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# Comparative Studies of the Chemical Nature of Ethanol Product of Selected Wood Species

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## Abstract

This thesis covers the process of making Ethanol from wood biomass instead of starchy biomass. The saw dust of Gmelina (*Gmelina arborea*), Eku (*Brachystegia euricoma*) and Mahogany *(Entandrophragma cylindricum*) was collected in a saw mill in Ore and used to produce ethanol by hydrolysis and fermentation processes. The density of each of the wood species was thereafter determined as 570 kg/m$^3$, 750 kg/cm$^3$ and 600 kg/cm$^3$ respectively. The yield of ethanol from Eku, Mahogany and Gmelina wood was determined as 50.61 g/l per 100 g of dry sawdust, 55.43 g/l per 100 g of dry sawdust and 53.01 g/l per 100 g of dry sawdust respectively. The density of the ethanol produced from the wood of Eku, Mahogany and Gmelina was 0.8033 g/cm$^3$, 0.7088 g/cm$^3$, and 0.8033 g/cm$^3$ respectively. These results were subjected to Analysis of Variance (ANOVA) and compared with conventional ethanol. The ANOVA result shows no significant difference among the ethanol yield and ethanol density obtained from the three wood species and that of the conventional ethanol. The ionic constituents of the ethanol of the three wood biomass was analyzed using Furrier Transform Infrared Spectrometric Analyzer (FTIR) and Atomic Absorption Spectrometric Analyzer (AAS). The AAS result shows that the ethanol obtained from the three wood species contains transition metals like Copper (Cu), Zinc (Zn), Cadmium (Cd) and Chromium (Cr) while the FTIR results show the presence of ethanol functional groups such as OH, Carbon to carbon single bond which are normal components of ethanol in the conventional ethanol as well as in the ethanol produced from each of the three wood species.

**Keywords:** Ethanol; Glucose; Cellulose; Hemicellulose; Lignin; Sulphuric acid; Caustic soda

## Chapter One: Introduction

For several decades, the major source of power in Nigeria apart from the irregular hydroelectric power source as well as the solar power source is the petroleum. The Crude oil is refined to obtain fractions like petroleum gas, petrol, kerosene, diesel etc., in refineries which are not available in our home countries. These petroleum fractions are used in driving engines, lighting of lamps and cooking gargets such as stoves, gas cookers etc. The petroleum fractions whose sources are from Nigeria and some other countries, are too costly due to high cost of exportation of the crude oil resource for refining in foreign countries and high cost of importation of the refined oil. Apart from the high cost of the crude oil fractions, incomplete combustion of the fractions of petroleum in engines brings about the release of a poisonous gas called carbon monoxide to the atmosphere. Inhalation of carbon monoxide by man and animals causes serious respiratory problems which cause drastic increase in the mortality rate of living organisms in the environment [1]. The petroleum fractions obtained from some other countries contain lead dust and sulphure impurities that are also injurious to man [2]. In order to check the adverse effects of petroleum fractions on lives, scientists discovered the use of ethanol, which burns completely with smokeless flame and without carbon monoxide, emission as fuel alternative instead of the common injurious and costly petroleum fraction [3].

As the production of ethanol increased, the effect of biofuel on agricultural markets and the environment became increasingly an important topic. Biofuel has the potential to displace petroleum as a transportation fuel at lower toxic emissions [3]. The evolution of new biofuel production technologies could help alleviate some of the concerns regarding the use of food for fuel by facilitating the use of non-food feedstock and could alleviate some of the environmental concerns associated with grain ethanol production. In particular, ethanol produced from wood species is believed to hold great promise in this regard. Hence there is the need for the development of an alternative means of ethanol derivation that reduces the competition between starch consumers and ethanol biofuel makers and in order to put an end to the use of hazardous and costly petroleum fractions.

This research therefore reveals a cost effective method of ethanol production from Eku *(Brachystegia euricoma)*, Mahogany (Sapele) *(Entandrophragma cylindricum)* and Gmelina *(Gmelina arborea)*; the yield and the chemical composition of the ethanol produced from the saw dust of three wood species: Eku *(Brachystegia eurycoma)*, Mahogany (Sapele) *(Entandrophragma cylindricum)* and Gmelina *(Gmelina arborea)* in comparison with that of conventional ethanol.

## Chapter Two: Literature Review

In order to satisfy the desire to make life better for man through science and technology and having in view the potentials ethanol has in this regard, there have been some concerted efforts directed at perfecting the fermentation techniques and feedstock used.

### Wood

Wood is a natural fibrous material produced by trees and it was one of the first structural materials discovered by man. It exhibits a lot of variation in properties in terms of durability, strength, density,

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moisture content, vessels and fibres properties [4]. Due to this diversity in characteristics, exploitation was selective and was limited to very strong and durable species like *Melicia exelsa*, *Khaya ivorensis*, *Afzelia africana*, *Nauclea diderrichii* [5,6] pointed out the need for more thorough investigation of the basic structure of woody elements from the tropics in a bit to get rid of diminishing traditional wood raw material resources.

## Physical properties of wood

Generally, the behaviour of wood is influenced by its physical properties. According to Panshin and Dezeeuw [7], the physical properties of wood are expressed in terms of cell wall substances that are present in given volume of wood, the amount of water present in the cell wall. It also includes the quantity of extraneous substances present, the arrangement and orientation of wall materials in the cell wall and the kind, size proportion and arrangement of the cell making up the wood (Xylem) tissue.

## Wood density

Density is defined as the amount of wood substance per unit volume [7,8]. Dinwoodie [9] reported that the density of wood is a function of the cell wall thickness and also depends on the level of cell wall development. Chafe [10] reported that high cellulose content in wood is an indication of high density. Density varies greatly depending on the anatomical structure of wood.

Variation in density occurs within as well as among species. The variability in density within tree species may be due to effect of tree age (age of cambium) while density variability among tree species usually occurs as a result of environmental, genetical and silvicultural effects [11].

There are several general pattern of density variation across the stem. Adequate explanation of density pattern must take into consideration, the difference between juvenile and mature wood. The presence of heartwood extractives affects the wood density. It has been explained that heartwood is slightly denser than the sapwood. However, extractives rarely add more than a few percent to the density [7].

Wood density was found to increase from the pith to the bark of tropical wet forest. The variation in density through particular cross section of the stem is less pronounced than along the height. Wood density variation is also affected by width of the annual rings. Panshin and Dezeeuw [7] summarized the radial trend in density by stating that density of wood is high at the pith, decreases outward for the first few years and later increases from the pith to the bark.

The most common trend in variation of density along the radial axes of tree is a general increase from the pith to the bark [12,13].

## Species description

**Mahogany *(Entandrophragma cylindricum)*:** Mahogany *(Entandrophragma cylindricum)* belongs the family-Meliaceae and it has the following vanacular names: Sapelli mahogany, sapele mahogany, West African cedar, scented mahogany (En). Sapelli, cédrat d'Afrique (Fr). Sapelli (Po). *Entandrophragma cylindricum* is widespread, occurring from Sierra Leone east to Uganda, and south to Congo and Cabinda (Angola) [14].

The wood, usually traded as 'sapelli', 'sapele', 'aboudikro' or 'assié', is highly valued for flooring, interior joinery, interior trim, panelling, stairs, furniture, cabinet work, musical instruments, carvings, ship building, veneer and plywood. It is suitable for construction of vehicle bodies, toys, novelties, boxes, crates and turnery. The bole is traditionally used for dug-out canoes. Wood that cannot be valorized as timber is used as firewood and for charcoal production [14].

In Central Africa the bark is used in traditional medicine. Bark decoctions or macerations are taken to treat bronchitis, lung complaints, colds, oedema and as anodyne, whereas bark pulp is applied externally to furuncles and wounds. Bark extracts have been used as protectant of stored maize. The tree is planted as roadside tree and ornamental shade tree. Caterpillars of the butterfly *(Imbrasia oyemensis)* are commonly found on the leaves; they are edible and in East Africa much sought after for human consumption [14].

Sapelli is one of the most important export timbers of tropical Africa. During the 1960s the main exporters were Côte d'Ivoire and Ghana. In 1963-1974 average annual exports from Côte d'Ivoire were 122,000 $m^3$ of logs and 15,700 $m^3$ of sawn wood. Average annual exports from Ghana in 1963-1967 were 48,000 $m^3$ of logs and 39,000 $m^3$ of sawn wood. In 1969-1970 Cameroon annually exported about 52,000 $m^3$ of logs per year, and Nigeria, Congo and the Central African Republic together about 60,000 $m^3$ per year.

Nowadays the wood is mainly harvested in Central Africa, with an export value of at least US$ 165 million in 2003, with exports mainly from Cameroon, the Central African Republic and Congo. The Central African Republic exported 41,000 $m^3$ of *Entandrophragma cylindricum* logs in 2003, at an average price of US$ 391/ $m^3$, and 29,000 $m^3$ of sawn wood, at an average price of US$ 473/ $m^3$. Congo exported 211,000 $m^3$ of logs in 2003, at an average price of US$ 224/ $m^3$, 221,000 $m^3$ in 2004, at US$ 219/ $m^3$, and 150,000 $m^3$ in 2005, at US$ 194/ $m^3$. Cameroon exported 108,000 $m^3$ of sawn sapelli wood in 2003, at an average price of US$ 806/ $m^3$, 120,000 $m^3$ in 2005 at US$ 350/ $m^3$, and 89,000 $m^3$ in 2006, at US$ 422/ $m^3$. In 2004 the export of veneer was 3000 $m^3$ from Ghana at an average price of US$ 870/ $m^3$ and 9000 $m^3$ from Congo at an average price of US$ 334/ $m^3$. Smaller amounts of plywood were exported from Ghana (1000 $m^3$ in 2004) and the Central African Republic, at an average price of US$ 347/ $m^3$ and US$ 372/ $m^3$, respectively [14].

The heartwood is pinkish brown when freshly cut, darkening upon exposure to reddish brown or purplish brown, and distinctly demarcated from the creamy white to pinkish grey, up to 10 cm wide sapwood. The grain is interlocked or wavy, texture fairly fine. Quarter-sawn surfaces are regularly striped or have a roe figure. The wood has a distinct cedar-like smell [14]. The wood is medium-weight, with a density of 560-750 kg/ $m^3$ at 12% moisture content. It air dries fairly rapidly, but it is liable to warping and distortion. Quarter-sawing before drying and careful stacking are recommended. Mild schedules are needed for kiln drying. The rates of shrinkage are medium to moderately high, from green to oven dry 3.5-7.6% radial and 4.3-9.8% tangential. Once dry, the wood is moderately stable in service. At 12% moisture content, the modulus of rupture is 95-184 $N/mm^2$, modulus of elasticity 8900-13,800 $N/mm^2$, compression parallel to grain 40-75 $N/mm^2$, compression perpendicular to grain about 8 $N/mm^2$, shear 7-18 $N/mm^2$, cleavage 15-20 N/mm, Janka side hardness 4180-6730 N and Janka end hardness 5650-7450 N [14]. The wood saws and works easily with both hand and machine tools. It has only slight blunting effects on cutting edges. In planning and moulding operations, a 15-20° cutting angle is recommended to avoid picking up of grain. Finishing gives usually good results, with a nice polish. The wood is not liable to splitting in nailing and screwing, with good holding properties. The gluing, staining and polishing properties are good, but the steam bending properties are poor. The wood is suitable for the production of both sliced and rotary veneer; steaming for 48-72 hours at 85°C

gives good results. It is moderately durable, being liable to powder-post beetle, pinhole borer and marine borer attacks and with moderate resistance to termites. The heartwood is resistant to preservatives, and the sapwood is moderately resistant [14].

The lactone entandrophragmin has been isolated from the heartwood and bark. It showed high toxicity to tadpoles. The bark also contains several acyclic triterpenoids, called sapelenins. Bark extracts showed inhibitory effects on the reproduction of the maize weevil Sitophilus zeamais. The tannin present in the bark has been used experimentally to produce tannin-formaldehyde resin, which can be used as lacquer, although the drying time was rather long, 5-7 hours [15]. The essential oil from the bark has been analyzed for trees originating from Cameroon and the Central African Republic. The major constituents were γ-cadinene (9-23%), α-copaene (7-22%) and T-cadinol (18-28%). The seeds contain about 45% oil. The fatty acid composition of the oil is characterized by the presence of about 50% cis-vaccenic acid, a rare isomer of oleic acid, that can be used in the industrial production of nylon-11. Other major fatty acids are stearic acid (16%), oleic acid (7%), linoleic acid (5%) and linolenic acid (6%) [15]. *Entandrophragma cylindricum* is a, dioecious large tree up to 55-65 m tall; bole branchless for up to 40 m, straight and cylindrical, up to 200-280 cm in diameter, with low, blunt buttresses up to 2 m high, rarely up to 4 m; bark surface silvery grey to greyish brown or yellowish brown, becoming irregularly scaly with scales leaving shallow pits with numerous lenticels, inner bark pinkish, soon becoming brown upon exposure, fibrous, with a strong cedar-like smell; crown rounded; young twigs brownish short-hairy, marked with lenticels. Leaves alternate, clustered near ends of twigs, paripinnately compound with 10-19 leaflets; stipules absent; petiole 5-13 cm long, flattened or slightly channelled, often slightly winged at base, rachis 7-17 cm long; petiolules 1-6 mm long; leaflets opposite to alternate, oblong-elliptical to oblong-lanceolate or oblong-ovate, 4-15 cm × 2-5 cm, cuneate to rounded and slightly asymmetrical at base, usually short-acuminate at apex, papery to thinly leathery, almost glabrous, pinnately veined with 6-12 pairs of lateral veins. Inflorescence an axillary or terminal panicle up to 25 cm long, short-hairy. Flowers unisexual, regular, 5-merous; pedicel 1-2.5 mm long; calyx cup-shaped, lobed to about the middle, 0.5-1 mm long, sparsely short-hairy outside; petals free, ovate, 3-4 mm long, sparsely short-hairy outside, greenish white; stamens fused into an urn-shaped tube c. 2 mm long, with 10 anthers at the slightly toothed apex; disk cushion-shaped, with 20 indistinct ridges; ovary superior, conical, 5-celled, style very short, stigma disk-shaped, with 5 lobes; male flowers with rudimentary ovary, female flowers with smaller, non-dehiscing anthers. Its fruit is a pendulous, cylindrical capsule 6-14 cm × 2.5-4 cm, brown to purplish black, dehiscing from the apex and base with 5 woody valves, up to 20-seeded with seeds attached to the upper part of the central column. Seeds 6-11 cm long including the large apical wing, pale brown. Seedling with epigeal germination, but cotyledons often remaining within the testa; hypocotyl 20-4 cm long, epicotyl 6-9 cm long; first 2 leaves opposite, simple.

Entandrophragma comprises about 10 species and is confined to tropical Africa. It belongs to the tribe Swietenieae and is related to Lovoa, Khaya and Pseudocedrela.

It has growth rings with one growth ring boundaries distinct, two growth ring boundaries indistinct or absent. It has diffuse-porous vessel with simple perforation plates and intervessel polygonal alternate pits. The size of its intervessel pits ranges from 4-7 μm. Its vessel-ray pits are distinctly bordered. Its average tangential diameter of vessel and vessel lumen is 100-200 μm and 47: 5-20 vessels per square millimeter respectively. It secrets gums and other deposits in its heartwood vessels, tracheids and fibres. It has thin to thick walled septate fibres with simple to minutely bordered pits although some non-septate fibres are also present. Its axial parenchyma cells are scanty [16,17].

Under natural conditions, seeds germinate abundantly, but mortality of seedlings is high, less than 1% reaching 10 cm stem diameter. Seedlings grow slowly, 20-40 cm/year. Root development takes considerable time. Seedlings up to 2 years old require light shade, but thereafter they should be gradually exposed to more light. They can survive for several years in the shade without significant growth, but when a gap is created in the forest providing enough light further development into a tree starts. The mean annual diameter increment for trees in the Central African Republic has been established at 3.9 mm, but the variability is large. For trees planted in lines in forest in Cameroon, the average annual height growth during 40 years was 30-50 cm and average annual diameter growth 4-8 mm. Trees planted in the open in Côte d'Ivoire reached an average height of 5.4 m and an average stem diameter of 10 cm after 7 years, with a survival rate of 74%.

Trees start flowering when 35-45 years old. Fruit production starts when trees have reached bole diameters above 50 cm. This has implications for forest management; minimum felling diameters should be well above 50 cm to allow natural regeneration. Research in Cameroon suggests that a reduction in the number of trees capable of producing seeds is the main limiting factor for regeneration after logging, rather than limits to pollen dispersal; there seems to be extensive pollen flow over larger distances. Trees can become over 500 years old.

*Entandrophragma cylindricum* trees lose their leaves for 0.5-1 month, or gradually change leaves during 2-3 months. In Liberia and Côte d'Ivoire trees are deciduous for a short period in October-November; flowering occurs near the middle of the dry season, in February-March. In the Central African Republic the trees change leaves from November to January. Mature fruits develop about 5 months after flowering. Fruits usually open on the tree and the seeds are dispersed by wind, although most seeds seem to fall close to the mother tree. Seed production is erratic. Although flowering may be common, fruit production is often irregular, e.g. 90% of trees with bole diameters above 50 cm flower each year in Cameroon, but only 50% of them develop fruits. In the Central African Republic 79% of the observed trees with bole diameter over 50 cm flowered in a 2 year period, and 76% fruited. *Entandrophragma cylindricum* is most common in semi-deciduous forest, particularly in regions with an annual rainfall of about 1750 mm, a dry period of 2-4 months and a mean annual temperature of 24-26°C. It tolerates dry forest better than other *Entandrophragma* spp. However, it can also be found in evergreen forest. In Uganda it occurs in rainforest at 1100-1500 m altitude, sometimes in thickets and gallery forest. It prefers well-drained localities. *Entandrophragma cylindricum* is characterized as a non-pioneer light demander, although it was indicated as exceptionally shade-tolerant after studies in DR Congo. Natural regeneration is often scarce in natural forest, but logging operations creating gaps may promote regeneration, larger gaps appearing more favourable. Natural regeneration in gaps created by selective logging in forest in Nigeria and Congo was very poor. Regeneration in forest in the Central African Republic 18 years after logging was inadequate, whereas a study in DR Congo showed that secondary forest resulting from the abandonment of slash-and-burn agriculture offers favourable conditions for regeneration [18]. 1000-seed weight is about 330 g. Fresh seeds may have a high germination rate of 80-95%. However, seeds lose their viability rapidly, often within 3 weeks. Germination starts 14-26 days after sowing. Soaking of the

seeds for one night is reported to speed up germination. Overhead shade is required for young seedlings. The seedlings perform poorly under full light conditions. Seeds can be stored for some time in sealed containers in a cool place, but insect damage, to which they are very susceptible, should be avoided, e.g. by adding ash. Cuttings 90-110 cm long have been used successfully for propagation [18]. Forests in the Central African Republic may contain 7 *Entandrophragma cylindricum* stems above 10 cm diameter per ha and a wood volume of 25 $m^3$/ha. In southern Cameroon the average density is up to one tree of more than 60 cm bole diameter per ha, and the average wood volume is up to 11.5 $m^3$/ha. In Côte d'Ivoire and Cameroon timber plantations of *Entandrophragma cylindricum* have been established, but only at a very small scale, with less than 10 ha and 425 ha, respectively. Line planting in the forest is practised in Cameroon [19]. *Hypsipyla robusta* shoot borers may severely attack young trees, often in their second or third year and when grown in full sunlight. Attacked stems often show poor growth and form. *Hypsipyla robusta* may also infest seeds. Several *lepidopterous* insects attack the leaves, fruits and seeds [20].

In the beginning of the 1990s in forests in Congo *Entandrophragma cylindricum* was selectively logged together with *Entandrophragma utile* (Dawe & Sprague) Sprague and *Triplochiton scleroxylon* K.Schum. in a rotation of 30-40 years and removing 1-2 trees/ha, with a wood volume of 10-15 $m^3$ [20]. Most freshly harvested logs float in water and can thus be transported by river. A test in Cameroon showed that 2% of the logs had sunken after 14 months in water. Common log defects which should be taken into consideration during processing are ring and cup shakes [20].

*Entandrophragma cylindricum* is the most common *Entandrophragma* species in much of its distribution area. In 1973 the total exploitable timber volume in Côte d'Ivoire, Cameroon, the Central African Republic and Congo together has been estimated at over 50 million $m^3$. However, the commercial interest in the valuable timber has resulted in extraction of large individuals from the forest in many regions. It is included in the IUCN Red list as vulnerable. In Cameroon the genetic diversity of populations in logged-over forest and non-disturbed forest was established by characterization of microsatellite loci. The populations showed the same high level of genetic diversity and a low genetic differentiation, indicating that genetic diversity is within rather than among populations [21]. *Entandrophragma cylindricum* provides one of the commercially most important timbers of Africa in terms of quantities produced as well as in terms of wood quality. This has resulted in heavy pressure on the populations, and in many regions *Entandrophragma cylindricum* is still not exploited on a sustainable basis. The low growth rates under natural conditions, the long time needed to reach maturity in terms of fruit production and poor dispersal ability of the seed seem to be serious drawbacks. It has been suggested that intensive silviculture, possibly involving the use of shifting cultivation in a taungya-like system, is needed to achieve sustainable management. *Entandrophragma cylindricum* does not seem to be a logical choice for planting in agroforestry systems because it grows too slowly [20].

**Eku *(Brachystegia eurycoma)*:** *Brachystegia eurycoma* is a woody legume that belongs to the family-Caesalpiniaceae (Leguminosae - Caesalpinioideae). *Brachystegia eurycoma* has a restricted area of distribution, occurring in southern Nigeria and western Cameroon, possibly also in Gabon. The wood of *Brachystegia eurycoma*, known as 'naga' or 'okwen' in trade, is used for construction, joinery and furniture. It is suitable for flooring, interior trim, interior carpentry, stairs, veneer and plywood. Traditionally, the bark has been used to make a coarse cloth used as protection against rain and as a shield against arrows. It is used to make containers. The seeds are spicy and consumed as condiment, and are used to prepare a flour, named 'achi', used to thicken soups. Igbo people in Nigeria use the plant as anthelmintic. The wood of Brachystegia eurycoma is mainly used and traded locally. It may occasionally be exported in mixed consignments. The heartwood *of Brachystegia eurycoma* is pinkish brown with vague bands and rather distant fine streaks, clearly demarcated from the whitish sapwood. The grain is usually interlocked, texture medium. There is no detailed information on physical and mechanical wood properties of *Brachystegia eurycoma*, but these are probably comparable to those of *Brachystegia cynometroides* Harms. The wood should be dried slowly and carefully to avoid defects; end splitting is common. It is hard to work with hand tools and has a blunting effect on saw teeth and cutting edges. To obtain a smooth surface, careful sanding and the use of a filler is required. The nailing and screwing properties are good, but pre-boring is needed to avoid splitting. Reports on the durability of the wood are contradictory; it is mostly considered non-durable, although wood extracts showed insecticidal and fungicidal activities. The heartwood is resistant to treatment with preservatives.

The seed flour used for thickening contains per 100 g: water 10-12 g, fat 13-14 g, protein 10-13 g, dietary fibre 1-2 g, carbohydrate 59-61 g and ash 1.5-4 g. As a thickening agent, 4-20 g of seed flour per litre water is used. Toasting the flour and addition of palm oil increases the viscosity of the solution, while salt has an opposite effect. Several commercial samples of 'achi' from Nigeria were found to contain aflatoxin B1-producing strains of the fungi *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus niger*. In a test in Nigeria, the seed mucilage was shown to be suitable as a tablet binder. The fatty acid composition of the seed oil is approximately: palmitic acid 26%, stearic acid 7%, lignoceric acid 13%, linoleic acid 6%, oleic acid 32% (total saturated fatty acids 59%, total unsaturated fatty acids 41%). Ethanol extracts of the wood, and to a lesser extent aqueous extracts, have shown an insecticidal effect against the termite *Amitermes evuncifer* and a fungicidal effect against *Fomes heterobasidium, Polyporus coridus* and *Daedalea daedaleopsis*; aqueous extracts inhibited the growth and cellulolytic activity of Bacillus subtilis. Aqueous extracts of the bark, and to a lesser extent ethanol extracts, inhibited the growth of several fungi. The root has been tested for its effect on the snail *Bulinus globulus*, but showed no effect.

Pollen counts in honey samples have indicated that *Brachystegia eurycoma* is an important bee plant. The yellow or reddish gum exuding from the bark hardens into a gutta-percha-like substance. It is a Medium-sized to fairly large tree up to 35 m tall; bole often low-branched and irregular, up to 200-250 cm in diameter, often with small buttresses; bark surface rough, grey to brown, flaking off in large patches, inner bark hard, fibrous, red, ripple-marked, darkening on exposure, exuding a reddish or yellowish gum; crown widely spreading and flattened, with spreading, twisted branches; twigs hairy but soon becoming glabrous. Leaves paripinnately compound with 4-6 pairs of leaflets; stipules early caducous; petiole 1.5-2.5 cm long, swollen at base, rachis 7–15 cm long; leaflets opposite, sessile, oblong-elliptical, up to 12 cm × 6 cm, basal leaflets smallest, upper ones largest, truncate to rounded and very asymmetrical at base, rounded or slightly notched at apex, thin-leathery, glabrous, pinnately veined with 6-8 pairs of lateral veins. Inflorescence a mostly terminal, up to 15 cm long panicle, short-branched, densely brown hairy, many-flowered. Flowers bisexual, slightly zygomorphic, small, at base with 2 oblong-obovate bracteoles. 7 mm long; pedicel very short sepals that are 3 mm long, slightly unequal, with hairy margins; petals absent; stamens 8-10, free. 9 mm long; ovary superior, ellipsoid, with short stipe, hairy, style slender, coiled. Fruit an

oblong to oblanceolate or obovate, flattened pod, 12-22 cm × 3.5-6 cm, at a right angle to the stipe, smooth but slightly wrinkled, dehiscent with 2 woody valves, 4-6-seeded. Seeds disk-shaped, 2 cm in diameter, shiny brown.

*Brachystegia eurycoma* flowers in April-May; fruits ripen in September-January. The fruits open explosively, throwing out the seeds. A trial in Nigeria showed that seedlings of *Brachystegia eurycoma* grow best on a fertile mixture of top-soil and river sand with watering intervals of up to 3 days. *Brachystegia* is a taxonomically difficult genus comprising about 30 species, distributed in mainland tropical Africa and South Africa, the majority of species occurring in southern tropical Africa, where they are characteristic of miombo woodland. *Brachystegia eurycoma* is most common in riverine forest, up to 1150 m altitude. It is locally quite abundant [22].

**Gmelina *(Gmelina arborea)*:** *Gmelina arborea (melina)* was introduced into reforestation programs in countries such as Myanmar and Bangladesh on early twentieth century. By the end of this century, the species had been introduced in several areas of the American tropical countries, Africa, and Asia [23-26]. Reforestation with this species is oriented to the production of raw material for sawlog, energy, and pulp production [27]. This artificial migration includes regions that vary from 100° W to 180° E latitude and from 23° N to 40° S longitude. The introduced sites have different ecological conditions compared to its natural habitat in Asia, mainly the ones related to the geography and precipitation. In Central America, for example, Gmelina has been grown in a different precipitation level, temperature and fertility sites. In Costa Rica, *G. arborea* is planted in a variety of sites and growth conditions for the production of raw material for sawlog.

The anatomical structure of secondary xylem is composed by different types of woody cells (vessel, fiber and radial and axial parenchyma), whose origins are in vascular cambium [28]. During their formation these cells are affected by many factors such as site, ecological conditions, management, genetics, and age for trees growing in plantation conditions. The anatomical features are modified within trees during their growth in order to adjust physiologic and water stress, then they maintain the existence of the species [6].

For Gmelina, many studies indicate that the variations in the anatomy of secondary xylem occurred in relation to tree age [29], growth conditions [30], growth rate [31], differences in site fertility [26], and water availability [32]. Based on a macroscopic examination of two Indian samples, [Ref. 33] has described in detail *G. arborea* wood. Other studies report the anatomical features variation. Chowdhury established three different porosities for different climatic conditions: diffuse, annular and semi-annular. The anatomical elements presented significant variation. vessels percentage was negatively correlated with latitude, longitude, growth rate and tree height [34]. The length and diameter vessels increased to increment distance from the pith [35]. Although vessels frequency decreased when pith distance or tree age increased [36,37]. Frimpong-Mensah found that cell wall thickness was significantly correlated with cambial age. Hughes and Esan found strong correlations between fiber length and tree age with distance from the pith in 9-year-old trees in Nigeria. Also in Nigeria, for 7 year-old trees, it was found that fiber length was different at four sites [38]. In contrast, Frimpong-Mensah found no variation in fiber length with cambial age in 20-year-old *G. arborea* trees in Ghana. Growth rate affects fiber dimensions too. Ref. [39] carried out a study on fiber length in 15-year-old trees and found that a high growth rate was strongly correlated with short fiber length.

The present research objective was to determine the differences in the anatomy of secondary xylem in melina trees growing in different geographic locations (latitude, longitude and altitude) and precipitation levels in the North and Northwest regions of Costa Rica.

## Lignocellulose material (Wood)

Lignocellulose materials refer to plants that are composed of cellulose, hemicellulose and lignin. The cellulose microfibrils (formed by ordered polymer chains that contain tightly packed, crystalline regions) are embedded within a matrix of hemicellulose and lignin. Covalent bonds between lignin and the carbohydrates have been suggested to consist benzyl esters, benzyl ethers and phenyl glycosides [40].

Lignin is primarily a structural material to add strength and rigidity to cell walls and constitutes between 15 wt% and 40 wt% of the dry matter of woody plants. Lignin is more resistant to most forms of biological attack than cellulose and other structural polysaccharides, [41-43] and plants with a higher lignin content have been reported to be more resistant to direct sunlight and frost [44]. *In vitro*, lignin and lignin extracts have been shown to have antimicrobial and antifungal activity, [45] act as antioxidants, [46] absorb UV radiation, [47,48] and exhibit flame-retardant properties [49].

Lignin is a cross-linked macromolecular material based on a phenylpropanoid monomer Structure. Typical molecular masses of isolated lignin are in the range 1000 g/mol to 20,000 g/mol, but the degree of polymerisation in nature is difficult to measure, since lignin is invariably fragmented during extraction and consists of several types of substructures which repeat in an apparently haphazard manner. The monomer structures in lignin consist of the same phenylpropenoid skeleton, but differ in the degree of oxygen substitution on the phenyl ring. The H-structure (4-hydroxy phenyl) has a single hydroxy or methoxy group, the G-structure (guaiacyl) has two such groups, and the S-structure (syringyl) has three. The polymerisation of the phenylpropanoid monomers is initiated by oxidases or peroxidases. While the precise mechanism is obscure, it is postulated that radical-radical combination of free radicals produced by enzymatic dehydrogenation is the key reaction, either under enzymatic control [50] or in a random 'combinatorial' manner (Figure 1) [51].

Both carbon-carbon and carbon-oxygen bonds between monomers are found in lignin. The most common functionality, accounting for about half the bonds between monomers in lignin from most sources, is a carbon-oxygen link between a p-hydroxy moiety and the β-end of the propenyl group (β-O-4) (Figures 2 and 3) [52-54].

## Cellulose

Cellulose was first discovered in 1838 by French chemist Anselme who isolated it from plant matter. He found that cellulose contains 44% to 45% carbon, 6 to 6.5% of hydrogen and the rest containing oxygen. Based on this data, the empirical formula was deduced to be $C_6H_{10}O_5$. However, the actual macromolecular structure of cellulose was still unclear [3]. Nelson and Cox (2010) recorded that Haworth proposed a chain-like macromolecular structure in the late 1920s and Staudinger delivered the final proof of the highly polymer nature of the cellulose molecule [3].

Cellulose is the one of the most abundant polymer on earth, which makes it also the most common organic compound. It is also referred to as fiber. Cellulose is a complex carbohydrate or polysaccharide. It is a linear chain of glucose molecules. It is an organic compound with the formula $(C_6H_{10}O_5)$ n.

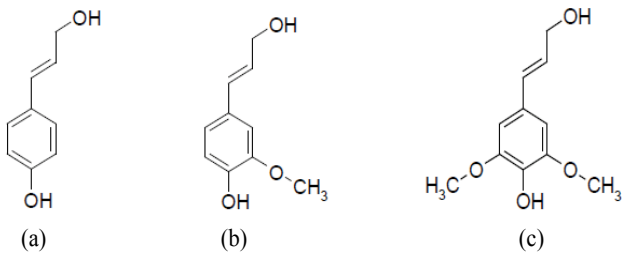


**Figure 1:** Monolignol monomer species. (a) *p*-coumaryl alcohol (4-hydroxyl phenyl, H), (b) coniferyl alcohol (guaiacyl, G), (c) sinapyl alcohol (syringyl, S) [27].

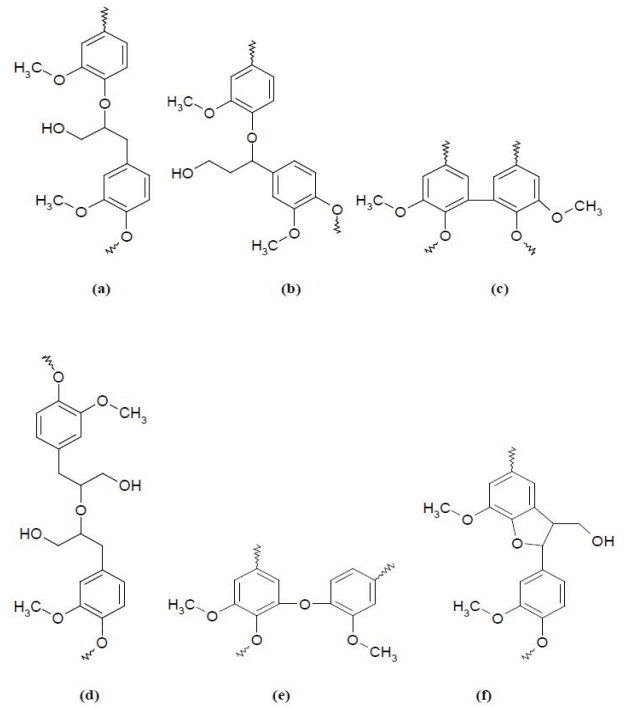


**Figure 2:** Significant lignin linkage structures. (a) β-O-4, (b) α-O-4, (c) 5-5, (d) β-β, (e) 5-O-5, (f) β-5 [27].

Each cellulose molecule is an unbranched polymer of 1000 to 1 million D-glucose units, linked together with β-1,4 glycosidic bonds. The acetyl linkage-beta makes it different from starch. The multiple hydroxyl groups on the glucose from one chain, one chain form hydrogen bonds with oxygen molecule on the same or on a neighbor chain, holding the chains firmly together side by side. Several of these polysaccharide chains are arranged in parallel arrays to form cellulose micro fibrils. The individual polysaccharide chains are bond together in the micro fibrils by hydrogen bonds. Micro fibrils of cellulose are extremely tough and inflexible due to the presence of hydrogen bonds. Micro fibrils have crystalline properties and are bundled together to form macro-fibrils. Although, starch has the same basic structure as cellulose. It is also a polysaccharide; the glucose subunits are bonded in such a way that allows the starch molecule to twist. In order words, the starch molecule is flexible, while the cellulose molecule is rigid. Cellulose from various sources are all the same at the molecular level, however, they differ in it crystalline structures and bindings by other biochemical (Figure 4) [3].

## Ethanol

Ethanol or ethyl alcohol has existed since the beginning of recorded history. The ancient Egyptians produced alcohol by naturally fermenting vegetative materials. Also in ancient times, the Chinese discovered the art of distillation, which increases the concentration of alcohol in fermented solutions. Ethanol was first prepared synthetically in 1826, through the independent effort of Henry Hennel in Britain and S.G in France. Michael Faraday prepared ethanol by the acid-catalyzed hydration of ethene in 1828, in a process similar to that used for industrial synthesis of ethanol today [55].

Ethanol was used as lamp fuel in the United States as early as 1840 but a tax levied on industrial alcohol during the Civil War made this use uneconomical. This tax was repelled in 1906. In 1907, Henry Ford re-introduced ethanol to the Americans motoring public by producing his first vehicle to run on ethanol. The first Ford Motor Company Automobile was designed to use corn alcohol called ethanol. The most common substrate used for nearly 99% of ethanol production in the United States today is starch from agricultural crops, primarily corn [3].

In 1940s the first fuel ethanol plant was built and operated by the U.S Army in Omaha, Nebraska, in order to produce fuel for the army and for regional fuel blending. Major quantities were not manufactured until the 1970s due to low cost of gasoline between 1940s and 1970s, however the ethanol industry began to remerge when ethanol was used as a fuel extender during gasoline shortages caused by the OPEC oil embargoes [3].

In 1980s, after investing heavily in renewable fuels in the 1970s, Brazil kept the program alive during the 1980s. With its robust ethanol programs, Brazil developed an extensive ethanol industry. By the mid-1980s, ethanol-only cars accounted for almost 90% of all new-auto sales in Brazil, making the country the biggest alternative fuel market in the world. In 1988 ethanol began to be added to gasoline for the purpose of reducing carbon dioxide emissions. By 2000, Brazil deregulated the ethanol market and removed its subsidies. However on market conditions, all fuels are required to be blended with 20 to 25 percent of ethanol [3].

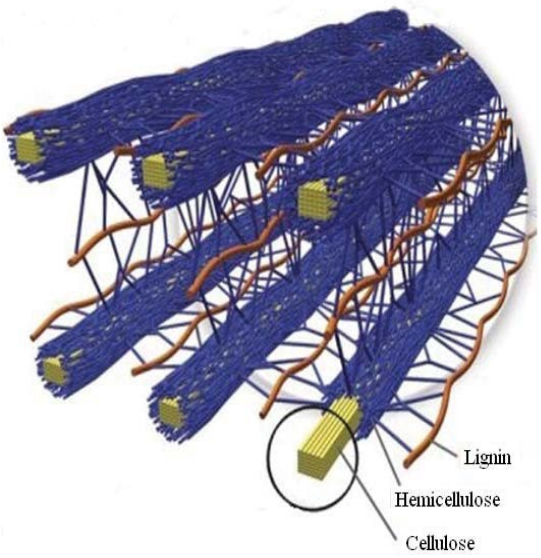


**Figure 3:** Schematic diagram of Lignocellulose structure [27].

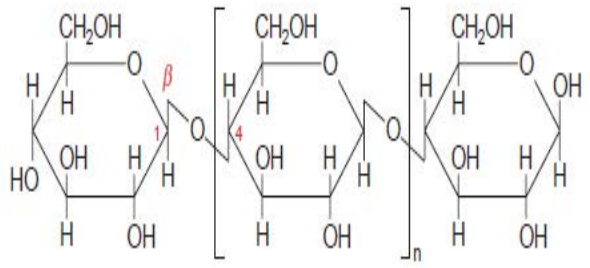

**Figure 4:** Cellulose structure [29].

As the production increased, the effect of biofuels on agricultural markets and the environment became increasingly an important topic. Biofuels have the potential to displace the use of petroleum as a transportation fuel at lower toxic emissions rate. The evolution of new biofuel production technologies could help alleviate some of the concerns regarding the use of food for fuel by facilitating the use of non-food feedstocks, and could alleviate some of the environmental concerns associated with ethanol produced from grains. In particular, ethanol produced from wood species is believed to hold great promise in this regard, even though there are currently no commercial scale plants in the United State and Nigeria [56].

## Ethanol synthesized from wood biomass

Ethanol from Wood biomass from forestry or agricultural waste is considered a way to prevent displacement of crops to feed humans. Corn-based ethanol has been blamed by some for higher food prices and shortages because food producers are at times forced to compete with energy companies for grain. Some also argue that the growing demand for such crops is also responsible for indirect land-use change, the destruction of rain forest and wet lands to make room for more farmland. The joint study sees cellulosic ethanol as a viable alternative for reducing oil dependences while protecting food crops. Corn-Stover and switch grass are very potential cellulosic feed stock [57].

According to Ref. [58], it was found that crop residues are likely the lowest cost biomass source. According to Atchison and Hettenhaus in 2003, over 240 million dry tons of corn Stover is produced each year in the United States. Brechbill and Tyner found through research that corn Stover collection risk soil loss from wind erosion and runoff from water erosion depending on the amount of corn stover collected [56].

Wood is the commonest lignocellulosic feedstock used to manufacture ethanol. Extraction of ethanol from wood alcohol dates as far back as 1819. According to Otulugbu a memorandum was published on wood alcohol by Braconnet, after which numerous attempts have been made on the distillation of wood alcohol. About eighty years after the memorandum by Braconnet, Simonsen in 1894 recommended the treatment of sawdust with dilute acids at high pressure of about 7-8 atm. It however did not become an industrial process because of excessive dilution of saccharine juices [3].

In 1899, the hydrolysis of wood was studied by Classen who recommended sulphric acid as the hydrolysis agent instead of sulphurous acid used by his predecessors because from his findings, volatile acids had better penetration of wood. Heating was carried out at 1500 C, 7 atm for 4 to 6 hrs; residuals are extracted by percolation and filtrate is neutralized and fermented. It was applied in America and abandoned due to corrosion, difficulty in stripping, consumption of coal and sulphuric acid [59].

Beginning in the 1910, two chemical engineers, Messrs Ewen and Tomlinson used the same procedure as Classen. They however improved the process by using a much shorter and wider converter (12 ft. by 18 ft.), and lining it with firebrick instead of lead 1 sulfur dioxide gas to the extent of one percent, of the weight of wood treated is introduced into the converter, and steam passed in until a pressure of 100 lb. is obtained. The steam is then turned off and the cylindrical converter slowly revolved for forty-five minutes, the temperature is raised as quickly as possible to the critical point, between 1350 C and 1630 C, above which there is an excessive destruction of sugar and production of fermentable substances. The filtrate or juice obtained is partly neutralized, filtered, cooled and sent on for fermentation using yeast as the source of enzyme. This was implemented on a large scale, in America for the manufacturing of ethanol from sawdust. Industrial yield, under normal conditions, reached 7.3 liters of 100-degree alcohol per 100 kilograms of dry wood, and the factory's annual production is 20,000 hectoliters of alcohol [3].

Before 1914, in France, alcohol manufacture from sawdust was studied and implemented industrially in a distillery in the Ardeche region. Due to the need for alcohol for national defense during the World War, Wood alcohol was reconsidered during the 1914-1918 war. Production of wood alcohol was achieved in Germany with either the classen or the Windesheim-ten-Doornkaat process. The later involved heating sawdust with dilute hydrochloric acid in the presence of catalysts (metallic salt), in rotatory autoclaves, at 7 to 8 atmospheres for 20 to 30 minutes. Yield is 6 liters of alcohol per 100 kilograms of dry matter, but it is surely possible to improve this [3].

The work of Otulugbu clearly shows that a research by Dubose led to the following conclusions:

I. In saccharifying sawdust with 2 parts of sulphuric acid (90-95% $H_2SO_4$) per 100 parts of dry sawdust, maximum yield is obtained with a pressure of 7.5 atmospheres; yield decreases above or below this pressure.

II. Conversion takes place as soon as 7.5 atmospheres is attained; in fact, maximum yield occurs within 15 minutes.

III. Increasing duration does not increase sugar content, but rather reduces it; the sawdust is destroyed and noxious secondary products are formed.

IV. With pine sawdust, yields of 22% to 23% sugar are obtained, giving on average 100 to 115 liters of 95% alcohol per ton of wood treated [60].

According to Otulugbu, Prodor presented a new process based on the hydrolysis of sawdust by cold hydrochloric acid, which considerably reduces destruction of glucose during hydrolysis. It is a continuous process and about 37% HCl used is recovered. The yield is said to be 250 liters of 100% alcohol per ton of dry sawdust. Residue contains non-fermentable pentose's, which can be converted to furfural, and lignin which, by dry distillation, gives as much methyl alcohol as would have been derived from all the wood from which it was extracted. As a matter of fact, we know that cellulose does not yield methyl alcohol on distillation. Process is still under economic and feasibility considerations. However, production of wood alcohol could only become economical if the wood, after decomposition, could be used for extraction of acetone and methyl alcohol by distillation, as intended in the Prodor process [61].

## Atomic absorption spectroscopy (AAS)

Atomic absorption spectroscopy (AAS) is a spectroanalytical procedure for the quantitative determination of chemical elements using the absorption of optical radiation (light) by free atoms in the gaseous state [62].

In analytical chemistry the technique is used for determining the concentration of a particular element (the analyte) in a sample to be analyzed. AAS can be used to determine over 70 different elements in solution or directly in solid samples used in pharmacology, biophysics and toxicology research [62].

Atomic absorption spectroscopy was first used as an analytical technique, and the underlying principles were established in the second half of the 19th century by Robert Wilhelm Bunsen [63] and Gustav Robert Kirchhoff, both professors at the University of Heidelberg, Germany [62].

The modern form of AAS was largely developed during the 1950s by a team of Australian chemists. They were led by Sir Alan Walsh [64]. at the Common wealth Scientific and Industrial Research Organization (CSIRO), Division of Chemical Physics, in Melbourne, Australia [62].

Atomic absorption spectrometry has many uses in different areas of chemistry such as:

- Clinical analysis: Analyzing metals in biological fluids and tissues such as whole blood, plasma, urine, saliva, brain tissue, liver, muscle tissue, semen
- Pharmaceuticals: In some pharmaceutical manufacturing processes, minute quantities of a catalyst that remain in the final drug product
- Water analysis: Analyzing water for its metal content.

The technique makes use of absorption spectrometry to assess the concentration of an analyte in a sample. It requires standards with known analyte content to establish the relationship between the measured absorbance and the analyte concentration and relies therefore on the Beer-Lambert Law [62].

In short, the electrons of the atoms in the atomizer can be promoted to higher orbitals (excited state) for a short period of time (nanoseconds) by absorbing a defined quantity of energy (radiation of a given wavelength). This amount of energy, i.e., wavelength, is specific to a particular electron transition in a particular element. In general, each wavelength corresponds to only one element, and the width of an absorption line is only of the order of a few picometers (pm), which gives the technique its elemental selectivity. The radiation flux without a sample and with a sample in the atomizer is measured using a detector, and the ratio between the two values (the absorbance) is converted to analyte concentration or mass using the Beer-Lambert Law (Figure 5) [62].

**Atomization in AAS analysis:** In order to analyze a sample for its atomic constituents, it has to be atomized. The atomizers most commonly used nowadays are flames and electrothermal (graphite tube) atomizers. The atoms are then irradiated by optical radiation. The radiation then passes through a monochromator in order to separate the element's specific radiation from any other radiation emitted by the radiation source, which is finally measured by a detector [62].

***Flame atomizer:*** The atomizers most commonly used nowadays are (spectroscopic) flames and electrothermal (graphite tube) atomizers. Other atomizers, such as glow-discharge atomizer, hydride atomizer, or cold-vapor atomizer might be used for special purposes [62].

The oldest and most commonly used atomizers in AAS are flames, principally the air-acetylene flame with a temperature of about 2300°C and the nitrous oxide system ($N_2O$)-acetylene flame with a temperature of about 2700°C. The latter flame, in addition, offers a more friendly environment, being ideally suited for analytes with high affinity to oxygen [62].

Liquid or dissolved samples are typically used with flame atomizers. The sample solution is aspirated by a pneumatic analytical nebulizer, transformed into an aerosol, which is introduced into a spray chamber, where it is mixed with the flame gases and conditioned in a way that only the finest aerosol droplets (<10 µm) enter the flame. This conditioning process is responsible that only about 5% of the aspirated sample solution reaches the flame, but it also guarantees a relatively high freedom from interference.

On top of the spray chamber is a burner head that produces a flame that is laterally long (usually 5-10 cm) and only a few mm deep. The radiation beam passes through this flame at its longest axis, and the flame gas flow-rates may be adjusted to produce the highest concentration of free atoms. The burner height may also be adjusted, so that the radiation beam passes through the zone of highest atom cloud density in the flame, resulting in the highest sensitivity.

The processes in a flame include the following stages:

- Desolvation (drying)-the solvent is evaporated and the dry sample nano-particles remain;
- Vaporization (transfer to the gaseous phase)-the solid particles are converted into gaseous molecules;
- Atomization-the molecules are dissociated into free atoms;
- Ionization-depending on the ionization potential of the analyte atoms and the energy available in a particular flame, atoms might be in part converted to gaseous ions.

Each of these stages includes the risk of interference in case the degree of phase transfer is different for the analyte in the calibration standard and in the sample. Ionization is generally undesirable, as it reduces the number of atoms that are available for measurement, i.e., the sensitivity.

In flame AAS a steady-state signal is generated during the time period when the sample is aspirated. This technique is typically used

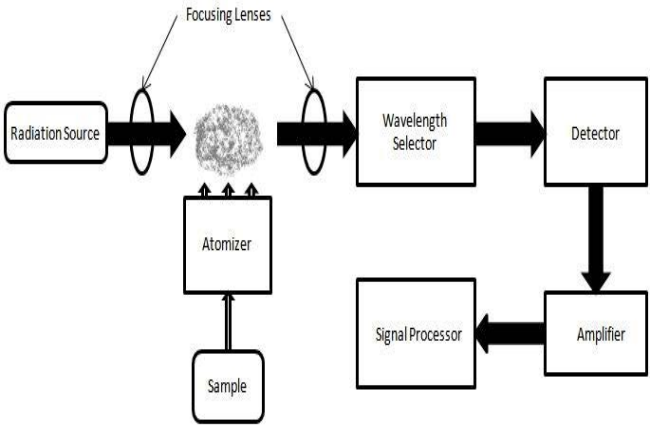


**Figure 5:** Atomic absorption spectrometer block diagram [62].

for determinations in the mg $L^{-1}$ range, and may be extended down to a few µg $L^{-1}$ for some elements.

***Electrothermal atomizers:*** Electrothermal AAS (ET AAS) using graphite tube atomizers was pioneered by Boris V. L'vov at the Saint Petersburg Polytechnical Institute, Russia, since the late 1950s, and further investigated by Hans Massmann at the Institute of Spectrochemistry and Applied Spectroscopy (ISAS) in Dortmund, Germany.

Although a wide variety of graphite tube designs have been used over the years, the dimensions nowadays are typically 20-25 mm in length and 5-6 mm inner diameter. With this technique liquid/dissolved, solid and gaseous samples may be analyzed directly. A measured volume (typically 10-50 µL) or a weighed mass (typically around 1 mg) of a solid sample are introduced into the graphite tube and subject to a temperature program. This typically consists of stages, such as:

- Drying - the solvent is evaporated
- Pyrolysis - the majority of the matrix constituents is removed
- Atomization - the analyte element is released to the gaseous phase
- Cleaning - eventual residues in the graphite tube are removed at high temperature.

The graphite tubes are heated via their ohmic resistance using a low-voltage high-current power supply; the temperature in the individual stages can be controlled very closely, and temperature ramps between the individual stages facilitate separation of sample components. Tubes may be heated transversely or longitudinally, where the former ones have the advantage of a more homogeneous temperature distribution over their length. The so-called Stabilized Temperature Platform Furnace (STPF) concept, proposed by Walter Slavin [65], based on research of Boris L'vov, makes ET AAS essentially free from interference. The major components of this concept are:

- Atomization of the sample from a graphite platform inserted into the graphite tube (L'vov platform) instead of from the tube wall in order to delay atomization until the gas phase in the atomizer has reached a stable temperature;
- Use of a chemical modifier in order to stabilize the analyte to a pyrolysis temperature that is sufficient to remove the majority of the matrix components;
- Integration of the absorbance over the time of the transient absorption signal instead of using peak height absorbance for quantification.

In ET AAS a transient signal is generated, the area of which is directly proportional to the mass of analyte (not its concentration) introduced into the graphite tube. This technique has the advantage that any kind of sample, solid, liquid or gaseous, can be analyzed directly. Its sensitivity is 2-3 orders of magnitude higher than that of flame AAS, so that determinations in the low µg $L^{-1}$ range (for a typical sample volume of 20 µL) and ng $g^{-1}$ range (for a typical sample mass of 1 mg) can be carried out. It shows a very high degree of freedom from interferences, so that ET AAS might be considered the most robust technique available nowadays for the determination of trace elements in complex matrices.

***Glow-discharge atomization:*** A glow-discharge (GD) device serves as a versatile source, as it can simultaneously introduce and atomize the sample. The glow discharge occurs in a low-pressure argon gas atmosphere between 1 and 10 torr. In this atmosphere lies a pair of electrodes applying a DC voltage of 250 to 1000 V to break down the argon gas into positively charged ions and electrons. These ions, under the influence of the electric field, are accelerated into the cathode surface containing the sample, bombarding the sample and causing neutral sample atom ejection through the process known as sputtering. The atomic vapour produced by this discharge is composed of ions, ground state atoms, and fraction of excited atoms. When the excited atoms relax back into their ground state, a low-intensity glow is emitted, giving the technique its name.

The requirement for samples of glow discharge atomizers is that they are electrical conductors. Consequently, atomizers are most commonly used in the analysis of metals and other conducting samples. However, with proper modifications, it can be utilized to analyze liquid samples as well as nonconducting materials by mixing them with a conductor (e.g. graphite).

***Hydride atomization:*** Hydride generation techniques are specialized in solutions of specific elements. The technique provides a means of introducing samples containing arsenic, antimony, tin, selenium, bismuth, and lead into an atomizer in the gas phase. With these elements, hydride atomization enhances detection limits by a factor of 10 to 100 compared to alternative methods. Hydride generation occurs by adding an acidified aqueous solution of the sample to a 1% aqueous solution of sodium borohydride, all of which is contained in a glass vessel. The volatile hydride generated by the reaction that occurs is swept into the atomization chamber by an inert gas, where it undergoes decomposition. This process forms an atomized form of the analyte, which can then be measured by absorption or emission spectrometry.

***Cold-vapor atomization:*** The cold-vapor technique an atomization method limited to only the determination of mercury, due to it being the only metallic element to have a large enough vapor pressure at ambient temperature. Because of this, it has an important use in determining organic mercury compounds in samples and their distribution in the environment. The method initiates by converting mercury into $Hg^{2+}$ by oxidation from nitric and sulfuric acids, followed by a reduction of $Hg^{2+}$ with tin(II) chloride. The mercury, is then swept into a long-pass absorption tube by bubbling a stream of inert gas through the reaction mixture. The concentration is determined by measuring the absorbance of this gas at 253.7 nm. Detection limits for this technique are in the parts-per-billion range making it an excellent mercury detection atomization method.

**Radiation sources:** We have to distinguish between line source AAS (LS AAS) and continuum source AAS (CS AAS). In classical LS AAS, as it has been proposed by Alan, the high spectral resolution required for AAS measurements is provided by the radiation source itself that emits the spectrum of the analyte in the form of lines that are narrower than the absorption lines. Continuum sources, such as deuterium lamps, are only used for background correction purposes. The advantage of this technique is that only a medium-resolution monochromator is necessary for measuring AAS; however, it has the disadvantage that usually a separate lamp is required for each element that has to be determined. In CS AAS, in contrast, a single lamp, emitting a continuum spectrum over the entire spectral range of interest is used for all elements. Obviously, a high-resolution monochromator is required for this technique, as will be discussed later.

**Plate 1:** Hollow cathode lamp (HCL) (Source: McCarthy [62]).

***Hollow cathode lamps:*** Hollow cathode lamps (HCL) are the most common radiation source in LS AAS. Inside the sealed lamp, filled with argon or neon gas at low pressure, is a cylindrical metal cathode containing the element of interest and an anode. A high voltage is applied across the anode and cathode, resulting in an ionization of the fill gas. The gas ions are accelerated towards the cathode and, upon impact on the cathode, sputter cathode material that is excited in the glow discharge to emit the radiation of the sputtered material, i.e., the element of interest. Most lamps will handle a handful of elements, i.e., 5-8. A typical machine will have two lamps, one will take care of five elements and the other will handle four elements for a total of nine elements analyzed.

***Electrodeless discharge lamps:*** Electrodeless discharge lamps (EDL) contain a small quantity of the analyte as a metal or a salt in a quartz bulb together with an inert gas, typically argon, at low pressure. The bulb is inserted into a coil that is generating an electromagnetic radio frequency field, resulting in a low-pressure inductively coupled discharge in the lamp. The emission from an EDL is higher than that from an HCL, and the line width is generally narrower, but EDLs need a separate power supply and might need a longer time to stabilize.

***Deuterium lamps:*** Deuterium HCL or even hydrogen HCL and deuterium discharge lamps are used in LS AAS for background correction purposes. The radiation intensity emitted by these lamps is decreasing significantly with increasing wavelength, so that they can be only used in the wavelength range between 190 and about 320 nm. The continuum radiation from the D2 lamp is passed through the flame alternately with the hollow-cathode beam. Since the atomic lines are very narrow, the D2 lamp is mostly absorbed by the background, whereas the hollow-cathode radiation is absorbed by the atoms. By comparing the radiant power of the two beams, the atomic absorption can be corrected for any backbround absorption.

***Continuum sources:*** When a continuum radiation source is used for AAS, it is necessary to use a high-resolution monochromator, as will be discussed later. In addition, it is necessary that the lamp emits radiation of intensity at least an order of magnitude above that of a typical HCL over the entire wavelength range from 190 nm to 900 nm. A special high-pressure xenon short arc lamp, operating in a hot-spot mode has been developed to fulfill these requirements.

**Plate 2:** Xenon lamp as a continuous radiation source (Source: McCarthy [62]).

***Spectrometer:*** As already pointed out above, there is a difference between medium-resolution spectrometers that are used for LS AAS and high-resolution spectrometers that are designed for CS AAS. The spectrometer includes the spectral sorting device (monochromator) and the detector.

### Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a technique which is used to obtain an infrared spectrum of absorption, emission, photoconductivity or Raman scattering of a solid, liquid or gas. An FTIR spectrometer simultaneously collects spectral data in a wide spectral range. This confers a significant advantage over a dispersive spectrometer which measures intensity over a narrow range of wavelengths at a time. FTIR has made dispersive infrared spectrometers all but obsolete (except sometimes in the near infrared), opening up new applications of infrared spectroscopy.

The goal of any absorption spectroscopy (FTIR, ultraviolet-visible ("UV-Vis") spectroscopy, etc.) is to measure how well a sample absorbs light at each wavelength. The most straightforward way to do this, the "dispersive spectroscopy" technique, is to shine a monochromatic light beam at a sample, measure how much of the light is absorbed, and repeat for each different wavelength. (This is how UV-Vis spectrometers works, for example.

Fourier transform spectroscopy is a less intuitive way to obtain the same information. Rather than shining a monochromatic beam of light at the sample, this technique shines a beam containing many frequencies of light at once, and measures how much of that beam is absorbed by the sample. Next, the beam is modified to contain a different combination of frequencies, giving a second data point. This process is repeated many times. Afterwards, a computer takes all these data and works backwards to infer what the absorption is at each wavelength.

The beam described above is generated by starting with a broadband light source-one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer-a certain configuration of mirrors, one of which is moved by a motor. As this mirror moves, each wavelength of light in the beam is periodically blocked, transmitted, by the interferometer, due to wave interference. Different wavelengths are modulated at different rates, so that at each moment, the beam coming out of the interferometer has a different spectrum.

As mentioned, computer processing is required to turn the raw data (light absorption for each mirror position) into the desired result (light absorption for each wavelength). The processing required turns out to be a common algorithm called the Fourier transform (hence the name, "Fourier transform spectroscopy"). The raw data is sometimes called an "interferogram" (Table 1).

## Chapter Three: Materials and Methods

### Collection of materials

Wood of Eku *(Brachystegia eurycoma)*, Gmelina *(Gmelina arborea)* and Mahogany *(Entandrophragma cylindricum)* was collected from Titilayo Saw Mill, in Ore, Ondo State.

### Experimental procedure

Three wood blocks of the dimension (2 cm × 2 cm × 2 cm) were extracted from the bole of the three wood species with the aid of a circular saw while other wood tissues of the three wood species were turned to dust with the aid of a sanding machine. Three saw dust

| Band position ($cm^{-1}$) | Functional group |
|---|---|
| 3450-3400 | O–H alcohol |
| 2930-2910 | C–H methyl and methylene groups |
| 1740-1730 | C=O carbonyls |
| 1640-1618 | C=C alkene |
| 1515-1504 | C=C aromatic |
| 1462-1425 | $CH_2$ cellulose, lignin |
| 1384-1346 | C–H cellulose, hemicellulose |
| 1206-1234 | O–H phenolic |
| 1170-1153 | O–H alcohols (primary and secondary) and aliphatic ethers |
| 910 | C=C alkenes |

**Table 1:** Assignment of the FTIR bands of functional groups in wood [71].

samples were extracted from each of the three wood species so that nine saw dust samples were used for the whole of the research work.

## Experimental procedure for ethanol production

With each of the nine saw dust samples, the various experimental steps are illustrated in the flow chart below Figure 6.

**Hydrolysis of lignocellulosic material using saw dust as a source material:** Each of the nine samples of saw dusts was sieved to create uniformity of particles with the aid of sieve with the mesh size of 200 picometre (pm). The saw dust samples were then air dried for 12 hours to remove moisture. Nine pieces of 250 ml conical flask were each filled with 100 g of dry sawdust from each of the nine saw dust samples and 300 ml of 18 M $H_2SO_4$ (sawdust to acid (w/v) ratio is 1:3) was added to each of them at standard conditions. In each of the nine pieces of 1000 ml beaker, 200 ml of distilled water was added and each of the nine acid solutions was poured respectively and stirred thoroughly.

**Neutralization of the acidic solutions:** 8.5 M NaOH (caustic soda/sodium hydroxide) solution was prepared by following the Mathematical formula as found in Ababio:

Concentration ($g/dm^3$)=Molar mass (g/mol) × Concentration ($mol/dm^3$)

Where: Concentration ($mol/dm^3$) of NaOH=8.5 M and the Molar mass of NaOH is calculated as 23+16+1=40 g/mol.

Therefore, Conc. ($g/dm^3$) of NaOH=8.5M × 40 g/mol=340 g/$dm^3$ and so, 8.5 M of NaOH was then prepared by weighing 340 g of NaOH pellets with the aid of the weighing balance and added to 1,000 $cm^3$ of distilled water, which was measured with the aid of the measuring cylinder of appropriate capacity, bearing in mind that 1 $dm^3$ of a substance is the same as 1000 $cm^3$. This newly prepared NaOH solution was added to each of the nine acidic solutions drop by drop until the pH of 4.8 was attained, as confirmed by the pH meter. The 8.5 M concentration of NaOH adhered to is in accordance to Otulugbu.

**Separation of cellulose and hemicellulose sugars from lignin in the hydrolysed lignocellulosic material:** The neutralized solution was poured on the open flat surface of Buchner funnel whose protruding end was placed in a conical flask. By so doing, the lignin component of the solution in form of black solid residue remained in the funnel, from where it was disposed while liquid filtrate of fermentable sugar solution was collected in the conical flask.

**Procedure for culturing *Saccharomyces*:** About 10 g of potato dextrose Agar (PDA) was dissolved completely in 250 ml of water in a conical flask. The mixture was covered with cotton wool and foil paper and then sterilized in an autoclave at 121°C for 5 minutes. On removal, it was allowed to cool and then poured into Petri dishes and set aside so as to solidify. The *Saccharomyces cerevisiae* cells were then introduced into the Petri dishes with the aid of a sterilized inoculating loop. The Petri dishes were sealed and kept in an incubator for 48 hours at a temperature of 250°C.

**Fermentation of the cellulose filtrate:** The cultures of *Saccharomyces cerevisiae* in the agar slant tubes were divided into nine portions and each of them was dissolved in 10 ml of distilled water containing a drop of tween80 oil (an oil whose presence aids the growth of yeast according to Tran et al. in a separate conical flask. 10 ml of the solution was then added to each of the nine filtrate samples to ferment them. Ethanol fermentation was performed in a shaker incubator at 150 rpm (revolution per minute) for 72 hours at 36°C to allow it to ferment completely.

**Purification:** The Ethanol solution in each of the nine conical flasks was distilled using a distillation bath at 78°C as water distilled at 100°C. A pure ethanol was then obtained in each of the nine bottle samples after the process of distillation.

**Determination of ethanol yield:** The distillate of the nine samples collected over a slow heat at 78°C was measured using measuring cylinder, and expressed as the quantity of ethanol produced in g/l by multiplying the volume of distillate collected at 78°C by the density of ethanol (0.8033 g/ml). g/l is equivalent to the yield of 100 g of dried substrate [66]. These ethanol yields were statistically analyzed, using ANOVA (Analysis of Variance) and compared.

**Determination of the density of ethanol produced from the three wood species:** The yield of ethanol from the three wood species as well as a conventional ethanol was separately weighed using electric balance

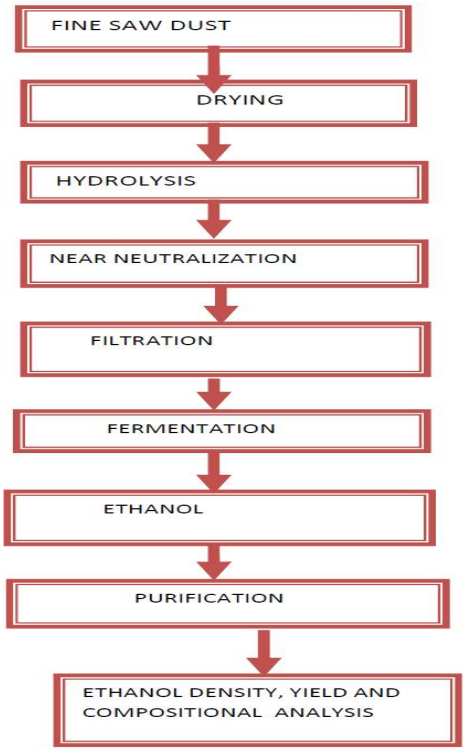


**Figure 6:** A flow chart showing the Sequence of production of ethanol from wood saw dust, Yield studies and Compositional Analysis.

while their volumes were measured using appropriate measuring cylinder. The densities were then determined using a formula below as shown by Anyahoha [67]:

Density (g/cm$^3$)=mass (g)/Volume (cm$^3$)

These densities were then statistically analyzed, using ANOVA (Analysis of Variance).

**Compositional analysis:** The compositional analysis of the synthesized cellulosic ethanol was carried out at the Centre for Energy Research and Development (CERD), Obafemi Awolowo University, Ile-Ife for AAS while that of FTIR was carried out as the Multidisciplinary Central Research Laboratory (MCRL) at University of Ibadan (U.I).

***Experimental procedure for atomic absorption spectrometry (AAS):*** The Atomic Absorption Spectrometer of the Centre for Energy Research and Development, Obafemi Awolowo University, Ile-Ife, Nigeria was used for the AAS analysis of the ethanol.

The AAS was used to determine the concentrations of Cadmion (Cd), Copper (Cu), Zinc (Zn), Chromium (Cr) present in the synthesized ethanol as well as in a sample of conventional/commercial ethanol.

The ethanol was aspirated into an air-acetylene flame, which caused evaporation of the solvent and vaporization of the free metal atoms; a process known as atomization. The concentration in part per million [PPM] of each of the metals present in the ethanol was measured with a conventional UV-visible dispersive spectrometer with photomultiplier detector.

***Experimental procedure for FTIR analysis:*** Fourier transform infrared, FTIR was conducted at the Multidisciplinary Central Research Laboratory, University of Ibadan, Ibadan.

It was performed on a Thermo Scientific-Nicolet 6700 FTIR spectrometer in attenuated total reflection infrared mode. The spectra were recorded in the wave number range of 4000 to 400 cm$^{-1}$ in the transmittance mode at a resolution of 4 cm$^{-1}$, with 64 scans per specimen.

### Experimental design

The experimental design for this research work is 2 × 3 factorial experiment in completely Randomized Design. (CRD).

Factor A (Wood species):

1) Eku *(Brachystegia euricoma)*

2) Mahogany *(Entandrophragma cylindricum)*

3) Gmelina *(Gmelina arborea)*

Factor B (Ethanol physical properties):

1) Ethanol yield

2) Ethanol density

### Statistical analysis

The data collected were analyzed using descriptive statistics, graphical representation and analysis of variance (ANOVA).

## Chapter Four: Results and Discussion

The table (Table 2) above show variation in wood density determined from the three wood species. The density of Eku *(Brachystegia euricoma)*, Mahogany *(Entandrophragma cylindricum)* and Gmelina *(Gmelina arborea)* is 600 kg/m$^3$, 750 kg/m$^3$ and 570 kg/m$^3$ respectively. According to the table, it is clearly shown that Mahogany wood has the highest density while Eku wood has the next highest density but Gmelina wood has the least density. The densities of the three wood species are approximately the same as determined by Meier.

| Wood species | Wood density (kg/m$^3$) |
|---|---|
| Eku (*Brachystegia euricoma*) | 600 |
| Mahogany (*Entandrophragma cylindricum*) | 750 |
| Gmelina (*Gmelina arborea*) | 570 |

**Table 2:** A table showing the wood density of the three wood species.

The above Table 3 shows the concentration in part per million [PPM] of Cadmion (Cd), Copper (Cu), Zinc (Zn) and Chromium (Cr) present in the ethanol produced from the three wood species and that of conventional ethanol. From the table, the concentration in part per million [PPM] of Cadmion (Cd), Copper (Cu), Zinc (Zn) and Chromium (Cr) in the ethanol produced from the wood of Eku in part per million [PPM] is 0.072 ± 0.0012, 0.587 ± 0.0009, 0.955 ± 0.0055, 0.104 ± 0.0003 respectively. The concentration in part per million [PPM] of Cadmion (Cd), Copper (Cu), Zinc (Zn) and Chromium (Cr) in the ethanol produced from the wood of Mahogany in [PPM] is 0.133 ± 0.0010, 0.889 ± 0.0010, 1.682 ± 0.0012 and 0.099 ± 0.0002 respectively. The concentration of Cadmion (Cd), Copper (Cu), Zinc (Zn) and Chromium (Cr) in part per million [PPM] in the ethanol produced from the wood of Gmelina is 0.100 ± 0.0028, 0.712 ± 0.0011, 1.103 ± 0.0009 and 0.099 ± 0.0002 respectively. The concentration of Cadmion (Cd), Copper (Cu), Zinc (Zn) and Chromium (Cr) in part per million [PPM] in the conventional ethanol is 0.011 ± 0.0022, 0.098 ± 0.0009, 0.077 ± 0.0009 and 0.033 ± 0.0017 respectively.

Onyekwelu et al. [68] prepared ethanol from the fruit pulp of *Gmelina arborea* of different ages and subjected the ethanol to spectrophotometry technique to determine the percentage alcoholic content according to AOAC [69] and a standard calibration curve of absorbance against alcoholic concentration was constructed. The results shows that the percentage ethanol yield was determined in percentage (%) and the absorbance as well as the concentration values of the ethanol standard varies from 0.01-0.05 and 0.09-0.19 respectively.

From the above table (Table 4), the ethanol yield of Eku, Mahogany and Gmelina is 50.61 g/l, 55.43 g/l and 53.01 g/l respectively. It can also be deduced from the table that the highest ethanol yield is obtained from the wood of Mahogany and followed by the wood of Gmelina while the least ethanol yield is obtained in the wood of Eku.

Ethanol yield in the three wood species is comparatively higher than that of grasses such as millet and guinea corn determined by Oyeleke and Jibrin [70]. This is so because wood biomass contains more celluloses and hemicelluloses which can serve as the source of fermentable monomeric sugars than grasses.

Table 4 shows the various values of density in g/cm$^3$ determined from the conventional ethanol and ethanol produced from the wood of Eku, Mahogany and Gmelina. From Table 4, the density of the conventional ethanol, ethanol from Eku, Mahogany and Gmelina is shown to be 0.8033 g/cm$^3$, 0.8033 g/cm$^3$, 0.7089 g/cm$^3$ and 0.8033 g/cm$^3$ respectively. It can be deduced that the density of the conventional ethanol is the same as the density of the ethanol produced from the wood of Gmelina and Eku while the least density was determined from the ethanol produced from the wood of Mahogany.

According to Onyekwelu et al. [68] the percentage ethanol yield of *Gmelina arborea* fruit pulp ranges from 1.45 ± 0.33 to 8.88 ± 0.20.

| | Cd | Cu | Zn | Cr |
|---|---|---|---|---|
| Ethanol produced from Eku wood | 0.072 ± 0.0012 | 0.587 ± 0.0009 | 0.955 ± 0.0055 | 0.104 ± 0.0003 |
| Ethanol produced from Mahogany wood | 0.133 ± 0.0010 | 0.889 ± 0.0010 | 1.682 ± 0.0012 | 0.178 ± 0.0004 |
| Ethanol produced from Gmelina wood | 0.100 ± 0.0028 | 0.712 ± 0.0011 | 1.103 ± 0.0009 | 0.099 ± 0.0002 |
| Conventional Ethanol | 0.011 ± 0.0022 | 0.098 ± 0.0009 | 0.077 ± 0.0009 | 0.033 ± 0.0017 |

**Table 3:** Concentration of minerals in [PPM] of the conventional ethanol and the ethanol produced from the three wood species.

| Conventional Ethanol | Ethanol Yield (g/l) | Density (g/cm$^3$) |
|---|---|---|
| Ethanol produced from Eku wood | 50.61 | 0.8033 |
| Ethanol produced from Mahogany wood | 55.43 | 0.7089 |
| Ethanol produced from Gmelina wood | 53.01 | 0.8033 |
| Conventional Ethanol | | 0.8033 |

**Table 4:** Ethanol yield and density of conventional ethanol and ethanol produced from the wood of Eku, Mahogany, Gmelina.

This is different from the result of ethanol yield determined in this work because of difference in parts of wood used as well as difference in methodology and unit of measurement (Figure 7).

Figure 7 above shows the interferogram of Ethanol produced from Gmelina. The figure shows the following peaks of wave numbers: 3361 cm$^{-1}$, 2365.71 cm$^{-1}$, 2093 cm$^{-1}$, 1643.28 cm$^{-1}$, 1261.91 cm$^{-1}$, 433.45 cm$^{-1}$. The wave number 3361 cm$^{-1}$ shows the presence of OH compound like Ethanol, Xylitol, Alcoholic Lignin, Sugars from Cellulose and Hemicellulose. The Wave number 2365.71 cm$^{-1}$ and 2093 cm$^{-1}$ show the presence of compounds with carbon to hydrogen bond (C-H) as in ethanol, Lignin, Cellulose and Hemicellulose sugars. The wave number 1643.28 cm$^{-1}$ shows the presence of compound that contain carbon to carbon double bond (C=C). This is common to phenolic compound like Lignin sub unit. The wave number 1261.91 cm$^{-1}$ shows the presence of Phenolic OH group like Lignin. These are in agreement with the specification made by Bodîrlău and Teacă [69].

The FT-IR of Ethanol produced from Mahogany, as illustrated in Figure 8 above, shows the following peaks of wave numbers: 3335 cm$^{-1}$, 2365.71 cm$^{-1}$, 2091 cm$^{-1}$, 1643.28 cm$^{-1}$, 424.11 cm$^{-1}$. The Wave number 3335 cm$^{-1}$ shows the presence of OH compound like Ethanol, Xylitol, Alcoholic Lignin, Sugars from Cellulose and Hemicellulose. The Wave number 2365.71 cm$^{-1}$ and 2091 cm$^{-1}$ show the presence of compounds with carbon to hydrogen bond (C-H) as in ethanol, Lignin, Cellulose and Hemicellulose sugars. The wave number 1643.28 cm$^{-1}$ Shows the presence of compound that contain carbon to carbon double bond (C=C). This is common to phenolic compound like Lignin sub unit. These are in agreement with the specification made by Bodîrlău and Teacă [71].

The FT-IR of Ethanol produced from Eku as illustrated in Figure 9 above, shows the following peaks of wave numbers: 3336 cm$^{-1}$, 2091 cm$^{-1}$, 1643.45 cm$^{-1}$, 1259.06 cm$^{-1}$, 415.54 cm$^{-1}$. The Wave number 3336 cm$^{-1}$ shows the presence of OH compound like Ethanol, Xylitol, Alcoholic Lignin, Sugars from Cellulose and Hemicellulose. The Wave number 2091 cm$^{-1}$ shows the presence of compounds with carbon to hydrogen bond (C-H) as in ethanol, Lignin, Cellulose and Hemicellulose sugars. The wave number 1643.28 cm$^{-1}$ Shows the presence of compound that contain carbon to carbon double bond (C=C). This is common to phenolic compound like Lignin sub unit. The wave number 1259.06 cm$^{-1}$ shows the presence of Phenolic OH group like Lignin. These are in agreement with the specification made by Bodîrlău and Teacă [71].

The FT-IR of conventional Ethanol as illustrated in Figure 10 above shows the following peaks of wave numbers: 3401 cm$^{-1}$, 2565.71 cm$^{-1}$, 2095 cm$^{-1}$, 1645.51 cm$^{-1}$, 1395.70 cm$^{-1}$, 433.45 cm$^{-1}$. The Wave number 3401 cm$^{-1}$ shows the presence of OH compound like Ethanol, Xylitol, Alcoholic Lignin, Sugars from Cellulose and Hemicellulose. The Wave number 2565.71 cm$^{-1}$ and 2095 cm$^{-1}$ show the presence of compounds with carbon to hydrogen bond (C-H) as in ethanol, Lignin, Cellulose and Hemicellulose sugars. The wave number 1645.51 cm$^{-1}$ Shows the presence of compound that contain carbon to carbon double bond (C=C). This is common to phenolic compound like Lignin sub unit. The wave number 1395.70 cm$^{-1}$ shows the presence of Hemicellulose carbon to hydrogen bond. These are in agreement with the specification made by Bodîrlău and Teacă [71].

From the four figures (i.e., Figures 7, 8, 9 and 10) above, it can be seen that the peak of wave number indicating the concentration of OH- functional group of conventional ethanol, ethanol produced from Gmelina, ethanol produced from Eku and ethanol produced from Mahogany is 3401 cm$^{-1}$ at the transmittance value of about 1%; 3361 cm$^{-1}$ at the transmittance value of about 0.5%; 3336 cm$^{-1}$ at the transmittance value of about 0.5% and 3335 cm$^{-1}$ at the transmittance value of about 0.5% respectively.

This shows that conventional ethanol has the highest alcoholic property, followed by ethanol produced from Gmelina. Ethanol produced from Eku has the next highest alcoholic property after Gmelina while ethanol produced from Mahogany has the least alcoholic property.

It is not surprising that conventional ethanol has the highest alcoholic properties because conventional ethanol is produced from starch source and starch has tendency to hydrolyze readily than lignocellulosic materials like wood as present in Gmelina, Mahogany and Eku. This is because of the presence of intermolecular hydrogen bonds and intramolecular hydrogen bonds in cellulose molecules especially in the crystalline regions [3].

Difference in the level of alcoholic properties of the ethanol produced from different wood biomass is due to difference in the rate of hydrolysis. Wood hydrolysis is a function of the wood density and extractive contents. Mahogany has the least degree of hydrolysis among Gmelina and Mahogany because it has the highest density of 750 kg/m$^3$ and this may be one of the reasons the ethanol produced from it has the least value of wave number indicating the presence of OH functional group compared to ethanol produced from Eku and Gmelina wood. Eku with the density 600 kg/m$^3$ is denser than Gmelina with the density of 570 kg/m$^3$ and hence, this may be one of the reasons the ethanol produced from shows the FT-IR spectra with a lesser wave number of alcoholic OH than the wave number of alcoholic OH in the ethanol produced from Gmelina (Table 5).

From the above Table 5, it can be deduced that there is no significant difference between the density and ethanol yield of Conventional ethanol as well as the density and the ethanol yield of the ethanol produced from the three wood species.

## Chapter Five: Conclusion and Recommendation

### Conclusion

Petroleum economy is enviable but has a lot of terrible associated problems. Nations that rely solely on petroleum economy often slum

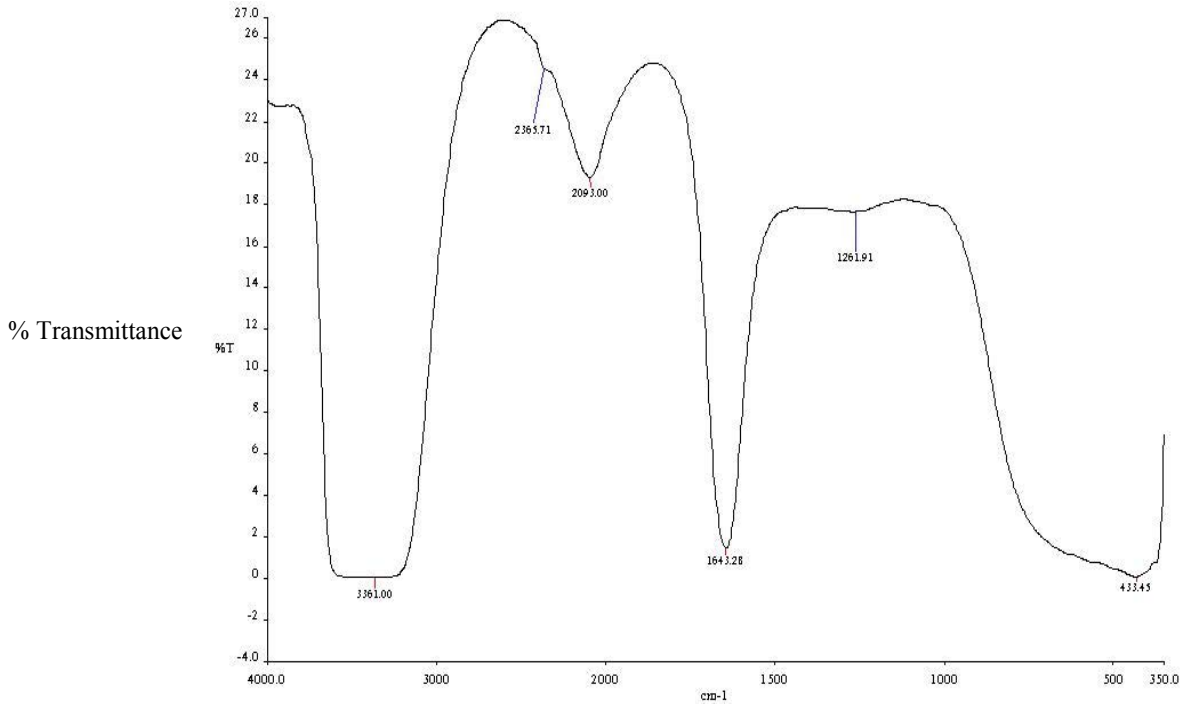


**Figure 7:** Interferogram of ethanol produced from *Gmelina* wood.

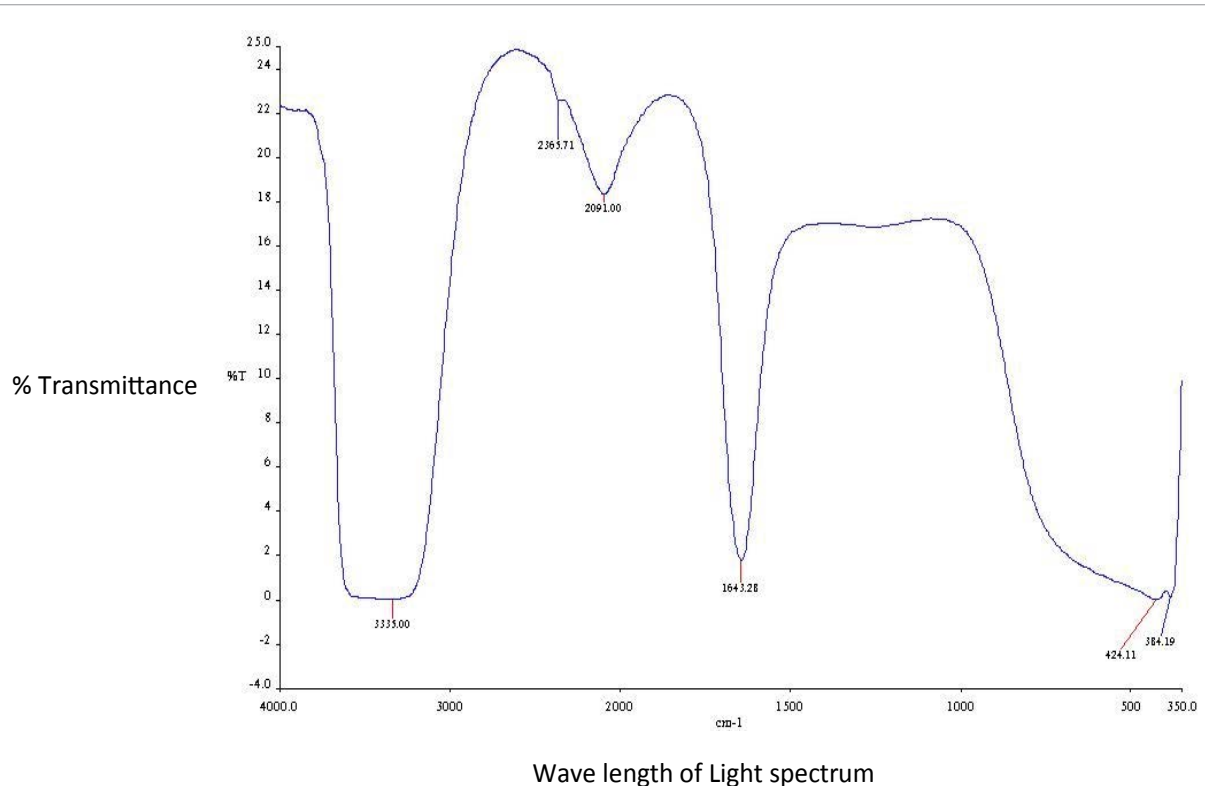


**Figure 8:** Interferogram of ethanol produced from Mahogany wood.

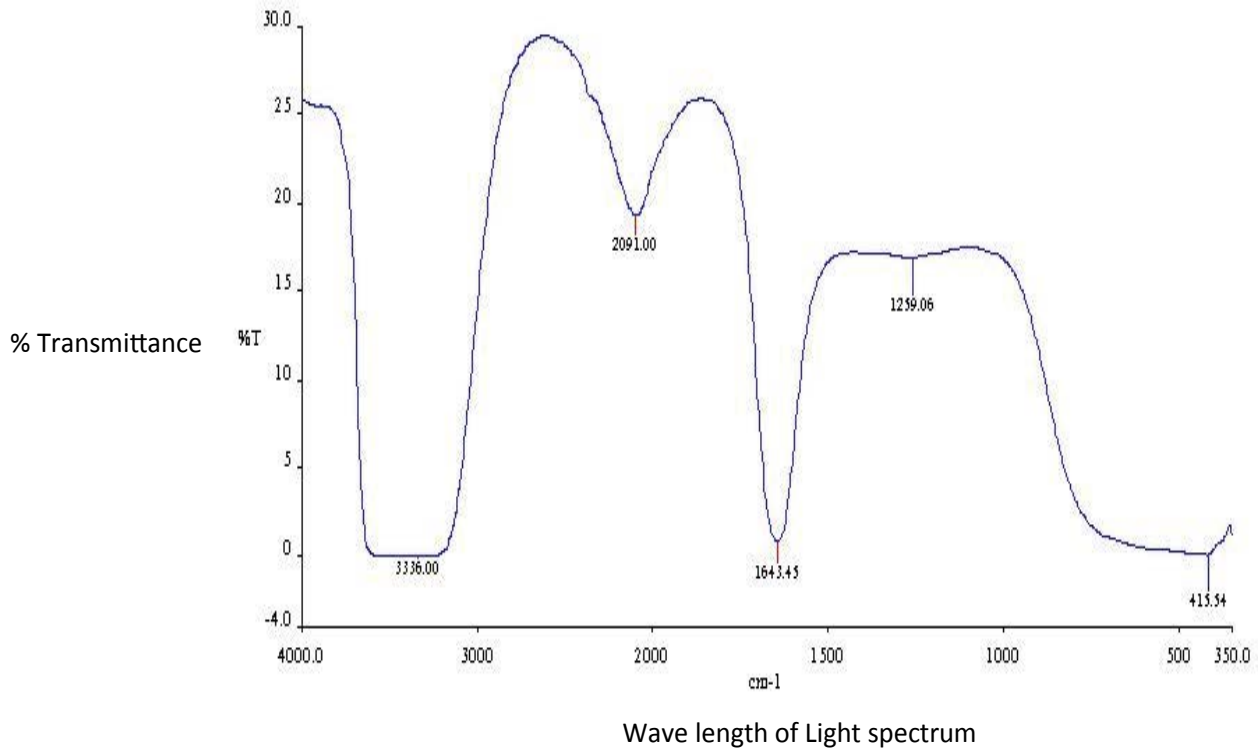


**Figure 9:** Interferogram of ethanol produced from Eku wood.

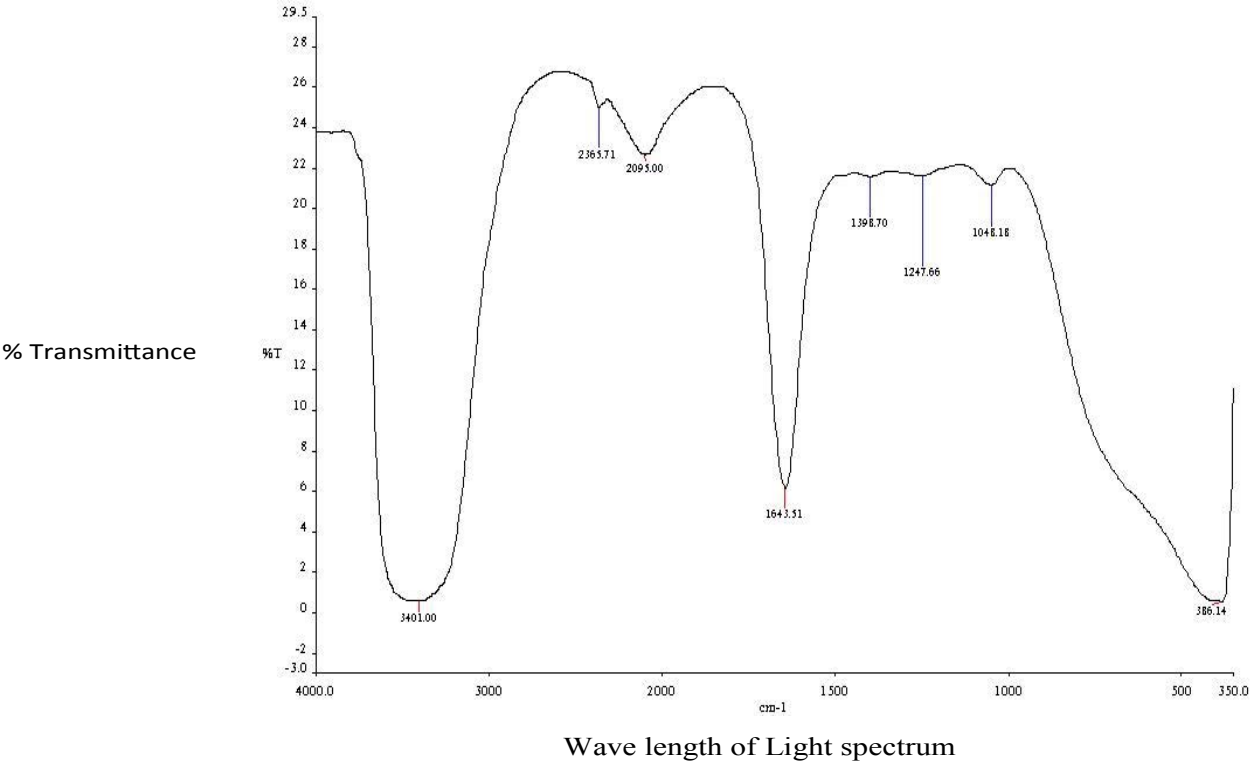


**Figure 10:** Interferogram of conventional ethanol.

| ANOVA | | | | | | |
|---|---|---|---|---|---|---|
| | | Sum of Squares | df | Mean Square | F | Sig. |
| Wood species | Between Groups | 7.333 | 12 | 0.611 | 0.655 | 0.747 |
| | Within Groups | 4.667 | 5 | 0.933 | | |
| | Total | 12.000 | 17 | | | |
| Physical properties | Between Groups | 4.500 | 12 | 0.375 | 0.0 | 0.0 |
| | Within Groups | 0.000 | 5 | 0.000 | | |
| | Total | 4.500 | 17 | | | |

**Table 5:** ANOVA Table comparing the physical properties (% yield and density) of conventional ethanol and ethanol produced from the wood of Eku, Gmelina and Mahogany.

into unforeseen fatal problems. Agro based economy is therefore recommended because of its lasting effects, particularly for the African nations that are agrarian.

The density of Eku *(Brachystegia euricoma),* Mahogany *(Entandrophragma cylindricum)* and Gmelina *(Gmelina arborea)* is 0.6 g/cm$^3$, 0.75 cm$^3$ and 0.57 cm$^3$ respectively.

The average ethanol yield of the wood of Eku *(Brachystegiaeurycoma)*, Mahogany *(Entandrophragma spp)* and *(Gmelina arborea)* is 50.61 g/l per 100 g of dry sawdust, 55.43 g/l per 100 g of dry sawdust and 53.01 g/l per 100 g of dry sawdust respectively.

The average density of the ethanol produced from Eku sawdust, Mahogany sawdust and Gmelina sawdust is 0.8033 g/cm$^3$, 0.7889 g/cm$^3$ and 0.8033 g/cm$^3$ respectively. With this unique densities, the ethanol from the three wood species are confirmed to be ethanol.

The ethanol from the three wood biomass contains the following metallic ions: $Cu^{2+}$, $Cd^{2+}$, $Zn^{2+}$ and $Cr^{2+}$. The ethanol from the three wood biomass contains the following functional groups: -OH, C-C, C-H by which they are actually confirmed to be real ethanol.

The higher the density of a wood biomass, the lesser its tendency to be hydrolyzed and produce ethanol and vice-versa. Waste wood biomass in form of saw dust can serve as the source of greenhouse free ethanol fuel as a good alternative to petroleum products whose cost of production is higher and also pollutes the air as it burns with smoky flames as well as a good alternative to ethanol produced from starchy Agricultural food stuffs which has been competing with man's food availability.

## Recommendation

The three wood biomass are recommended for ethanol production due to their high ethanol yield instead of depending on feed stock raw materials in order to save them for food security and continuity.

Low density wood species are better utilized in ethanol production than high density wood species because of their ease of hydrolysis.

The density of ethanol is the same as 0.8033 g/cm$^3$, no matter the type of raw material from which the ethanol is produced.

Transition metals like Cu, Cd, Cr, Zn play important roles in binding enzymes to substrate sugars during the production of ethanol. They are readily present in the wood biomass of Eku *(Brachystegia eurycoma)*, Mahogany *(Entandrophragma spp)* and *(Gmelina arborea)*. For more efficient production of ethanol, the addition of transition metals to the substrate or the use of the sawdust of any of the three wood species like Eku *(Brachystegia eurycoma)*, Mahogany *(Entandrophragma spp)* and *(Gmelina arborea)* is therefore recommended.

Ethanol from wood biomass is therefore recommended as a good fuel alternative to costly and smoky petroleum fuel.

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