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**Isolation and Characterization of Lignocellulosic Polymers from  
Wood Pulp of *Khaya anthotheca***

عزل وتشخيص بوليمرات اللجنوسليلوز من نشارة خشب الموهقتى

A dissertation Submitted in Partial Fulfillment of the Requirements of Bachelor Degree  
in Chemistry

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## **Dedication**

This research work is dedicated to our respective parents who have been our constant source of inspiration. They have given us the drive and enthusiasm and without their love and support this project would not have been made possible.

To our families whom support us all the time.

## **Acknowledgement**

First of all we would like to thank **Allah** (God) who gave us strength, blessing, and courage during this study and during all of our life.

We would like to express our deepest and profound gratitude to our supervisor doctor Essa Esmail Mohammed Ahmed for his guidance, encouragement, and endless support during our research study. We earned a lot throughout your supervision. We really feel that words will not express our appreciation to whatever you have done for us.

Special thanks for those teachers whom helped us in lots of time during this study, Ahmed, Munzir.

Also we would like to thank all our colleagues, friends, and the academic staff in the department of chemistry, college of science at SUST, Sudan.

## Abstract

The objective of this study was to isolate and characterize lingo-cellulosic polymers (lignin, hemicelluloses, and cellulose) from wood pulp of *Khaya anthotheca*.

Concentrated sulfuric acid was used to isolate lignin from the other constituents, whereas for isolation of holocellulose, sodium chloride and glacial acetic acid were used and finally cellulose was separated from hemicellulose using alkaline hydrolysis in aqueous solution of sodium Hydroxide. The results have shown that the percentages of lignin, hemicelluloses, and cellulose are: 23%, 32%, and 42%, respectively.

FTIR spectra of the isolated lignin, cellulose, and holocellulose have displayed their characteristic absorption bands. FTIR spectrum of lignin have displayed the characteristic aromatic (C=C) absorption band between 1500-1600  $\text{cm}^{-1}$  together with other stretching and bending vibrations of -OH, -CH, -C-O functional groups. On the other hand, the FT-IR spectrum of holocellulose demonstrated the presence of the characteristic carbonyl absorption peak of hemicellulose around 1736  $\text{cm}^{-1}$  with the stretching and bending absorption peaks of -OH, -CH, and -C-O. Finally, the spectrum of cellulose proved the disappearance of -C=O peak of hemicellulose and only -OH, -CH, -C-O absorption bands were appeared.

## مستخلص البحث

هدفت هذه الدراسة الى عزل وتشخيص بوليمرات الليجنوسيليلوز (لجيين ،هيمي سيليلوز ، سيليلوز) من نشارة خشب المهوقني.

استخدم حمض الكبريتيك المركز لعزل اللجيين من باقي المكونات الأخرى بينما الهولوسيليلوز باستخدام كلوريد الصوديوم وحمض الخل الثلجي و أخيرا عزل السليلوز من الهولو سيليلوز بالتحلل في القاعدي باستخدام محلول هيدروكسيد الصوديوم .

بينت النتائج ان نسبة اللجيين والهيمي سيليلوز والسيليلوز (٢٣%، ٣٢%، ٤٢%) على التوالي .

أطياف الاشعه تحت الحمراء للبوليمرات المعزوله واعطت الامتصاصات المميزة لها ،

طيف الاشعه تحت الحمراء للجيين اعطى امتصاص للرابطة الثنائيه الاروماتيه في المدى ١٦٠٠ - ١٥٠٠

مع وجود امتصاصات تمثل الشد والانحناء لمجموعات الهيدروكسيل ،  
-OH, -CH, -C-O

في الجانب الاخر فان طيف الاشعه تحت الحمراء للسيليلوز ،للهولوسيلولوز ،بين ظهور امتصاص مجموعه الكاربونيل المميزة للهيمي سيليلوز حوالي ١٧٤٦ مع وجود امتصاصات أخرى

أخيرا فان طيف السيليلوز أوضح اختفاء امتصاص مجموعه الكاربونيل ووجود امتصاصات  
-OH, -CH, -C-O

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# Chapter one

## Introduction and literature review

### 1.1 Natural Fibers: general introduction

Fibers are a class of hair-like material that are continuous filaments or are in discrete elongated pieces, similar to pieces of thread. They can be spun into filaments, thread, or rope. They can be used as a component of composites materials. They can also be matted into sheets to make products such as paper or felt. Fibers are of two types: natural fiber and man- made or synthetic fiber. Figure 1.1 shows the classification of natural fibers.

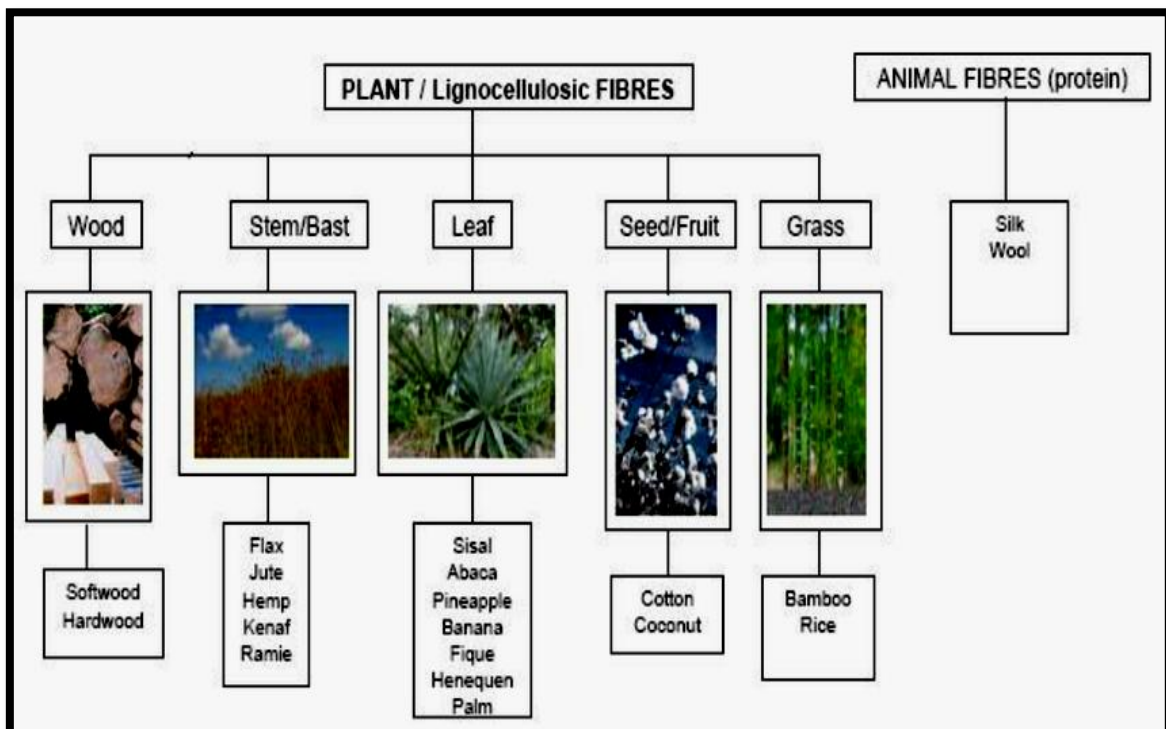


Figure 1.1: Classification of natural fibers

Natural fibers include those made from plant, animal and mineral sources. They can be classified according to their origin into:

## **1.2 Vegetable fibers**

Vegetable fibers are generally comprised mainly of cellulose: examples include cotton, jute, flax, ramie, sisal, and hemp. Cellulose fibers serve in the manufacture of paper and cloth. This fiber can be further categorized into the following:

- i) Seed fiber** : Fibers collected from seeds or seed cases. E.g. cotton and kapok .
- ii) Leaf fiber**: Fibers collected from leaves. e.g. sisal and agave .
- iii) Bast fiber or skin fiber**: Fibers are collected from the skin or bast surrounding the stem of their respective plant. These fibers have higher tensile strength than other fibers. Therefore, these fibers are used for durable yarn, fabric, packaging, and paper. Some examples are flax, jute, kenaf, industrial hemp, ramie, rattan, soybean fiber, and even vine fibers and banana fibers .
- iv) Fruit fiber**: Fibers are collected from the fruit of the plant, e.g. coconut (coir) fiber .
- v) Stalk fiber**: Fibers are actually the stalks of the plant. E.g. straws of wheat, rice, barley, and other crops including bamboo and grass. Tree wood is also such a fiber .

The most used natural fibers are cotton, flax and hemp, although sisal, jute, kenaf, and coconut are also widely used. Hemp fibers are mainly used for ropes and aerofoils because of their high suppleness and resistance within an aggressive environment. Hemp fibers are, for example, currently used as a seal within the heating and sanitary industries .

## **1.3 Animal fibers**

Animal fibers generally comprise proteins; examples include silk, wool, angora, mohair and alpaca.

**i) Animal hair (wool or hairs):** Fiber or wool taken from animals or hairy mammals. E.g. sheep's wool, goat hair (cashmere, mohair), alpaca hair, horse hair, etc.

**ii) Silk fiber:** Fiber collected from dried saliva of bugs or insects during the preparation of cocoons. Examples include silk from silk worms.

**iii) Avian fiber:** Fibers from birds, e.g. feathers and feather fiber .

#### **1.4 Mineral fibers**

Mineral fibers are naturally occurring fiber or slightly modified fiber procured from minerals. These can be categorized into the following categories:

**i) Asbestos:** The only naturally occurring mineral fiber. Varieties are serpentine (chrysotile) and amphiboles (amosite, crocidolite, tremolite, actinolite, and anthophyllite).

**ii) Ceramic fibers:** Glass fibers (Glass wool and Quartz), aluminum oxide, silicon carbide, and boron carbide.

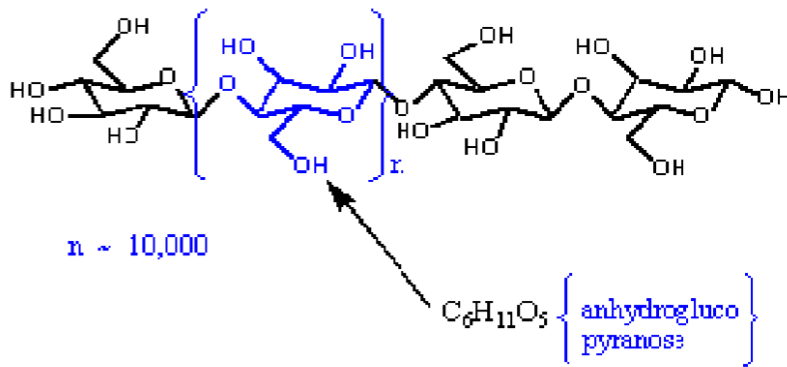
**iii) Metal fibers:** Aluminum fibers. [1]

#### **1.5 Cellulose: structure, properties and applications**

Cellulose is the most abundant polymer on Earth and hence can be regarded as a very important raw material for several purposes. Virtually all the natural manifestations of cellulose are in the form of semi-crystalline fibres whose morphology and aspect ratio can vary greatly from species to species. Cellulose is a linear polymer made of the monomer D-glucose units that are linked successively through glycosidic linkage in the  $\beta$ -configuration

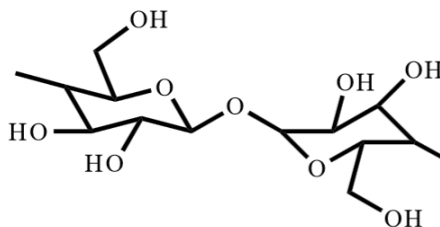
between carbon 1 and carbon 4 of adjacent unit to form a polymeric chain

Figure 1.2



**Figure 1.2: Structure of Cellulose**

Because of the  $\beta$ -configuration of the intermonomer links, the glucose units effectively alternate up and down in the chain, as a result of that cellobiose Figure 1.3 is considered to be the repeating unit of cellulose, on which a syndiotactic configuration of the macromolecule is formed.



**Figure 1.3: Structure of glucose unit.**

The size of the cellulose molecule occurring in nature is indicated by its degree of polymerization (DP) and is dependent heavily on its source, pure cellulose inevitably loses some of its molecular weight (MW) during isolation and purification. The DP of cellulose typically ranges from 1,000 to 10,000, and cellulose chains are composed entirely of anhydrous D-glucose units. Cellulose is unlike starch, no coiling occurs, and the molecule adopts an extended stiff rod-like conformation. The multiple hydroxyl groups on the glucose residues

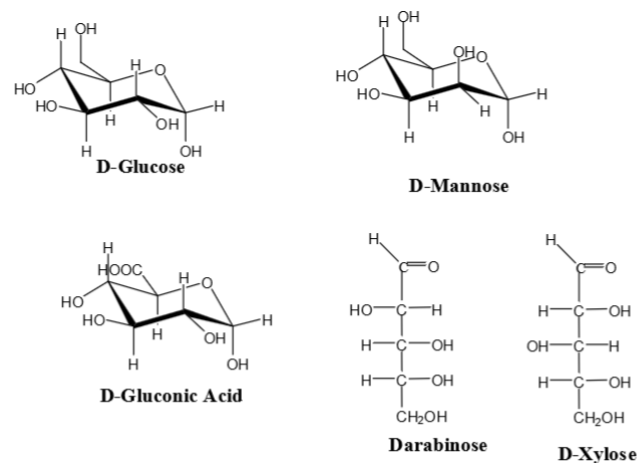
from one chain form hydrogen bonds with oxygen molecules on adjacent chain, holding. The chains firmly together side-by-side and forming micro-fibrils with high tensile strength. Compared to starch, cellulose is composed of two regions (crystalline and amorphous) while starch is an amorphous cellulose. Cellulose is very stable, It has no taste, odorless, hydrophilic, insoluble in water and in most organic solvents, chiral, and is biodegradable. The uniqueness of the chemical structure and macromolecules configurations make cellulose rigid. Being natural polymers cellulose and cellulose derivatives are generally recognized as safe nontoxic material, noncarcinogenic, biocompatible, and in no way harmful in the biological environment. Cellulose in its polymeric form can be employed for manufacture of paper and industrial feedstocks or converted into large number of derivatives with unlimited number of commercial applications, and also in the production of ethanol when chemically or enzymatically hydrolyzed into glucose. For instance cellulose acetate is a plastic collodion useful in medicinal applications. Cellulose ethers like hydroxypropylmethyl cellulose (HPMC) are generally applied to many areas of industry and domestic life. Its spectrum of applications ranging from auxiliaries in large-scale emulsion or suspension polymerization, through to additives for paints and wall papers adhesives, to viscosity enhancers in cosmetics and food-stuffs. The worldwide industrial manufacture of cellulose ethers has presently arrived at a level of over half a million tons annually. The most important properties of cellulose ethers are their solubility, chemical stability and non-toxicity. Cellulose also is the raw material in the manufacture of nitrocellulose (cellulose nitrate) which was historically used in smokeless gunpowder and as the base material for celluloid used for photographic and movie films until the mid 1930s.

Another important application for cellulose is in the laboratory as the stationary phase for thin layer chromatography. Cellulose insulation made from recycled newsprint is becoming popular as an environmentally preferable material for building insulation. Cellulose is also used to make hydrophilic and

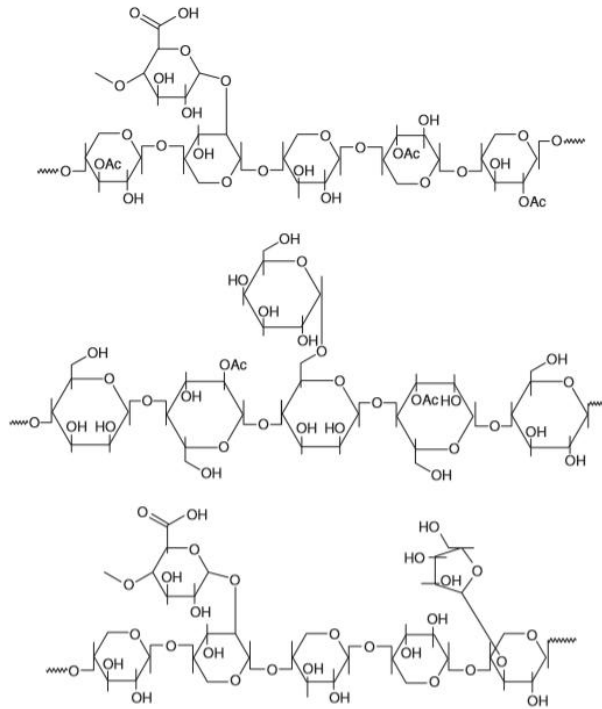
superabsorbent sponges, as well as water-soluble adhesives and binders such as methyl cellulose and carboxymethyl cellulose which are used in wallpaper paste. Cellulose fibers are also used in liquid filtration, sometimes in combination with diatomaceous earth or other filtration media, to create a filter bed of inert material. Despite the fact that humans (and many other animals) cannot digest cellulose (meaning that their digestive systems cannot break it down into its basic constituents), cellulose is a very important part of the healthy human diet. This is because it forms a major part of the dietary fiber that we know is important for proper digestion. Since human bodies cannot break cellulose down and it passes through our systems basically unchanged, and that makes called bulk or roughage that helps the bowel movements. In fact, crystallite cellulose is added to some foods to reduce the caloric value. Another important form of cellulose is microcrystalline cellulose and powdered cellulose. Microcrystalline cellulose (MCC) is partially depolymerized cellulose prepared by treating cellulose with hydrochloric acid. The microcrystalline cellulose occurs as a fine, white, odorless crystalline powder usually used as inactive ingredients fillers in medicine tablets and as thickeners and stabilizers in processed foods. Microcrystalline cellulose used as an excipient to assist in the flow lubrication, and bonding properties of the ingredients to be tableted; to improve the stability of the drugs in tablet form; and especially to provide for rapid disintegration in the stomach. It also contributes stability to many pharmaceutical formulation. When properly dispersed in water to formed stable colloidal gels and dispersions, MCC can be used in cosmetics and pharmaceuticals for both creams and solid suspensions. It is also used to prepare reduced-lipid or lipid-free ice cream and mayonnaise like products and low-and no-oil pourable salad dressings, it also strengthens and stabilizes foams. [2]

## 1.6 Hemicelluloses

The second main component of wood is hemicelluloses; that is group of heteropolysaccharides built up of different types of a monosaccharides. The chains of hemicellulose are shorter than those of cellulose, with a degree of polymerization of about 100 to 200. Like cellulose, most hemicelluloses function as supporting material in the cell walls. Hemicelluloses are relatively easily hydrolyzed by acids to their main monomers consisting of glucose, mannose, xylose, galactose arabinose and rhamnose. The structures of these monosugars are shown in Figure1.4 In addition some hemicelluloses contain uronic acids. The compositions and structures of the hemicelluloses depend on the source. [2]



**Figure 1.4: Structure of mono sugars.**

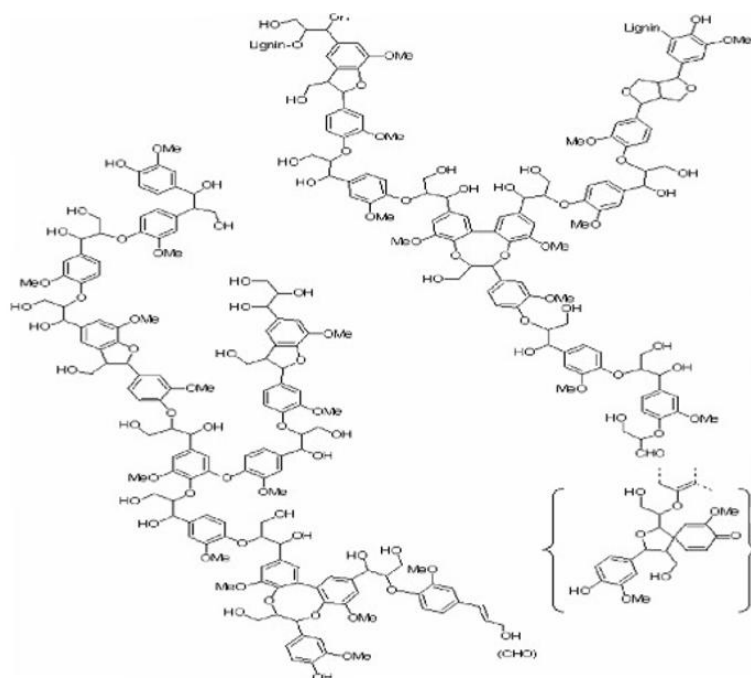


**Figure 1.5: Hemicellulose structure. [2]**

### 1.7 Lignin

The third main component of wood is lignin, the material that binds the cellulose fiber together in wood and it differs from cellulose and hemicelluloses in many ways. It has no obvious repeating unit building up of its structure and the structure of lignin can in the broadest sense be described as three dimensional net work as shown in Figure 1.6

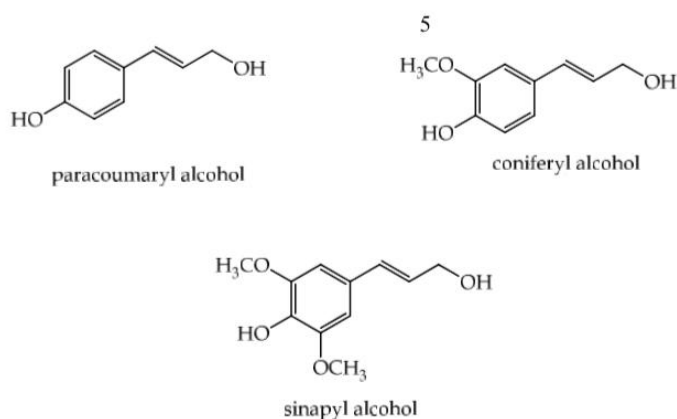




**Figure 1.6: Structure of Lignin.**

The lignin is built up of hydroxyphenylpropane units and is phenolic in character. The hydroxyphenylpropane units are connected by various types of bonds, of which arylglycerol-arylethers (-O-4) are the most frequent. The chemical structure of lignin is irregular in the sense that the structural elements are not linked to each other in any systematic order. Lignin is a result of the radical polymerization of three Hydroxy phenyl propane units shown in Figure 1.7. Lignin is one of the most predominant biopolymers present in plants. Together with cellulose and hemicelluloses, lignin builds up the cell wall in an arrangement which is regulated on the nano-scale and results in lignin-carbohydrate network structures. Lignin is present in all vascular plants making it second to cellulose in abundance among polymers in nature. Since lignin, like many other biomass components, is formed via the photosynthesis reaction, it is renewable and it has been estimated that the annual production of lignin on earth is in the range of 5–36 tons in woody plants from the gymnosperm and angiosperm phylum, the lignin content is in the order of 15–40 per cent whereas in herbs, the lignin content is less than 15 per cent. Low lignin content is usually also encountered in annual plants. Some representative values for the content of lignin in

various types of commercially important plants are given. many softwood and hardwood species, together with certain types of annual plants, have commercial interest as a source of cellulose fibres for the production of paper and board products. [2]



**Figure 1.7: Structure of Hydroxy phenyl propane units.**

Thus, in technical fibre liberation processes, such as alkaline or sulphite pulping, huge quantities of lignin are dissolved as alkali lignin and liginosulphonates, respectively. With few exceptions, these lignins are, however, never isolated, but burnt together with other wood constituents liberated in the pulping liquor in order to produce the steam required for the process. In all commercial pulping processes, as well as in emerging processes, such as wood hydrolysis for the production of biofuels, the lignin is structurally altered in comparison to the native lignin. Broadly, these types of lignin can be described as being heterogeneous polyphenols with molecular masses in the range of 100–300,000 often with a high degree of polydispersity. In growing plants, on the other hand, the lignin constitutes an integral part of the cell walls with chemical linkages to all types of polysaccharide constituents present. For spruce wood, it has been shown that the major portion of lignin is covalently linked to the hemicelluloses ( i.e. xylan and glucomannan) with a minor amount being linked to cellulose. The concentration of lignin is, however, not evenly distributed throughout the cell wall and, despite a high concentration of lignin in the middle lamella, the predominant portion is located in the S2 layer of the secondary wall due to its large

relative volume. A detailed analysis of the lignin distribution in two wood species, one softwood and one hardwood, has been published. All attempts to isolate lignin from wood or other types of biomass must be preceded by some mechanical disintegration of the material. Usually, intensive milling of the material is employed whereby the structural integrity (i.e. cell types), cell layers and any inhomogeneities at the macromolecular level, is eliminated and, from such materials, only an average lignin structure can be obtained. Despite these drawbacks, almost all present knowledge about the structure of lignins is based on comprehensive milling of the plant material, followed by solvent extraction with dioxane and (sometimes) further purification to yield low to moderate yields of lignin. In most isolation procedures, the lignin contains minor impurities of carbohydrates.[3]

### **1.8 *Khaya anthotheca* (Mahogany)**

Deciduous forest trees 60 m high. Stems buttressed to a high of 4 m. Bark grayish brown, flaking into small irregular scales; slash bright crimson exuding red sap. Branches grey, glabrous with shield-like leaf scars. Leaves pinnate, clustering at end of branches, spirally arranged, 12.5 – 20 cm long; petioles 7cm long pinnate pairs 4-5; leaflets opposite or subopposite, 11-14\*4-4.5 cm elliptic to ovate –elliptic, coriaceous, glabrous, 0.5-1cm long, new foliage pink or copper colour. inflorescence stiff auxiliary panicles up to 30 cm long; flowers white to cream, tetramerous (rarely 5-merous);ovary with an orange red disc at the base. Fruit globose , grey to light brown,woody,4-valved capsules, 6-8 cm across ,dehiscing long- intrudingly ; seeds dorso-ventrally fiat ,oblong, light brown , 1.5 - 2.5 \*1 - 1.7cm (including the narrow flat wings). Flowers May; fruits October. On alluvial soils along river banks and valleys in broad leaved high rainfall savanna in equatorial (katire) and the eastern bank of the White Nile. *k.grandifolila* deciduous to evergreen large trees 20 – 35 m high with slightly crooked buttressed stem. Bark light grey, smooth at top of the bole cracking into irregular scales towards the base; girth 3 m; slash dark red with lighter streaks . Branches grey, glabrous with white round lenticels and prominent shield – like leaf scars . leaves pinnate, clustered at ends of branches , spirally arranged ,large50 – 60 cm long with

petioles 10 – 20 cm long ;pinnate pairs 5 – 7;leaflets broadly elliptic to ovate elliptic,20 – 25\*5 – 10cm , opposite or sub-opposite , coriaceous and glabrous 9 cm long; new foliage pink. [4]

### **1.9 Objective**

The objective of the present study is isolate and characterize lignocellulosic polymers (lignin, cellulose and hemicelluloses) from *Khaya anthotheca*.

## **Chapter two**

### **Materials and methods**

#### **2.1 Sample collection and pretreatments**

The sample was collected from industrial area (it is a wooden waste remaining during furniture processing)-Omudurman.

#### **2.2 Chemicals**

Ethanol – n-hexane - sulphuric acid( 98% ,Alpha INDA)– sodium hydroxide (Assay = 97%, CDH-India) – acetic acid( 99.7% ,Reagent DUKSAN) - glacial acetic acid(99.7%,DUKSAN) – sodium chloride(Egypt B.p93) – acetone(99.0% CDH).

#### **2.3 Methods**

##### **2.3.1 Extractable content**

The air dried sample of 5 g was weighed in an extraction thimble and placed in Soxhlet extraction unit. A mixture of ethanol and toluene was used as solvent and extraction process continued for a period of five hours. After extraction the sample was rinsed with ethanol and hot water and dried up to constant weight at the temperature of 60°C. The extractable were calculated as a percentage of the oven dried test sample and the method has been repeated for each sample.[]

##### **2.3.2 Determination of lignin content**

Two grams of the extracted sample (previous method) were placed in a flask and 15 ml of 72% sulphuric acid was added. The mixture was stirred frequently for two and half hours at 25°C and 200 ml of distilled water were added to the mixture. Then the mixture was boiled for next two hours and cooled. After 24 hours, the lignin was transferred to the crucible and washed with hot water repeatedly until becoming acid free. The collected lignin was dried at 105°C and cooled down in desiccator and weighed. The drying and weighing were repeated until constant weight.[]

##### **2.3.3 Determination of holocellulose content**

Three grams of air dried fiber were weighed and placed in an Erlenmeyer flask and then, 160 ml of distilled water, 0.5 ml of glacial acetic acid and 1.5 g of sodium chloride were added successively. The flask was placed in water bath and heated up to 75°C for an hour and then additional 0.5 ml of glacial acetic acid and 1.5 g of sodium chloride were added. The additions of acetic acid and sodium chloride were repeated two times hourly. The flask was placed in an ice bath and cooled down below 10°C. The holocellulose was filtered and washed with acetone, ethanol and water respectively and at the end; sample was dried in an oven at 105°C before weighed.[]

#### **2.3.4 Determination of $\alpha$ - cellulose content**

Two grams of holocellulose were placed in a beaker and 10 ml of sodium hydroxide solution (17.5%) was added. The fibres were stirred up by glass rod so that they could be soaked with sodium hydroxide solution vigorously. Then sodium hydroxide solution was added to the mixture periodically (once every five minutes) for half an hour and the mixture temperature was kept at 20°C. About 33 ml of distilled water was added in the beaker and kept it for an hour. The holocellulose residue was filtered and transferred to the crucible and washed with 100 ml of sodium hydroxide (8.3%), 200 ml of distilled water, 15 ml of acetic acid (10%) and again water successively. The crucible with  $\alpha$ -celluloses was dried and weighed.[]

#### **2.4 FT-IR measurements**

A few milligrams of the samples (wood fiber, lignin, holocellulose, and cellulose) was taken and mixed thoroughly with KBr powder and pressed into a disc shape. The disc was placed in IR spectrometer and the spectrum and the spectrum was recorded in the range 500 -4000  $\text{cm}^{-1}$ .

## Chapter three

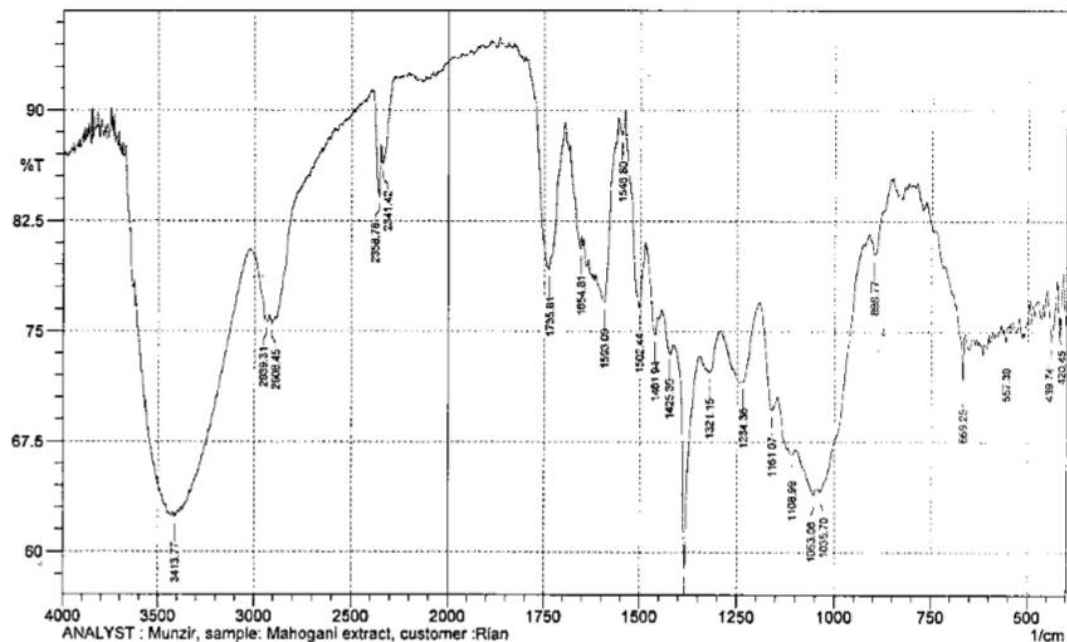
### Results and Discussion

**Table 3.1:** Percentages of Cellulose, lignin, and hemicelluloses

Component	Percentage %
Cellulose	42
Lignin	23
Hemi-cellulose	32

### 3.2 FT-IR measurements

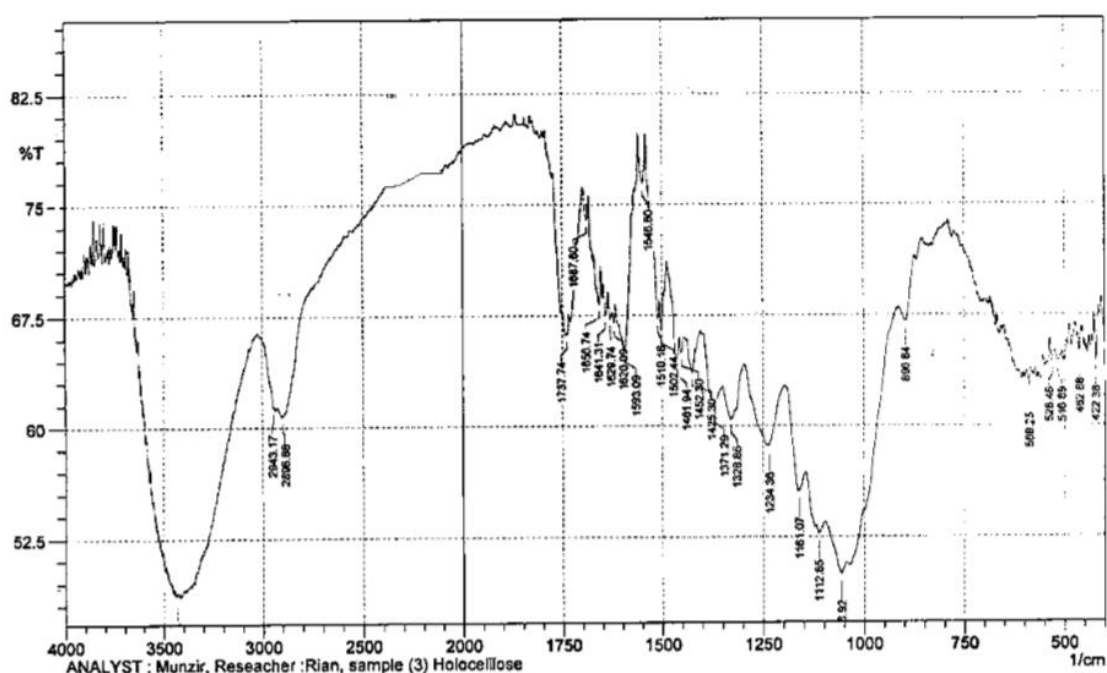
FTIR analyses were carried out to confirm the functional groups of lignocellulosic polymers. Figure 3.1 represents the FTIR spectrum of Mahogany wood pulp.



**Figure 3.1:** FTIR spectrum of Mahogany wood pulp.

As can be seen from the spectrum, the peak at  $3414\text{ cm}^{-1}$  is due to O-H stretching vibration, whereas the two peaks at  $2939\text{ cm}^{-1}$  and  $2908\text{ cm}^{-1}$  are

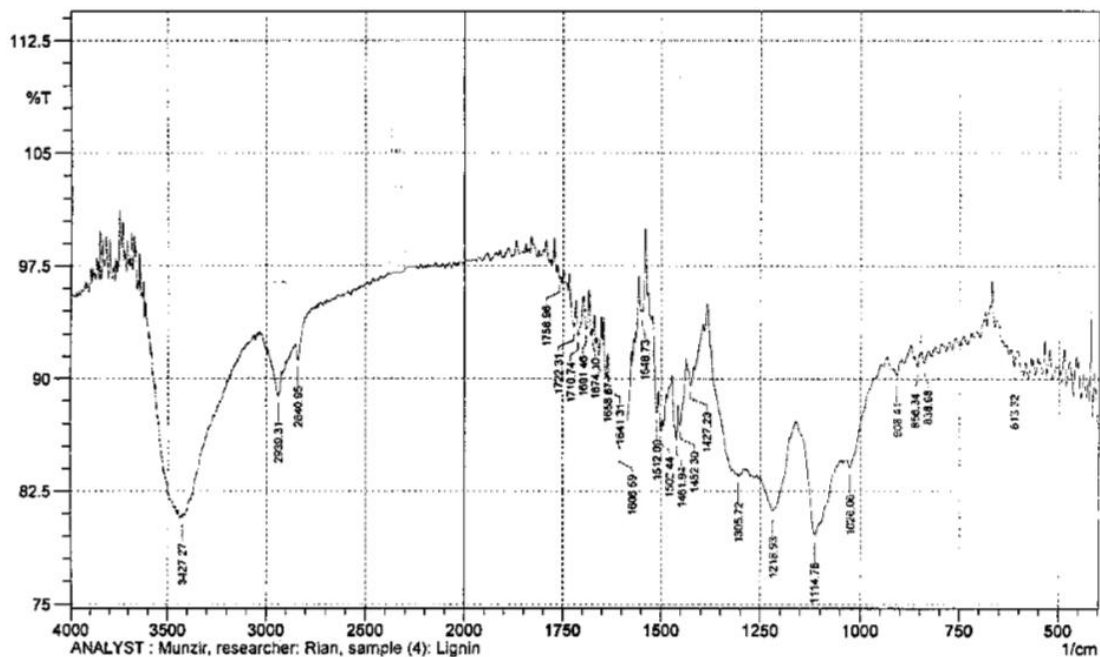
attributed to absorption bands of C-H ( $sp^3$  hybridized) stretching vibration of alkanes. The peak at  $1736\text{ cm}^{-1}$  is due to carbonyl group stretching vibration and the peaks around  $1502\text{ cm}^{-1}$  and  $1655\text{ cm}^{-1}$  are characteristic bands of aromatic C=C stretching vibration. The peaks at  $2359\text{ cm}^{-1}$  and  $2341\text{ cm}^{-1}$  are characteristic bands of adsorbed water, and the peak at  $1234\text{ cm}^{-1}$  is due to C-O stretching vibration, and the peaks at  $1425\text{ cm}^{-1}$ ,  $1462$  are characteristic band of C-H bending vibrations.



**Figure 3.2: FTIR spectrum for Holocellulose.**

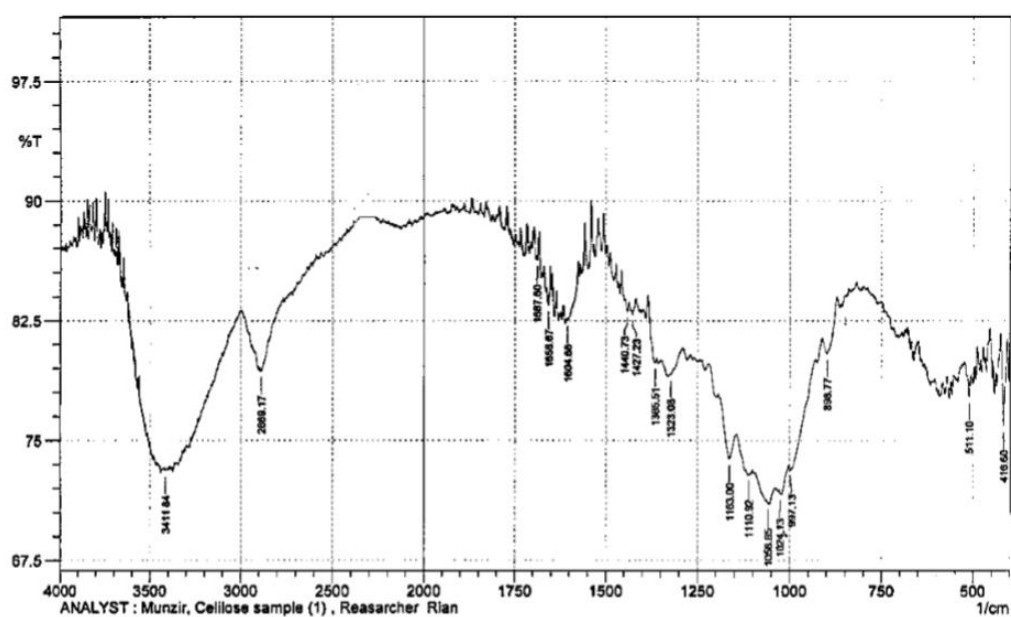
As can be seen from the spectrum Figure 3.2, the peak at  $3450\text{ cm}^{-1}$  is due to O-H stretching vibration, whereas the peaks at  $2943\text{ cm}^{-1}$  and  $2896\text{ cm}^{-1}$  are attributed to absorption bands of C-H ( $sp^3$  hybridized) stretching vibration of alkanes, and the peak at  $1738\text{ cm}^{-1}$  is due to carbonyl group stretching vibration, and the peaks around  $1425\text{ cm}^{-1}$  and  $1328\text{ cm}^{-1}$  are characteristic as the absorption bands of C-H bending vibrations, whereas the peak at  $1234\text{ cm}^{-1}$  is due to C-O stretching vibration.





**Figure 3.3: FTIR spectrum of lignin.**

As can be seen from the spectrum (Figure 3.3), the peak at 3427.27 is due to O-H stretching vibration, whereas the two peaks at 2939  $\text{cm}^{-1}$  and 2842  $\text{cm}^{-1}$  are attributed to absorption bands of C-H ( $\text{sp}^3$  hybridized) stretching vibration of alkanes, the peaks at 1512  $\text{cm}^{-1}$  and 1502  $\text{cm}^{-1}$  are characteristic bands of aromatic ring, the peak at 1219  $\text{cm}^{-1}$  is due to C-O stretching vibration, The peak at 1641  $\text{cm}^{-1}$  is due to adsorbed water.



**Figure 3.4: FTIR spectrum for cellulose.**

As can be seen from the spectrum, the peak at  $3412\text{cm}^{-1}$  is due to O-H stretching vibration, whereas the peak at  $2889\text{ cm}^{-1}$  is characteristic as C-H stretching vibration, and the two peaks at  $1386\text{cm}^{-1}$  and  $1323\text{ cm}^{-1}$  are attributed bands of C-H bending vibration, and the peak at  $1163\text{ cm}^{-1}$  is due to C-O stretching vibration.

## **Conclusion**

The three natural polymers (lignin, cellulose, and hemicellulose) were isolated from wood pulp waste. The percentages were determined and the identification was carried out using Infra-Red Spectroscopy.

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