture content can be stored for a longer period with lower chances of microbial attack and growth. Moisture content of 16.35 % and 18.67 % were observed for *C. odorata* lignin and *T. diversifolia* lignin respectively. These high moisture content values in this study imply that these lignins are more susceptible to bacterial attack.

Bulk density of a sample gives a measure of how dense a sample is. Bulk density of 0.538 g/ml was observed for *T. diversifolia* lignin which is higher than 0.439 g/ml for *C. odorata* lignin. The values of various properties of dried *C. odorata* and *T. diversifolia* plants are also shown in Table 1.

A*cid insoluble and acid soluble lignin***:** Acid insoluble lignin (AIL) also known as klason lignin is a measure of lignin purity since lignin isolation process is based on lignin insolubility in acid media. So, it can be assumed that the measurement of the acid insoluble lignin is related to the lignin purity. The *C. odorata* lignin has the higher AIL value of 33.65 % than *T. diversifolia* lignin which has 21.96 %. These results are in agreement with their ash content in which *C. odorata* lignin with the lower ash content of 4.22 % exhibited the higher AIL.

Acid soluble lignin (ASL) is probably composed of two components; lignin degradation products and secondarily formed hydrophilic materials such as lignin-carbohydrate (Yasuda *et al.*, 2001). The *T. diversifolia* lignin has higher acid soluble lignin value of 4.42 % than 2.10 % obtained for *C. odorata* lignin. This also supports the acid insoluble lignin results which in turn reflect on their purity. These results are presented in Table 1.

Table 1: Physico-chemical properties of raw and isolated Lignins of two plants

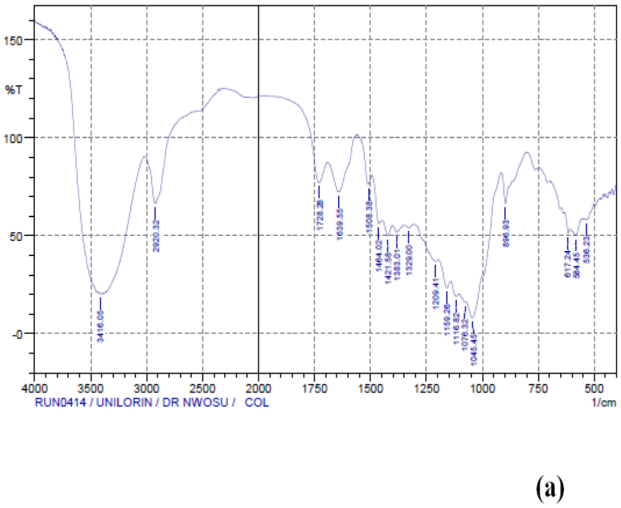
Physico-chemical properties	Raw stem plants		Isolated lignin	
	Chromolaena odorata	*Tithonia diversifolia*	*Chromolaena odorata*	*Tithonia diversifolia*
Yeild (%)	-	-	15.12	7.40
pH	6.97	5.24	4.57	4.28
Conductivity (µS/cm)	818.67	995.67	419	639
Ash content (%)	2.51	9.25	4.22	9.56
Moisture content (%)	12.18	14.26	16.35	18.67
Bulk density (g/ml)	0.249	0.469	0.439	0.538
Crude fibre (%)	36.97	16.56	-	-
Acid insoluble lignin (%)	-	-	33.65	21.96
Acid soluble lignin (%)	-	-	2.10	4.42

Each measurement is replicated 3 times; the deviation between the experimental values does not exceed 5% and – indicates not applicable

FT-IR analysis: The infrared spectra of *C. odorata* and *T. diversifolia* lignin samples obtained by the alkali pretreatment process are presented in Figure 2 and their corresponding band assignments are given in Table 2. Strong and broad band at 3416 and 3441 cm^{-1} observed in *C. odorata* lignin and *T. diversifolia* lignin spectra respectively are attributed to OH stretching. The bands at 2922 and 2920 cm^{-1} correspond to the vibration of C-H bond in methyl and methylene groups. The asymmetric deformation of this bond also produced a band at around 1464 and 1462 cm^{-1}.

Three typical vibrations that normally appeared in aromatic compounds such as lignin, are exhibited around 1597, 1510 and 1423 cm^{-1}. The vibration at around 1728 and 1710 cm^{-1} are associated with C=O bond stretching in unconjugated ketones, carbonyl and ester groups while the vibration at around 1639 cm^{-1} is related to the C=C bond stretching of aromatic ring.

The most significant bands in lignin spectra were those that correspond to its main substructures, guaiacylpropane (G), syringylpropane (S) and p-hydroxyphenylpropane (H) - such as the peak around 1329 cm^{-1} that was related to the breathing of the syringyl ring with C-O stretching and the bands at around 1265 cm^{-1} (shoulder) and 1226 cm^{-1} that were associated to the breathing of the guaiacyl ring with C-O stretching. Around 1159 and 1157 cm^{-1} is a vibration caused by the deformation of the bond C-H in guaiacyl substructures while the same linkage but in syringyl substructures appeared at around 1116 and 1112 cm^{-1}. The vibration at around 1026 cm^{-1} was due to the deformation of the aromatic C-H linkages in guaiacyl substructures. It can also be related to the deformation of the bond C-O in primary alcohols. Similar bands have been reported for lignin (Mohamad *et al.*, 2006; Khan and Ashraf, 2006).



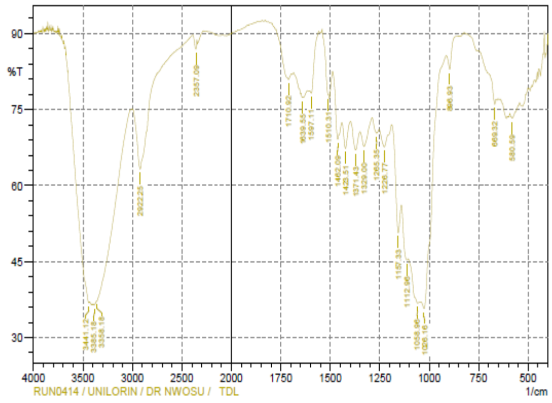


Fig 2: FTIR spectra of (a) *Chromolaena odorata* and (b) *Tithonia diversifolia* lignins

Table 2: Assignments of FT-IR absorption bands (cm^{-1})

Lignin sample		
Absorption bands (cm^{-1})		Assignments
COL	TDL	
3416	3441	Phenolic OH group
2920	2922	CH bond in CH_3 and CH_2
1728	1710	C=O bond stretching in unconjugated ketones, carbonyl and ester groups
1639	1639	C=C bond stretching of aromatic ring
1508,1421	1597,1510	Characteristics of aromatic rings due to aromatic skeletal vibrations
1464	1462	CH bond deformation
1329	1329	Breathing of the syringyl ring with C-O stretching
1209	1265	Breathing of the guaiacyl ring with C-O stretching.

SEM analysis: This analytical technique was used for investigating the surface morphology of the isolated lignins in order to have an insight into their applicability in industries in terms of adsorption capacity. Figures 3 and 4 depict SEM images for *C. odorata* and *T. diversifolia* lignins and are observed to exhibit porous surface at higher magnification. The pores observed from SEM images are having diameter in micrometer (µm) range. These pores are considered as channels to the microporous network. This suggests that *C. odorata* and *T. diversifolia* lignins might be used as biosorbents for removal of organics even without modification.

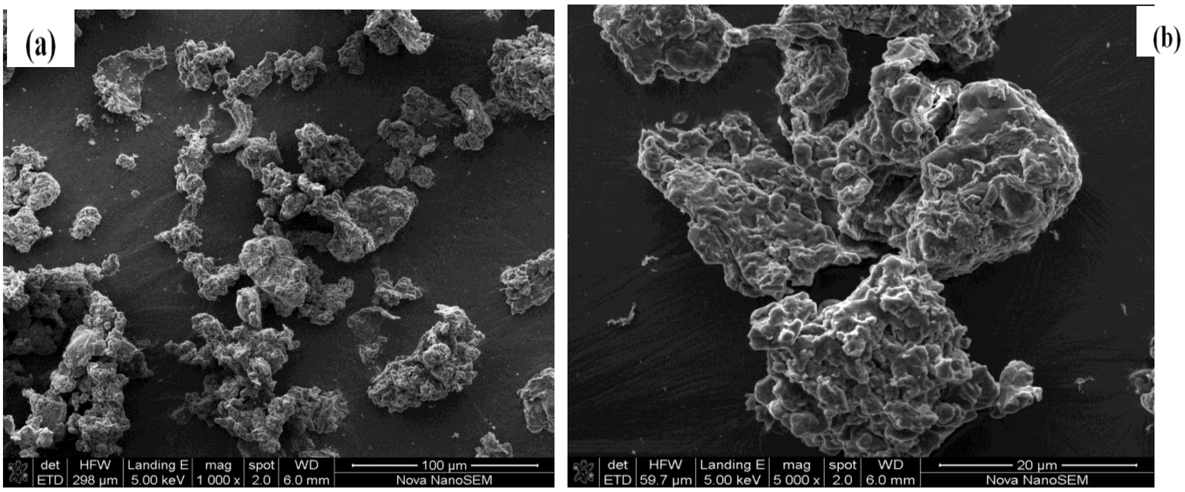


Fig 3: SEM images of CO lignin (a) X 1000 (b) X 5000

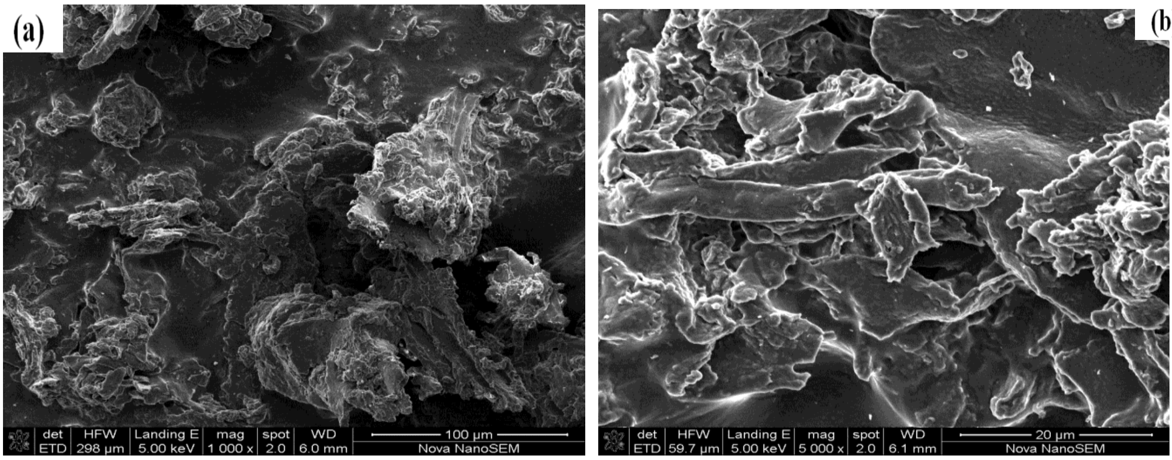


Fig 3: SEM images of TD lignin (a) X 1000 (b) X 5000

Conclusion: Lignin was extracted from *C. odorata* and *T. diversifolia* stems by soda pulping. *C. odorata* gave higher lignin yield than *T. diversifolia* while the *C. odorata* lignin contains lower ash and higher acid insoluble lignin than *Tithonia diversifolia* lignin. This shows that *C. odorata* has higher degree of purity than *T. diversifolia* lignin. The analyses by FTIR of the two lignins isolated from *C. odorata* and *T. diversifolia* confirmed that both are of the HGS type, which are dominant in softwood lignin. The FTIR spectra also reveal the presence of hydroxyl (OH) and carbonyl (CO) functional groups for both lignin types. The SEM images show that the isolated lignins are having pores in micrometer range which suggests their applicability for adsorption of pollutants from solution.

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