

# Qualitative and quantitative determination of lignin in different types of Iraqi *Phoenix dactylifera* Date palm pruning woods

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## Abstract

The target of this study is to find analytical data base for Iraqi phoenix date palm pruning woods, which produced by pruning process at the season of date palm production. Lignin has been extracted and purified for five types of Iraqi date palm using Klason lignin method. The weight of the extracted lignin ranged from (0.473 g – 0.600 g) and the lignin % ranged from (23.5 – 30). The other ingredients (waxes, oils, resin, and proteins of wood gums) % ranged from (11.2 – 53.5). FT– IR Characterization showed that the (-OH) phenolic group appear in Digal lignin structure only and dis appear in other lignin samples, and the (4-O-5 inter monomeric lignin linkage) showed moderate to weak intensity peaks for all studied samples except the Humrani sample at (1271 cm<sup>-1</sup>) has a strong intensity peak. Also (DODO inter monomeric lignin linkage) showed strong intensity peaks for each (Humrani at 1116 cm<sup>-1</sup>, and Tibarzal at 1111 cm<sup>-1</sup>). UV – Vis. Characterization showed that the lowest absorption maximum (267 nm) corresponds to Basrawi lignin sample, While the highest absorption maximum (297 nm) corresponds to Tibarzal lignin sample.

**Keywords:** Qualitative and quantitative determination, pruning woods, lignin, *Phoenix dactylifera*.

## 1. Introduction

Date palm (*Phoenix dactylifera* L.) is one of the oldest fruit crops grown in the arid regions of the Arabian Peninsula, North Africa, and the Middle East. The most probable area of origin of the date palm was in the country of Iraq. The earliest record from Iraq (Mesopotamia) shows that date culture was probably established as early as 3000<sub>BCE</sub>. Because of the long history of date culture and the wide distribution and exchange of date cultivars, the exact origin of the date is unknown, but it most likely originated from the ancient Mesopotamia area (southern Iraq) [1].

Date palm tree concentrated in Basra Governorate, recent statistic which is considered the largest date palm forest in the world. It is estimated that the number of date palm trees in this Governorate exceeds 13 million with over 400 varieties and cover an area over 50.000 hectares [2].

Despite the large number of Iraqi date palm types, although a little knowledge about these types is yet known. Also the local names of these deferent types may slightly defer from that known in Arab home land. A few studies about these types were published. Lignin, being one of the most abundant families of polymers, is an enormous renewable resource and has capture the attention technologist and entrepreneurs [3].

Despite the huge amount of studies concerning lignin structure and polymerization degrees, although the problem still unsolved to date [4]. Lignin has been extensively reported to be a cross-linked network polymer with many deferent structure [5]. Lignin molecules are derived mainly from three phenylpropane monomers: *p*-coumaryl alcohol, coniferyl alcohol, and synapyl alcohol (Fig. 1). These monolignols are polymerized by a radical coupling process that links them by carbon-carbon or ether bonds.

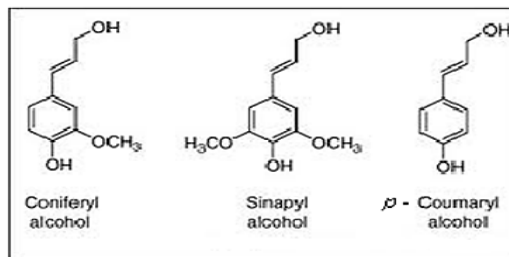


Fig.1. The major building blocks of lignin.

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  $\beta$ -O-4,  $\alpha$ -O-4,  $\beta$ -5, 5-5, 4-O-5,  $\beta$ -1, and  $\beta$ - $\beta$

(Fig. 2). Though these are the dominant linkages, at least 20 different linkage types have been identified [7]. The ether type linkages are known to dominate in native lignin, estimated to make up approximately one half to two thirds of the total number of native plant lignin linkages. Monolignols can be trifunctionally linked, forming branch points within the polymer and giving it a network-like structure. Given the variety of linkages that occur, lignin molecules cannot be depicted as a series of regular, defined repeating units, as traditional polymers are. In contrast, lignin is a highly irregular, complex polymer [6].

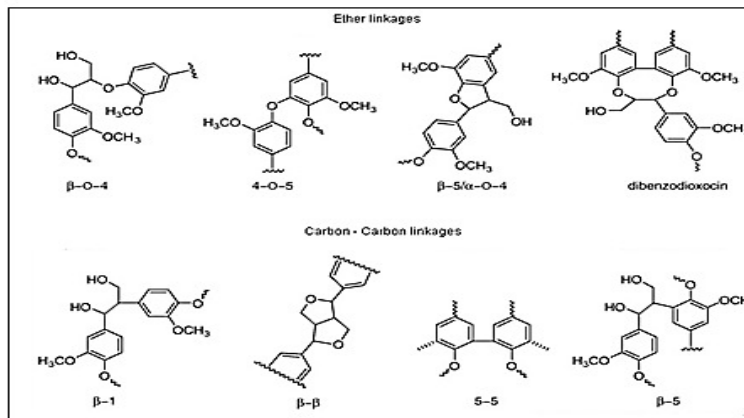


Fig. 2. Common inter monomeric linkages in a lignin molecule

Models have been proposed for lignin from several different sources, though due largely to lignin's complicated nature and the difficulties inherent in lignin analysis, no complete structure of a lignin molecule has ever been identified. The models that have been developed are only representations drawn from analyses of the relative proportions of each lignin unit type and each linkage type (Fig. 3) [6].

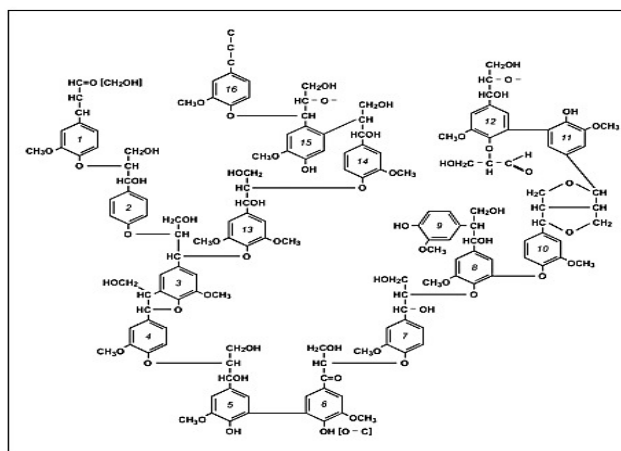


Fig. 3. A structural model of softwood lignin [8]

Recent study has reported a detailed characterization of lignin structure [9]. <sup>13</sup>C NMR. study analyzing the residual lignin after Kraft pulps had lower contents of  $\beta$ -O-4-structures and higher contents of condensed structure [10]. 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 [11].

This research is dealing with the 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 – Digal, Phoenix – Zahdi, Phoenix –Basrawi, Phoenix – Humrani, Phoenix – Tibarzal,) 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, [12] 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, and then extracted with 400 cc. of 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  $20 \pm 1$  °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).

### 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 ( 0.473 g – 0.600 g), and the lignin % ranged from (23.5 – 30). Also the highest lignin % was in Digal Pruning Wood, and the lower lignin % was in Tibarzal Pruning Wood. The other ingredients (Waxes, Oils, resins and proteins of wood Gums)% ranged from (11.2 – 53.5), where the lowest % was for Basrawi Pruning Wood, and the highest % was for Zahdi 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) which derived from Figs. (5 – 8).

The stretching vibration absorption of (-OH) group showed convergent peaks values with strong intensities for all studied samples except the Digal sample which showed a moderate intensity peak at ( $3392\text{ cm}^{-1}$ ), which indicates that all studied samples contain this group in the lignin structure at high concentrations, except the Digal sample which have lower concentrations. Also the stretching vibration absorption of (- C-H) aliphatic group showed convergent peaks values for all studied samples, but the first three samples (Zahdi at  $2927\text{ cm}^{-1}$ , Basrawi at  $2921\text{ cm}^{-1}$ , and Humrani at  $2937\text{ cm}^{-1}$ ) have a strong intensity peaks. The others (Digal at  $2922\text{ cm}^{-1}$ , Tibarzal at  $2941\text{ cm}^{-1}$ ) have a moderate intensity peaks. It can be considered that the first three samples contains higher concentrations of monomers in lignin structure than that of the other two samples. The stretching vibration absorption of Conjugated carbonyl group showed strong intensity peak for Tibarzal sample at ( $1705\text{ cm}^{-1}$ ), and have a varying intensities of the other samples, Which could means its presence at a high concentration in Tibarzal sample. The stretching vibration absorption of aromatic rings and (-C=C-C-and -C=C-C) or ( $\beta$ -1,  $\beta$ - $\beta$ , 5-5, and  $\beta$ -5 inter monomeric lignin linkage) showed convergent peaks values with strong intensities for all studied samples. Which may explain the existence of these linkages frequently in lignin structure. (C-H) deformation and aromatic ring vibration showed convergent peaks values with strong intensities for all studied samples. Bending vibrations absorptions of (-OH) phenolic group showed absorption peak at ( $1373\text{ cm}^{-1}$ ) with moderate intensity for Digal sample only while the other samples have no absorption, this means that the (-OH) phenolic group appear in Digal lignin structure only and dis appear in other 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 moderate to weak intensities for all studied samples except the Humrani sample at ( $1271\text{ cm}^{-1}$ ) has a strong intensity peak, and this means that (4-O-5 inter monomeric lignin linkage) probability is higher in Humrani lignin structure.

The stretching vibration absorption of cyclic ether large ring stretching (DODO inter monomeric lignin linkage) showed different peaks values for all studied samples with strong intensities for each (Humrani at  $1116\text{ cm}^{-1}$ , and Tibarzal at  $1111\text{ cm}^{-1}$ ), this indicates its strong existence probability in these two types. The other three samples (Digal at  $1109\text{ cm}^{-1}$  and  $1056\text{ cm}^{-1}$ ) showed moderate intensity peak, (Basrawi at  $1114\text{ cm}^{-1}$  and  $1089\text{ cm}^{-1}$ )

showed weak intensity peak, and Zahdi at ( $1120\text{ cm}^{-1}$ ) showed strong intensity peak, but at ( $1101\text{ cm}^{-1}$ ) showed weak intensity peak. This indicates its lower existence probability in these types. The stretching vibration absorption of alkyl substituted ether ( $\text{O}-\text{CH}_3$  or  $\text{O}-\text{CH}_2$ ) two convergent peaks values with moderate to weak intensities for all studied samples, except Tibarzal at ( $1166\text{ cm}^{-1}$ ) showed one absorption peak with moderate intensity. This means that the alkyl substituted ether ( $\text{O}-\text{CH}_3$  or  $\text{O}-\text{CH}_2$ ) dis appear in all studied samples lignin structures due to the probability of free radical intermediate formation from the ether ( $\text{O}-\text{CH}_3$  or  $\text{O}-\text{CH}_2$ ) 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.

### 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  $\lambda$  – maxes and absorbencies derived from Figs. (9 – 13).

Two regions of peaks were obtained in every Klason Lignin samples which had the absorption maximum at wavelength of (207 – 214) nm, and (267 – 297) nm. The appearance of these characteristic peaks in the lignin spectrum originated from non-condensed phenolic groups (aromatic ring) in lignin [13] 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. Which is in a good agreement with the suggestion of FT – IR spectrum data in (Table 2). The lowest absorption maximum (267 nm) corresponds to Basrawi lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a lowest existence probability. While the highest absorption maximum (297 nm) corresponds to Tibarzal lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a higher existence probability.

### 3. Conclusions

Many facts have been discovered in this study, which showed the different nature of the studied Phoenix date palm types. The highest lignin % was in Digal Pruning Wood, and the lowest lignin % was in Tibarzal Pruning Wood. The lowest % of the other ingredients (Waxes, Oils, resins and proteins of wood Gums) was for Basrawi Pruning Wood, and the highest % was for Zahdi Pruning Wood. FT – IR Characterization showed that the (-OH) phenolic group appear in Digal lignin structure only and dis appear in other 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, and the (4-O-5 inter monomeric lignin linkage) probability is higher in Humrani lignin structure. Also (DODO inter monomeric lignin linkage) showed strong existence probability in Humrani, and Tibarzal samples. UV – Vis. Characterization showed that the lowest absorption maximum (267 nm) corresponds to Basrawi lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a lowest existence probability. While the highest absorption maximum (297 nm) corresponds to Tibarzal lignin sample, indicates that the Cyclic ether large ring (DODO inter monomeric lignin linkage) has a higher existence probability.

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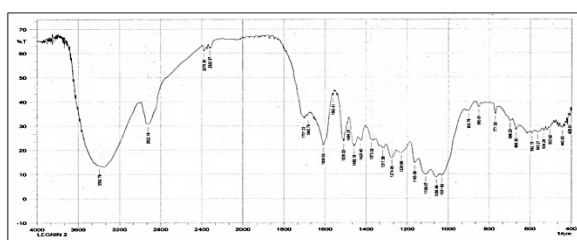


Fig. 4. FT – IR Spectrum of Phoenix –DIGAL pruning lignin

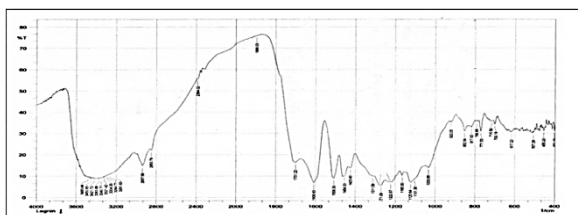


Fig. 5. FT – IR Spectrum of Phoenix –ZAHDI pruning lignin.

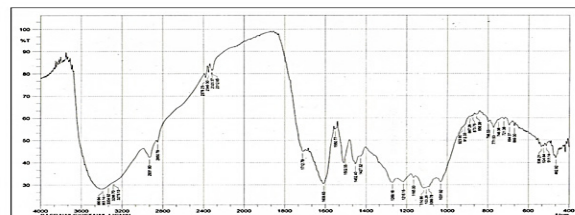


Fig. 6. FT – IR Spectrum of Phoenix –BASRAWI pruning lignin.

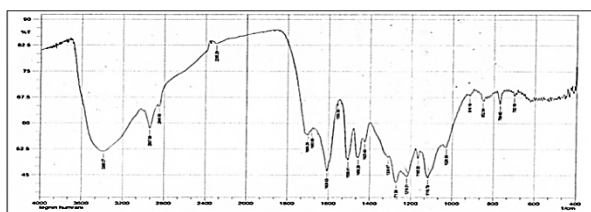


Fig. 7. FT – IR Spectrum of Phoenix –HUMRANI pruning lignin.

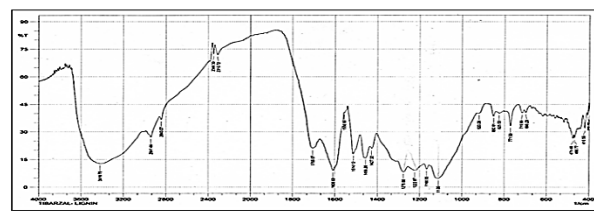


Fig. 8. FT – IR Spectrum of Phoenix –TIBARZAL pruning lignin.

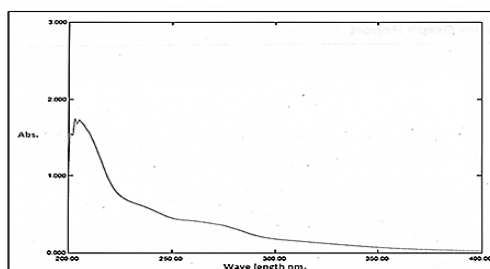


Fig. 9. UV-Vis. Spectrum of Phoenix –DIGAL pruning lignin

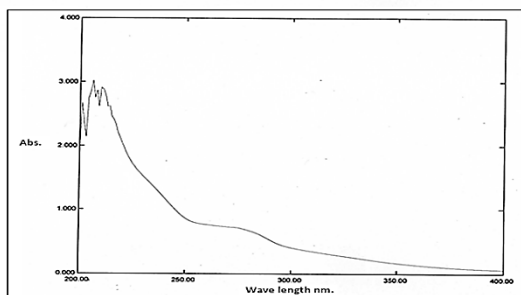


Fig. 10. UV-Vis. Spectrum of Phoenix –ZAHDİ pruning lignin.

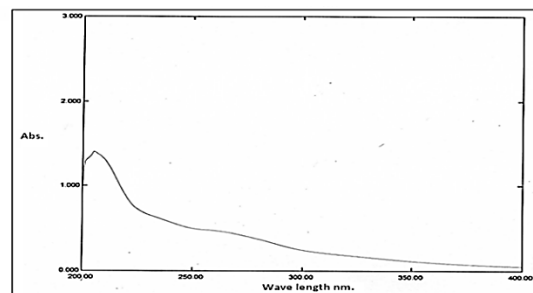


Fig. 11. UV-Vis. Spectrum of Phoenix –BASRAWI pruning lignin

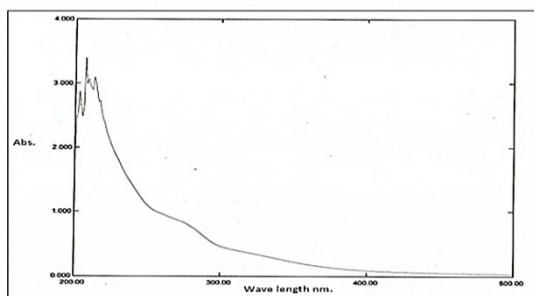


Fig. 12. UV-Vis. Spectrum of Phoenix –HUMRANI pruning lignin

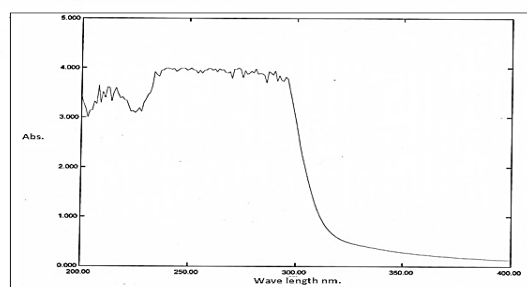
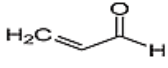
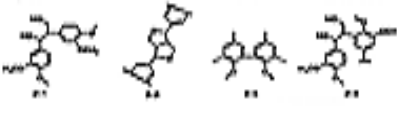
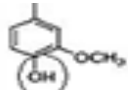
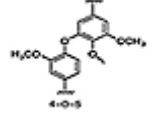
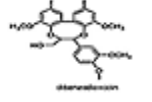
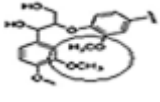



Fig. 13. UV-Vis. Spectrum of Phoenix –TIBARZAL pruning lignin.

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 <sub>2</sub> SO <sub>4</sub> (g.) | Waxes, resins and proteins of wood Gums % | Wood Lignin % |
|-------------------|-----------------------|--|--|---|---------------|
| Phoenix-Digal     | 2                     | 1.180  | 0.600  | 41.0                                      | 30.0          |
| Phoenix -Zahdi    | 2                     | 0.930  | 0.597  | 53.5                                      | 29.9          |
| Phoenix-Basrawi   | 2                     | 1.776  | 0.560  | 11.2                                      | 28.0          |
| Phoenix -Humrani  | 2                     | 1.631  | 0.500  | 18.4                                      | 25.0          |
| Phoenix -Tibarzal | 2                     | 1.420  | 0.473  | 29.0                                      | 23.5          |

Table 2 -FT-IR peaks of lignin and its inter monomeric linkages.

| Vibrational groups and remarks   | Inter monomeric lignin linkage structure  | Phoenix-Digal lignin                        | Phoenix - Zahdi lignin                      | Phoenix-Basrawi lignin                      | Phoenix - Humrani lignin                    | Phoenix - Tibarzal lignin                   |
|--|---|---|---|---|---|---|
|  |   | Peak frequency wave number $\text{cm}^{-1}$ | Peak frequency wave number $\text{cm}^{-1}$ | Peak frequency wave number $\text{cm}^{-1}$ | Peak frequency wave number $\text{cm}^{-1}$ | Peak frequency wave number $\text{cm}^{-1}$ |
| -OH stretching vibration   |   | 3392 (m)                                    | 3340 - 3363 (s)                             | 3388 (s) and 3398 (s)                       | 3358 (s)                                    | 3419 (s)                                    |
| C-H stretching vibration   |   | 2922 (m)                                    | 2937 (s)                                    | 2921 (s)                                    | 2937 (s)                                    | 2941 (m)                                    |
| Conjugated carbonyl stretching   |    | 1701 (w) and 1685 (w)                       | 1701 (m)                                    | 1712 (w)                                    | 1699 (m)                                    | 1705 (s)                                    |
| aromatic rings and (-C=C-C-and -C=C-C=C-) or ( $\beta$ -1, $\beta$ - $\beta$ , 5-5, and $\beta$ -5 inter monomeric lignin linkage) |    | 1608 (s) and 1508 (s)                       | 1608 (s) and 1508 (s)                       | 1608 (s) and 1502 (s)                       | 1608 (s) and 1506 (s)                       | 1608 (s) and 1514 (s)                       |
| C-H deformation and aromatic ring vibration  |   | 1458 (m)                                    | 1454 (s)                                    | 1452 (s)                                    | 1456 (s)                                    | 1456 (s)                                    |
| Bending vibrations of (-OH) phenolic bonds   |  | 1373 (w)                                    | ---   | ---   | ---   | ---   |
| Aromatic ether aryl (4-O-5 inter monomeric lignin linkage)   |  | 1274 (m) and 1228 (m)                       | 1271 (m) and 1222 (w)                       | 1269 (m) and 1215 (m)                       | 1271 (s) and 1219 (m)                       | 1276 (m) and 1222 (w)                       |
| Cyclic ether large ring stretching (DODO inter monomeric lignin linkage)   |  | 1109 (m) and 1056 (m)                       | 1120 (s) and 1101 (w)                       | 1114 (w) and 1089 (w)                       | 1116 (s)                                    | 1111 (s)                                    |
| Alkyl substituted ether (O - CH <sub>3</sub> or O - CH <sub>2</sub> stretch)   |  | 1163 (m) and 1031 (w)                       | 1165 (w) and 1033 (w)                       | 1165 (w) and 1031 (m)                       | 1165 (m) and 1028 (m)                       | 1166 (m)                                    |
| Vinyl ether ( in phase C-O - C stretch)  |  | 850 (w)                                     | 852 (w)                                     | 856 (w)                                     | 852 (w)                                     | 850 (w)                                     |

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

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-Digal      | 207 - 210               | 1.744 - 1.720  | 270                   | 0.395          |
| Phoenix -Zahdi     | 207 - 213               | 3.031 - 2.875  | 273                   | 0.718          |
| Phoenix-Basrawi    | 207                     | 1.355          | 267                   | 0.465          |
| Phoenix - Humrani  | 207 - 210               | 3.406 - 3.078  | 272                   | 0.875          |
| Phoenix - Tibarzal | 207 - 214               | 3.615 - 3.538  | 297                   | 3.807          |