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Isolation and Characterization of Lignin from Iraqi Date Palm Phoenix Dactylifera L. Frond Bases as a Good and Cheap Source of Lignin

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Abstract
This study aimed to find analytical data base for Iraqi phoenix date palm pruning woods. Lignin has been extracted for five types of Iraqi date palm using Klason lignin method. Weight of extracted lignin ranged from (0.341 g – 0.56 g), and lignin % ranged from (17.1– 28.2). (Waxes, oils, resin, and proteins of wood gums) % ranged from (23.5– 32.6). FT– IR Characterization showed that the (-OH) phenolic dis appear in all studied lignin samples, except Sukkari lignin showed weak intensity peak. The (4-O-5 inter monomeric lignin linkage) showed strong intensity peaks for Bream and Saif, samples, moderate intensity for Saada, and weak intensity peaks for Sukkari and Makawi. Also aromatic rings (β-1, β-β, 5-5, and β-5 inter monomeric lignin linkage) showed strong intensity peaks for all studied samples. UV – Vis. Characterization showed that the lowest absorption maximum (261 nm) corresponds to Saada lignin sample, While the highest absorption maximum (288 nm) corresponds to Bream lignin sample.

Keywords: Iraqi Date, Phoenix dactylifera L., frond bases, lignin, Isolation and characterization.

1. Introduction

Lignin is a heteropolymer that is thought to form in the cell wall by combinatorial radical coupling of monolignols. It is a complex aromatic polymer in which the cell wall polysaccharides (mainly cellulose and hemicelluloses) and cell wall glycoproteins are embedded [1-3]. It is synthesized from the oxidative coupling of p-hydroxycinnamyl alcohol monomers and related compounds. The main units in the polymer, p-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units are derived from the mono lignols p-coumaryl, coniferyl and sinapyl alcohol, monomers differing in the number of methoxyl substituents on the aromatic ring (Figure 1).

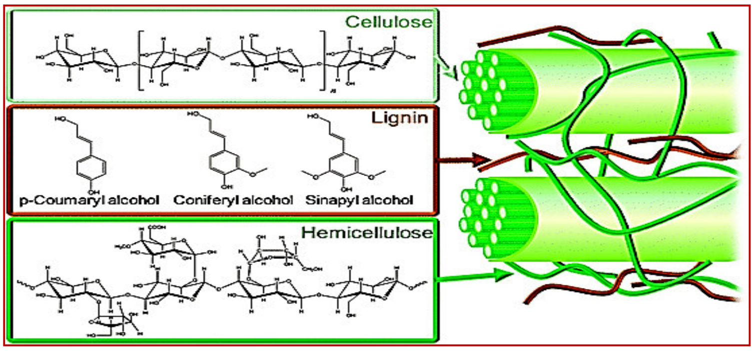


Figure 1: The major building blocks of lignin.

The relative abundance of these main units varies among plant species, tissues, cell types, and developmental stages [4]. A linkage may occur at any of several different locations on each phenolic unit, causing many different linkage types to be possible. The most common linkage types found in a lignin molecule are *β*-O-4, *α*-O-4, *β*-5, 5-5, 4-O-5, *β*-1, and *β*-*β* (Figure 2). Though these are the dominant linkages, at least 20 different linkage types have been identified [5].

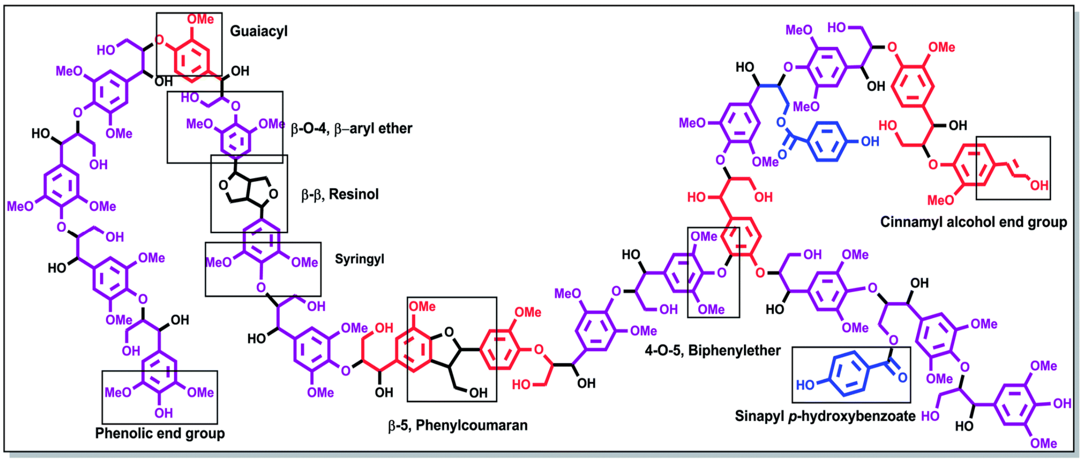


Figure 2. Common inter monomeric linkages in a lignin molecule

Lignin hinders the release of mono saccharides during enzymatic hydrolysis of cell wall polysaccharides–a process called saccharification– which is necessary for the production of second-generation biofuels and materials from biomass-derived sugars [6, 7]. Lignin chemical reactivity also is a critical barrier to wood-pulp production, because lignin removal from wood is either initiated by chemical degradations or, in most cases, accomplished entirely through chemical reactions [8-10]. Lignin is a non-fermentable oxygenated aromatic polymer comprising 15%-25% of the dry weight of hardwoods [11]. As part of the overall goal of converting biomass into ethanol for use as a liquid fuel, the utilization of lignin as a liquid fuel has been investigated [12]. In zmintegrated ethanol plant, lignin could be used as the source of process fuel, giving it a value of about $0.025/ lb [13]. This task studies the conversion of lignin into an oxygenated high-octane blending agent for gasoline or ethanol that would contribute more positively to the overall economics of biomass conversion to liquid fuels [14]. During the first decay of this century the specialists announced that relaying on fossil fuels is reaching a critical level and it has now become a matter of international security to develop new sources of energy for the future. They suggested two main ways to alleviate the problems that arise as a result of reliance on fossil fuels. One method is to improve fuel efficiency and promote conservation. Another solution is to develop alternative, renewable fuels such hydrogen for use in fuel cells, solar, wind, nuclear, and biofuels. One biomass fuel of particular interest is biodiesel, which is produced from the Trans esterification reaction of vegetable oil [15]. The forest bio refinery concept involves converting a pulp mill into a multipurpose biofuels, biomaterials, and bio power production facility in which these products are produced in an environmentally compatible and sustainable manner. A key challenge in this process is the recovery of lignin from process streams such that it can be utilized in a variety of innovative green chemistry processes. In the case of a future commercial cellulitis ethanol plant, since only the carbohydrate fraction is utilized during the fermentation step, the production of ethanol based on lingo cellulosic materials will bring about the coproduction of significant amounts of under-utilized lignin Due to these reasons it is an excellent idea to examine the potential to convert ethanol organosolv lignin from a low grade fuel to potential fuel precursors or other value added chemicals [16]. The study of Lignin and Cellulose content in some parts of date palm Phoenix dactylifera L Cultivars Hillawi and Barhi showed the highest content of lignin appear in frond bases it had (37.63%), keeping in mind that ,there are about 20 million date palm trees in Iraq and every tree produces about (40Kgm) of soft wood as waste each year this means that around (800,000 ton) of soft wood making it a very good and cheap source to produce lignin [17]. The aim of this work is to determine lignin, and (wax, oil, resins, possibly some portions of wood gums) percentages in other five different types of Iraqi Phoenix dactylifera Date palm pruning woods and characterization of lignin using U.V., and FT-IR spectrum [18]. Recent study has reported a detailed characterization of lignin structure [19]. ^{13}C NMR. study analyzing the residual lignin after Kraft pulps had lower contents of β-O-4-structures and higher contents of condensed structure [20]. There are several methods to isolate lignin from wood ,generally, where lignin is isolated either by removing non-lignin or lignin components .Usually H_2SO_4 is used to isolate lignin [21]. This study includes determination of lignin, and (wax, oil, resins, possibly some portions of wood gums) percentages in five different types of Iraqi Phoenix dactylifera Date palm pruning woods and characterization of lignin using UV- Vis., and FT-IR spectrum.

2. Materials and method

2.1. Quantitative determination of lignin, oil, waxes, gum, and protein of Iraqi date palm pruning woods.

Five types of Iraqi date palm pruning woods (Phoenix – Bream, Phoenix – Saif, Phoenix – Sukkari, Phoenix – Saada, Phoenix – Makawi,.) were examined by Klason Lignin or sulfuric acid Lignin method. Wood samples were extracted with alcohol – benzene which employed to remove materials, such as waxes, oils, some resins, and possibly some portions of wood gums to avoid the presence of these foreign materials in the lignin residue, [22] this pretreatment method summarized by extracting the wood samples with a minimum boiling – point solution of alcohol- benzene as shown below: Approximately 2 grams of air-dried pruning wood powder (60 to 100 mesh) are weighed in a tared alundum crucible. The crucible and its contents are dried to constant weight at 105° C, cooled, and weighed. The material is then extracted for 4 hours in a Soxhlet apparatus with a minimum boiling solution of alcohol-benzene. The solvent is removed by suction, the residue washed with alcohol by suction to remove the benzene, Extracted by 400 ml hot water in a water bath for 3 hours, filtered, washed with hot water, then with alcohol, and finally dried. (Washing the residue with alcohol aids in the removal of the pruning wood powder from the crucible after drying.) The dried residue is transferred to a glass Stoppard weighing bottle, and weighed to calculate the percentage of waxes, oils, protein and gums in each sample, as shown in (Table 1). Then the dried residue stirred, well mixed at room temperature and hydrolysis with 25 cc. of 72 percent sulfuric acid, and maintained at that temperature by keeping it in a bath at 20 ± 1 °C for 2 hours. The resulting, mixture is transferred to an Erlenmeyer flask, diluted with water to make a 3 percent acid solution by adding 575 ml of water, and then boiled for 4 hours under .a reflux condenser . The hydrolyzed residue is filtered on a tared alundum. Crucible, washed free of acid by means of hot water, dried, and weighed. The lignin content (lignin %) was calculated on the basis of the oven-dry sample as shown in (Table 1).

Table 1. Quantitative determination data of different types of IRAQI Phoenix Date - Palm Wood Pruning Lignin.

Sample name	Weight of sample (g.)	Wt. of sample after treatment with Benzene- alcohol mixture (g.)	Wt. of sample after treatment with 72% H_2SO_4 (g.)	Waxes, Oils, Resins, and Gums %	Wood Lignin %
Phoenix -Bream	2	1.360	0.341	32.0	17.1
Phoenix- Saif	2	1.500	0.45	25.0	22.5
Phoenix –Sukkari	2	1.530	0.55	23.5	27.5
Phoenix - Saada	2	1.347	0.56	32.6	28.2
Phoenix- Makawi	2	1.500	0.356	25.0	17.8

2.2. Lignin characterization.

The five isolated dried lignin samples were characterized by FT –IR spectroscopic analysis (Shimadzu FTIR Spectrometer – 30 000:1/ IRAff), and UV-Vis. spectrophotometric analysis (UV – 1800 Shimadzu Spectrophotometer).

3. Results and discussion

3.1. Quantitative determination of lignin.

(Table 1) showed that the weight of extracted lignin from these five different types of IRAQI Phoenix Date - Palm Pruning (2g.) Wood samples ranged from (0.341 g – 0.56 g), and the lignin % ranged from (17.1– 28.2). Also the highest lignin % was in Saada Pruning Wood, and the lowest lignin % was in Bream Pruning Wood. The other ingredients (Waxes, Oils, resins and proteins of wood Gums) % ranged from (23.5– 32.6), where the lowest % was for Sukkari Pruning Wood, and the highest % was for Saada Pruning Wood.

3.2. Characterization of lignin.

3.2.1. FT – IR Characterization.

The lignin samples extracted from Iraqi pruning date palms under study showed convergent peaks of absorption on FT-IR spectrum but with different values can be interpreted as shown in (Table 2) deriving from Figures. (3 – 7). The stretching vibration absorption of (-OH) group showed convergent peaks values with strong intensities for all studied samples which indicates that all studied samples contain this group in the lignin structure at high concentrations. Also the stretching vibration absorption of (- C-H) aliphatic group showed convergent peaks values for all studied samples, but the three samples (Bream at 2937 cm^{-1}, Saif at 2939 cm^{-1}, and Saada at 2939 cm^{-1}) have a strong intensity peaks. The others (Makawi at 2941cm^{-1}) has a moderate intensity peak, (Sukkari at 2962cm^{-1}) has a weak intensity peak. It can be considered that the three samples contain higher concentrations of monomers in lignin structure than that of the other two samples. The stretching vibration absorption of conjugated carbonyl group showed convergent peaks values for all studied samples with low to moderate intensity peak. Which could means its presence at a low to moderate concentration in these samples. The stretching vibration absorption of aromatic rings and (-C=C-C-and –C=C-C=C-) or (β-1, β-β, 5-5, and β-5 inter monomeric lignin linkage) showed convergent peaks values with strong intensities for all studied samples, except Sukkari showed weak intensity peak at 1610 cm-1.This may explain the existence of these linkages frequently in all studied samples, except Sukkari lignin structure. (C-H) deformation and aromatic ring vibration showed convergent peaks values with strong intensities for all studied samples, except Makawi showed moderate intensity peak at 1456 cm-1. Bending vibrations absorptions of (-OH) have no absorption peaks for all studied samples, except Sukkari showed weak intensity peak at 1375cm-1, this means that the (-OH) phenolic group disappear in all lignin samples due to the probability of free radical intermediate formation from the (-OH) phenolic group site which consume these groups configures the ether linkages. The aromatic ether aryl (4-O-5 inter monomeric lignin linkage) showed convergent peaks values with strong intensity peaks for (Bream at 1273 cm-1, and 1219 cm-1), and (Saif at 1273 cm^{-1}, and 1222 cm-1) samples, moderate intensity for (Saada at 1278 cm^{-1}, and weak intensity at 1217 cm^{-1}), and weak intensities for (Sukkari at 1269 cm^{-1}, and at 1219 cm^{-1}), and (at 1271 cm^{-1}, and at 1209 cm^{-1}). This means that (4-O-5 inter monomeric lignin linkage) probability is higher in Bream, and Saif lignin's structures. The stretching vibration absorption of cyclic ether large ring stretching (DODO inter monomeric lignin linkage) showed convergent peaks values with strong intensities for (Bream at 1120 cm-1, and weak intensity at 1105cm-1), and (Saif at 1114 cm-1) samples. Weak intensities for (Sukkari at 1114 cm-1, and 1097 cm-1), and Makawi at 1118 cm-1, and 1083 cm-1), and moderate intensity for (Saada at 1093 cm-1). This indicates its strong existence probability in Bream, and Saif types. The stretching vibration absorption of alkyl substituted ether ($O - CH_3$ or $O - CH_2$) showed two convergent peaks values with moderate and weak intensities for all studied samples. This means that the alkyl substituted ether ($O - CH_3$ or $O - CH_2$) dis appear in all studied samples lignin structures due to the probability of free radical intermediate formation from the ether ($O - CH_3$ or $O - CH_2$) site which consume these groups configures the other types of ether linkages. The stretching vibration absorption of vinyl ether (in phase C- O - C stretch.) showed convergent peaks values, with weak intensities for all studied sample, Which could means its presence at a lower concentrations in all probable lignin structures.

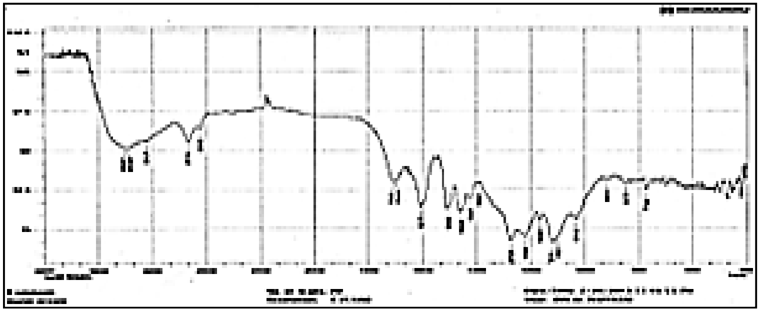


Figure 3. FT – IR Spectrum of Phoenix – BREAM pruning lignin.

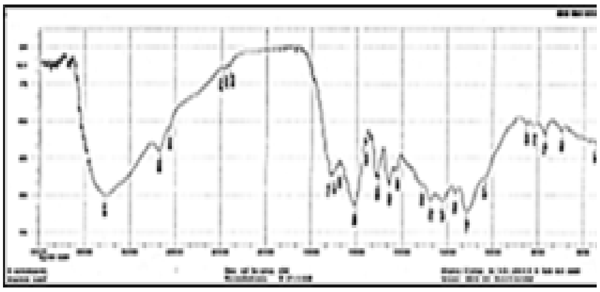


Figure 4. FT – IR Spectrum of Phoenix – SAIF Pruning lignin.

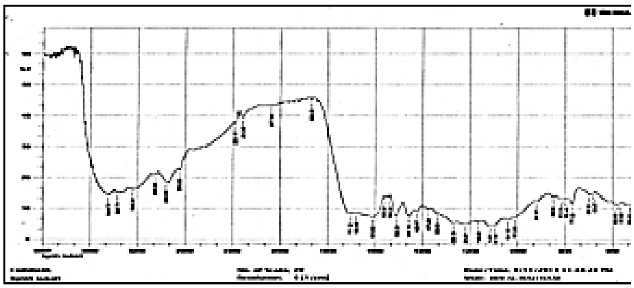


Figure 5. FT – IR Spectrum of Phoenix – SUKKARI Pruning lignin.

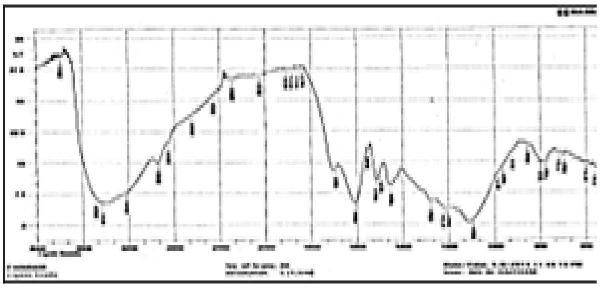


Figure 6. FT – IR Spectrum of Phoenix – SAADA

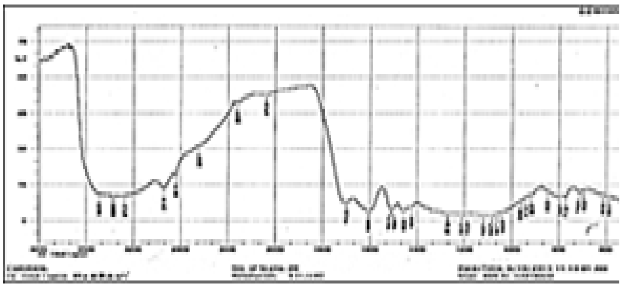
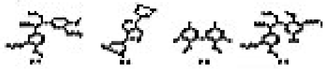
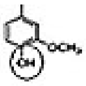
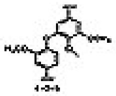
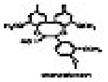


Figure 7. FT – IR Spectrum of Phoenix – MAKAWI

Pruning lignin. Pruning lignin.

Table 4. FT-IR peaks of lignin and its inter monomeric linkages.

Vibrational groups and remarks	Inter monomeric lignin linkage structure	Phoenix - Bream lignin	Phoenix - Saif lignin	Phoenix - Suklari lignin	Phoenix-Saada lignin	Phoenix – Malawi lignin
		Peak frequency wave number cm^{-1}	Peak frequency wave number cm^{-1}	Peak frequency wave number cm^{-1}	Peak frequency wave number cm^{-1}	Peak frequency wave number cm^{-1}
-OH stretching vibration		3376 (s)	3421 (s)	3367(s)	3427(s)	3369 (s)
C-H stretching vibration		2937 (s)	2939 (s)	2962 (w)	2939(s)	2941 (m)
Conjugated carbonyl stretching		1708 (m)	1716 (m)	1701 (w)	1701 (m)	1701 (m)
aromatic rings and (-C=C-C- and –C=C-C=C-) or (β-1, β-β, 5-5, and β-5 inter monomeric lignin linkage)		1608(s) and 1504(s)	1608 (s) and 1505 (s)	1610 (w) and 1508 (s)	1608 (s) and 1502 (s)	1608 (s) and 1506 (s)
C-H deformation and aromatic ring vibration		1456 (s)	1456 (s)	1458 (s)	1452 (s)	1456 (m)
Bending vibrations of (-OH) phenolic bonds		—	—	1375 (w)	—	—
Aromatic ether aryl (4-O-5 inter monomeric lignin linkage)		1273 (s) and 1219 (s)	1273 (s) and 1222 (s)	1269 (w) and 1219 (w)	1278 (m) and 1217 (w)	1271 (w) and 1209(w)
Cyclic ether large ring stretching (DODO inter monomeric lignin linkage)		1120 (s) and 1105(w)	1114 (s)	1114 (w) and 1097(w)	1093(m)	1118 (w) and 1082(w)
Alkyl substituted ether (O - CH_3 or O – CH_2 stretch)		1165 (m)	1166 (m)	1165 (w)	—	—
Vinyl ether (in phase C - O - C stretch)		846(w)	852 (w)	850 (w)	856(w)	848 (w)

Abbreviations used in the table; w, weak, m, moderate, s, strong.

3.2.2. UV – Vis. Characterization.

A five samples of Klason Lignin of the studied Iraqi Phoenix date palm pruning woods were dissolved in ethanol (80%) to prepare five solutions of (100 mg / L) concentration in 10 ml volumetric flasks. UV-Vis. scanning spectrum has been recorded, (Table 3) show the spectral data of λ – maxes and absorbencies derived from Figs. (8 – 12). Two regions of peaks were obtained in every Klason Lignin samples which had the absorption maximum at wavelength of (203 – 228) nm, and (261 – 288) nm. The appearance of these characteristic peaks in the lignin spectrum originated from non-condensed phenolic groups (aromatic ring) in lignin [23] for the absorbance maximum values at short wavelengths, and Cyclic ether large ring (DODO inter monomeric lignin linkage) for the absorbance maximum values at long wavelengths. This is in a good agreement with the suggestion of FT – IR spectrum data in (Table 2). The lowest absorption maximum (261 nm) corresponds to Saada lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a lowest existence probability. While the highest absorption maximum (288 nm) corresponds to Bream lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a higher existence probability.

Table 3 The UV absorbance of Klason Lignin of the studied Iraqi Phoenix date palm pruning woods.

Klason Lignin	Short wavelengths (nm).	Absorbance (A)	Long wavelengths (nm)	Absorbance (A)
Phoenix -Bream	227	2.394	288	0.875
Phoenix- Saif	203	1.748	275	1.198
Phoenix – Sukkari	223	2.616	272	1.000
Phoenix - Saada	220	1.947	261	0.601
Phoenix- Makawi	228	1.828	275	0.803

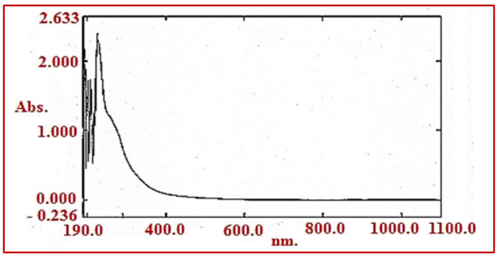


Figure 8. UV-Vis. Spectrum of Phoenix – BREAM Pruning lignin

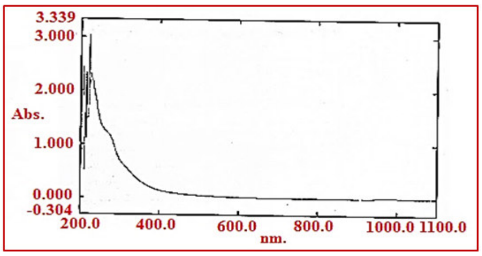


Figure 9. UV-Vis. Spectrum of Phoenix – SAIF pruning lignin.

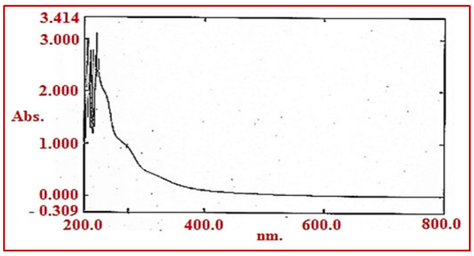


Figure 10. UV-Vis. Spectrum of Phoenix SUKKARI pruning lignin

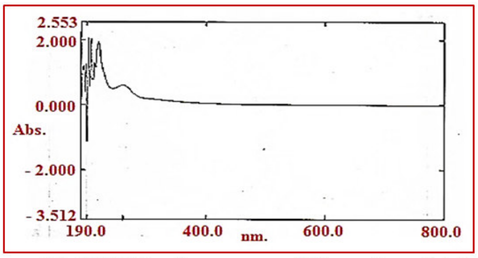


Figure 11. UV-Vis. Spectrum of Phoenix – SAADA pruning lignin

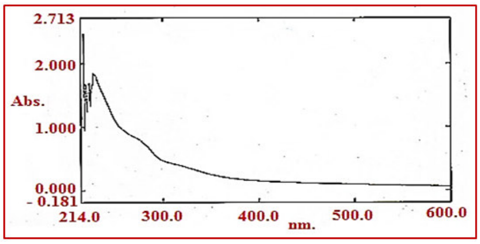


Figure 12. UV-Vis. Spectrum of Phoenix MAKAWI pruning lignin.

3. Conclusions

Different nature of studied Phoenix date palm pruning woods has been discovered. Highest lignin % was in Saada Wood, and lowest lignin % was in Bream Wood. FT – IR spectrums showed that (-OH) phenolic group disappear in all samples, except Sukkari lignin showed weak intensity peak, due to the probability of free radical intermediate formation from (-OH) phenolic group site. UV – Vis. Spectrums showed that lowest λ max. (261 nm) corresponds to Saada lignin, indicates that cyclic ether large ring (DODO) has lowest existence probability. While the highest λ max. (288 nm) corresponds to Bream lignin, indicates that cyclic ether large ring (DODO) has highest existence probability.

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