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# Characterization of lignin isolated from Iranian *Fagus Orientalis* wood

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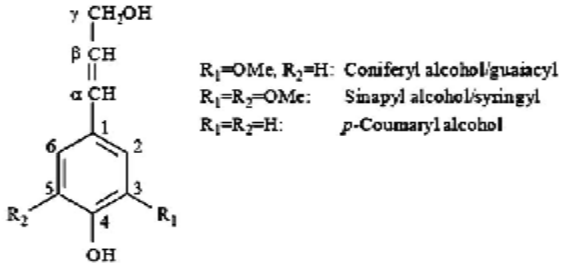
**Abstract:** Lignin isolated from *Fagus Orientalis* by acidolytic dioxane method were characterized using alkaline nitrobenzene oxidation, elemental analysis, molecular weight analysis, GC-MS chromatography, FT-IR and HNMR spectroscopy**.** The results showed that $M_w$ (weight average molecular weight) and $M_n$ (number average molecular weight) of the lignin respectively is 15986 g/mol and 9746.9 g/mol. The ratio of S unit to G unit is 1.9 and $C_9$ formula of *Fagus Orientalis* lignin is $C_9H^{al}_{2.78}H^{ph}_{2.1}O_{2.144}(OH)^{ph}_{0.673}(OH)^{al}_{1.055}(OCH_3)_{1.66}$.

**Keywords:** Lignin; Fagus Orientalis; Acidolytic Dioxane Method

## Introduction

Secondly only to cellulose, lignin is the most abundant biopolymers on earth. It is estimated that the planet contains $13 \times 10^{11}$ metric tons [1]. Lignin constitutes approximately 30% of the dry weight of softwoods and about 20% of the hardwoods [2]. Lignification is associated with the development of vascular systems in plants, providing resistance to biodegradation and environmental stress such as changes in the balance of water and humidity [3]. Industrially, in pulping process, chemical separation of lignin from cellulose results in the production of vast amounts of lignin as a by products. Lignin is a large, cross-linked macro molecule with molecular masses in excess of 10,000 u. It is relatively hydrophobic and aromatic in nature. Different types of lignin have been described depending on the means of isolation. There are three monolignol monomers, methoxylated to various degrees: *p*-coumaryl alcohol (**P**), coniferyl alcohol (**G**) and sinapyl alcohol (**S**) [4] (Fig. 1). *Fagus Orientalis* is second important tree in north of IRAN and cover about 25% of the forest.

**Figure 1**. Monolignol monomer in lignin polymer

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## Material and Methods

### Sample Preparation

A 55 years old *Fagus Orientalis* wood cut, chipped and milled to 40 mesh size and extracted with ethanol: toluene, ethanol and hot water solvent for preparation of extractive free wood powder. The extraction of dioxan lignin was performed with dioxan: HCl 2 mol/l (9:1) at reflux temperature for 3 h. After evaporation of the solvent, raw extracted lignin purified by bjorkman method. Purified lignin acetylated and characterized with alkaline nitro benzene oxidation, elemental analysis, methoxy content analysis, molecular weight analysis, GC-MS chromatography, FTIR and $^1$H-NMR spectroscopy.

### Nitrobenzene Oxidation

Alkaline nitrobenzene oxidation of *Fagus Orientalis* was carried out according to Mun's method [5]. 1g of a dried extractive free wood meal (40-60 mesh), 35ml of 2M NaOH and 2 ml of freshly distilled nitrobenzene are placed in a 50-ml stainless steel bomb. The bomb is sealed tightly with a screw cap fitted with a Teflon gasket and heated at 170 C for 2.5h in an electrically preheated. The bomb is shaken occasionally and after the heating period, the oxidation mixture is transferred to a liquid-liquid extractor and extracted continuously with $CHCl_3$ for 4h to remove nitro benzene reduction products and excess nitro benzene. The oxidation mixture is acidified to pH=3-4 with conc. HCl and further extracted continuously with $CHCl_3$ for 48h. The solvent from the second $CHCl_3$ solution is removed at 40 C under reduced pressure to abtain the nitrobenzene oxidation mixture. The mixture was then dissolved in 10ml $CH_2Cl_2$ to obtain the nitrobenzene oxidation mixture used for GC-MS analysis.

### Acetylation of Lignin

100 mg Purified lignin was added in 2ml of dry pyridine-acetic anhydride (1:1) for 72 h. The solution was added to a 10-fold volume of ice-cold water where upon the acetylated sample was recovered as a precipitate, which was lignin by successive washing with water and dry.

**Molecular Weight**

The weight average ($M_w$) and number average ($M_n$) molecular weight *Fagus Orientalis* acetylated lignin were determined by GPC on an Agilent plgel column. The samples were dissolved in tetrahydro furan (THF) and 20µl was injected to the column. The column was operated at 30°C and eluted with THF at a flow rate of 1ml/min. The column was calibrated using polystyrene standards.

**GC-MS Analysis**

GC-MS analysis was conducted using a Agillent gas chromatograph equipped with capillary column (30m×0.5mm), Column temperature was programmed to increase from 50 to 260°C at the rate of 6°C/min. Column flow rate was 10 ml/min and split ratio was 10.

**Elemental and Methoxyl content Analysis**

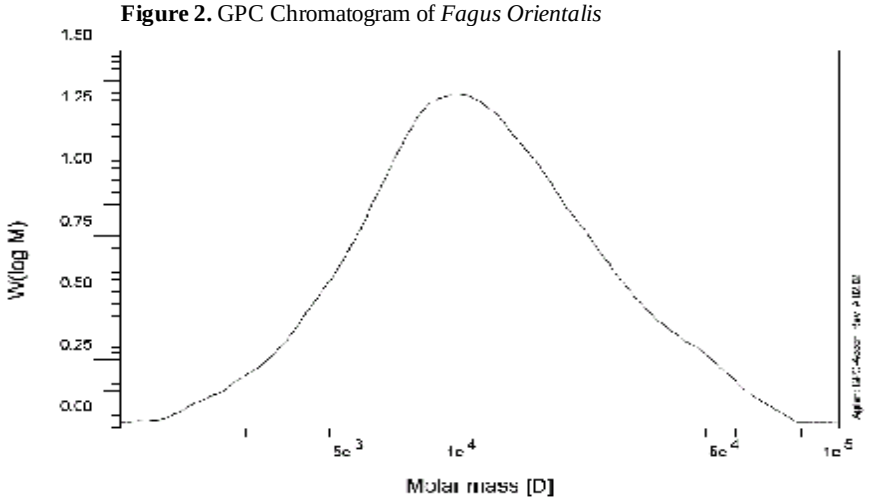
C, H and N analysis of purified lignin samples were carried out using C, H, N analyzer and O content was determined by difference. The methoxyl content in lignins was determined in accordance of JIS P8013 1972 standard method.

**Spectroscopy**

*FTIR*: Infrared spectra were recorded by using a Shimadzu FTIR spectrometer model 8201PC [6]. The dried samples were embedded in KBr pellets in the concentration of about 1mg/100mg KBr. The spectra were recorded in the absorption band mode in the range 4000–400 $cm^{-1}$.

$^1$*H-NMR*: Spectra of 100mg acetylated lignin solution in 0.5ml $CDCl_3$ were recorded in a Bruker 400 spectrometer. TMS Solvent was used as internal standard (7.25ppm).

**Figure 2.** GPC Chromatogram of *Fagus Orientalis*




**Results and Discussion**

**Molecular weight**

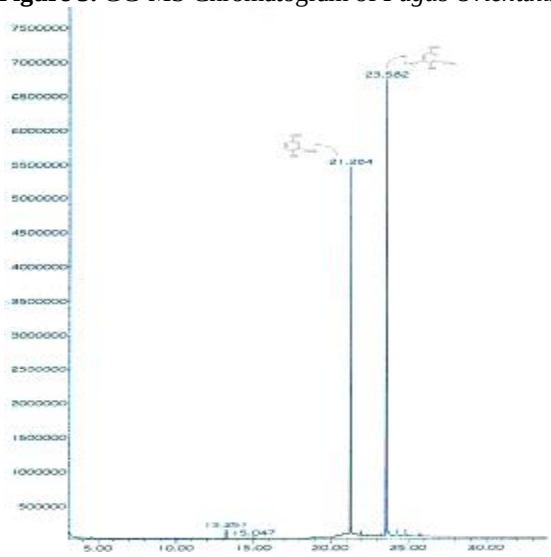
The weight average ($M_w$) and number average ($M_n$) molecular weight of *Fagus Orientalis* lignin are 15986 g/mol and 9746.9 g/mol respectively (Fig. 2). The polydispersity of lignin (Mw/Mn) was about 1.64.

**Alkaline nitrobenzen oxidation**

Fig. 3 shows the GC chromatogram of *Fagus Orientalis* lignin. Procedures for analyzing lignin by alkaline nitrobenzene oxidation can be used to obtain information about the composition of the original lignin polymer. In this oxidation, three constitutive monomeric lignin units *p*-hydroxyphenyl, guaiacyl and syringyl produce the corresponding *p*-hydroxybenzaldehyde,

vanillin, syringaldehyde. Alkaline nitrobenzene oxidation showed that the syringaldehyde (S) was the predominant, which comprised 66%. Vanillin appeared as the second major degradation products resulting from the non condensed guaiacyl (G) units. Vanillin percentage was 34. The relative ratio of S to G was 1.94. These results shows that ratio of OCH3/C9 in the lignin is about 1.66. It has good agreement with other hardwood lignin.

**Figure 3.** GC-MS Chromatogram of *Fagus Orientalis*




## $^1$H-NMR Spectroscopy

Table 1. lists the position of signal assigned by [7] and their proton number per C9 unit calculated from the integration of $^1$H-NMR spectra.

The area ratio of free aliphatic and phenolic hydroxyl groups were determined from the corresponding acetate signals. The number of (OH)al/C9 and (OH)ph/C9 was 1.055, 0.67 respectively. The ratio of $(OH)_{al}/(OH)_{ph}$. was 1.57. The lignin showed two peaks in the aromatic proton region, which corresponded to guaiacyl units (δ 6.9) and syringyl units (δ 6.6). There is stronger peak in syringyl units region (δ 6.6) than guaiacyl units region (δ 6.9) and the ratio is 1.9. This ratio is in agreement with GC analyze results (1.94).

## FTIR Spectroscopy

FTIR spectra were recorded and the assignment made by Faix (1991). The band at 1600cm$^{-1}$ was assigned to the aromatic skeletal vibrations, 1507cm$^{-1}$ assigned to the aromatic skeletal vibrations coupled with C-H in plane deformations, 1460cm$^{-1}$ assigned to C-H deformations (asymmetric in methyl, methylene and methoxyl group).

**Figure 4.** $^1$H-NMR Spectra of *Fagus Orientalis*

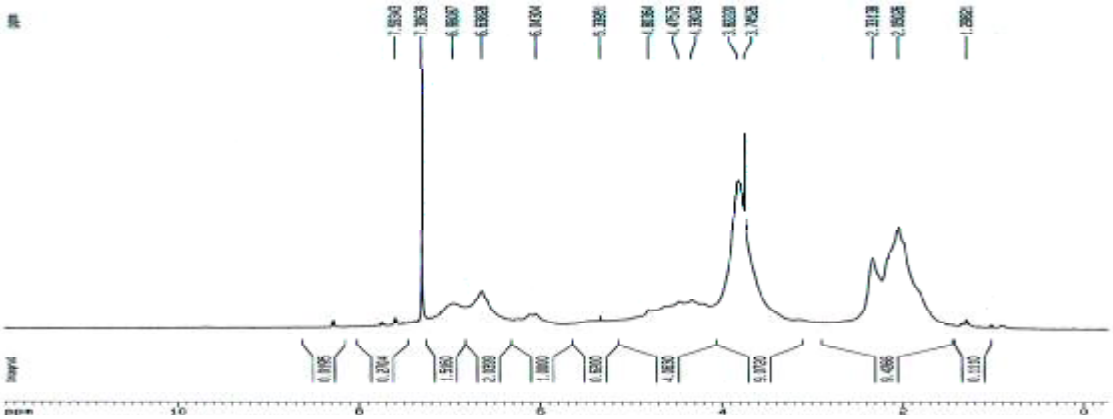


**Figure 5.** FTIR Spectra of *Fagus Orientalis*

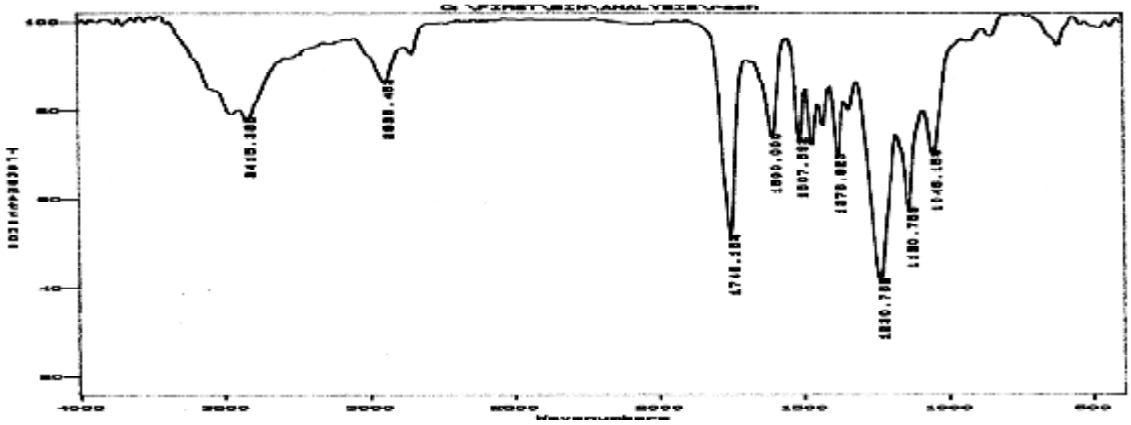


**Table 1:** [1]H-NMR data of the acetylated *Fagus Orientalis* lignin

| Region δ (ppm) | Attribution | Area (arbitrary units) | % area / total area |
|---|---|---|---|
| > 7.5 | Other | 0.29 | 1.05 |
| 6.95 | Aromatic region in G. | 1.51 | 5.49 |
| 6.63 | Aromatic region in S. | 2.04 | 7.42 |
| 5.75-6.25 | Cyclic benzylic region | 1 | 3.64 |
| 3.95-5.20 | Aliphatic region | 4.06 | 14.76 |
| 3.55-3.95 | Methoxyl | 9.072 | 32.98 |
| 2.20-2.50 | Aromatic acetoxyl region | 3.676 | 13.36 |
| 1.60-2.20 | Aliphatic acetoxyl region | 5.75 | 20.90 |
| <1.60 | Nonoxygenated aliphatic region | 0.111 | 0.4 |

## Elemental analysis and $C_9$ formula

The average $C_9$ formula was calculated from the elemental analysis, methoxyl content and HNMR Spectra [8]. The results of elemental analysis for *Fagus Orientalis* purified dioxan lignin was 5.9% H, 55.75% **C**, 38.4% **O**. The number of methoxyl groups per **C**9 unit was 1.66. The $C_9$ formula for *Fagus Orientalis* wood calculated as $C_9H^{al}_{2.78}H^{ph}_{2.1}O_{2.144}(OH)^{ph}_{0.673}(OH)^{al}_{1.055}(OCH_3)_{1.66}$.

## Conclusions

It was concluded that dioxan lignin extracted from *Fagus Orientalis* was syringyl–guaiacyl type. The polydispersity of lignin (Mw/Mn) was about 1.64. The methoxyl content of these lignins was similar to hardwood ligninas (1.66/**C**9) and **C**9 formula is: $C_9H^{al}_{2.78}H^{ph}_{2.1}O_{2.144}(OH)^{ph}_{0.673}(OH)^{al}_{1.055}(OCH_3)_{1.66}$.

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