



# Bioactive Constituents of Essential Oil from *Khaya senegalensis* (Desr.) Bark Extracts

Blessing Ikoojo Omotoyinbo<sup>1,\*</sup>, Ayoola Ebenezer Afe<sup>2</sup>, Olawale Solomon Kolapo<sup>1</sup>,  
Oluseyi Valerie Alagbe<sup>1</sup>

<sup>1</sup>Department of General Studies, Chemistry Unit, Federal College of Agriculture, Akure, Nigeria

<sup>2</sup>Department of Biochemistry, Federal University of Technology, Akure, Nigeria

## Email address:

blessingebiloma@gmail.com (B. I. Omotoyinbo)

\*Corresponding author

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**Abstract:** The use of plants in traditional medicines has been a common practice in the medical care of many human cultures and dates back to several thousands of years and pre-dates the introduction of antibiotics and other related drugs due to their great source of phytochemicals which are exploited in all medical systems. This study was aimed at characterizing using Gas Chromatography-Mass Spectrometry (GC/MS) technique, the chemical constituents of the essential oil extracted from the bark of *Khaya senegalensis* Desr. using methanol-chloroform. Eight compounds were identified which include: Sulfurous acid, decylpentyl ester (0.51%), n-Hexadecanoic acid (12.08%), 1-Pentadecanol (1.84%), 13,16-Octadecadienoic acid, methyl ester (1.71%), Oleic acid (39.16%), Octadecanoic acid (21.9%), Dodecanoyl chloride (3.93%) and cis-11-hexadecenal (18.88%). The presence of these compounds in the bark of *Khaya senegalensis* might amongst many other bioactive constituents be responsible for the medicinal properties these plant parts exhibit and is being used for in herbal medicines.

**Keywords:** *Khaya senegalensis*, GC/MS Analysis, Essential Oil, Traditional Medicine

## 1. Introduction

Human existence has been hinged on nature as sources of medicine, shelter, clothing, food stuffs and means of transportation from ancient times. In fact, plants and their medicinal properties have continually played a dominant role in the healthcare system of many developing countries. These traditional medicine systems are majorly formed from plants and have been in existence for thousands of years; and plants remain to offer mankind with new medicines [1, 21]. Plant extracts, in form of concoction, decoction and infusion have been used to treat a variety of diseases and infections. They are especially efficacious against certain health challenges that have shown resistance to orthodox medicine. More so, demands of traditional herbal medicines are increasing by the day from both the developing and the developed countries of the world [5]. Plants are not only indispensable in health care, but form the best hope of source for safe future

medicines [8], in many climes and especially developing societies that can not afford the towering cost of the highly evolving orthodox health care.

Mahogany trees belong to the *Meliaceae* family and represent one of the most economically important tree species in the world [13, 16]. Mahoganies indigenous to Africa comprise several species. However, the most common species in Nigeria and several West African countries is *Khaya senegalensis* (common name: Dry-zone mahogany). It is an evergreen tree, which grows up to 40 m high in climates ranging from the Savannah to the humid forests [14]. The bark extract is used for treating jaundice, dermatoses, hookworm infection and malaria [7, 14]. It is reported that the stem bark of *Khaya senegalensis* possesses anti-sickling [6], anti-hyperglycemic effects [11], antimicrobial effects [18], antifungal effects [22], antiprotozoal effects [9], antihelminthic effects [3, 15] and anti-cancer effects [4, 20], as well as free radical scavenger activities [12]. These and

many more health benefits of this plant part is undoubtedly a function of the secondary metabolites which exist as volatile organic compounds (VOCs) stored in the bark of such trees, hence, giving it the widely attributed medical usage by traditional medicine users in several cultures. Some of these groups of VOCs are sometimes released under different conditions by plants, based on exposure to varying abiotic factors after mechanical damage of tissues or biotic factors such as herbivores [23]. This study was carried out to investigate and ascertain the chemical constituents of the essential oil obtained from *Khaya senegalensis* stem bark with the aid of GC-MS technique.

## 2. Materials and Method

### 2.1. Collection of Plant Material

The stem bark of *Khaya senegalensis* was acquired from *Oja Oba* market beside the Kings' Palace in Akure. They were further identified and authenticated at the Department of Crop Soil and Pest management, Federal University of Technology Akure, Ondo State Nigeria.

### 2.2. Preparation of Powder and Extract

The plant material which was shade dried and ground into fine powder was extracted by the modified Bligh and Dyer procedure as reported by Sanni and Omotoyinbo [19].

Firstly, 10 g of powdered sample were shaken with 40ml methanol–chloroform (2:1; v/v), for 20 minutes. The homogenate was also filtered and the residue was shaken with 40 ml of methanol–chloroform (2:1; v/v) and 5 ml of water for 20 minutes. This was followed by another round of filtration and the filtered residue was washed with 5 ml of methanol–chloroform (2:1; v/v), after which the combined filtrates were poured in a Separatory funnel then 5 ml of chloroform and 6 ml of water was added and the phases were allowed to separate. The chloroform layer was withdrawn, diluted with benzene and concentrated in rotary evaporator.

The residual lipids were immediately dissolved in 1 ml chloroform–methanol (1:1) for further analysis.

### 2.3. GC-MS Analysis and Identification of Components

A qualitative characterization analysis of all the possible phytochemicals present in extracted fraction was carried out using GC-MS using scan mode. This analysis of *Khaya senegalensis* stem bark extract was performed using 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies). The stationary phase of separation of the compounds was HP-5 capillary column coated with 5 % Phenyl Methyl Siloxane (30 m length x 0.32 mm diameter x 0.25  $\mu$ m film thickness) (Agilent Technologies). The carrier gas was Helium used at constant flow of 1.49 mL/min at an initial nominal pressure of 1.49 psi and average velocity of 44.22 cm/sec. 1  $\mu$ L of the samples were injected in splitless mode at an injection temperature of 300°C. Purge flow was 15 mL/min at 0.75 min with a total flow of 16.67 mL/min; gas saver mode was switched on. Oven was initially programmed at 40°C (1 min) then ramped at 12°C/min to 300°C (10 min). Run time was 32.67 min with a 3 min solvent delay. The mass spectrometer was operated in electron-impact ionization mode at 70eV with ion source temperature of 230°C, quadrupole temperature of 150°C and transfer line temperature of 300°C. Scanning of possible phytochemical compounds was from  $m/z$  45 to 550 amu at 2.00s/scan scan rate and was identified by comparing measured mass spectral data with those in *NIST 14 Mass Spectral Library* and literature.

Prior to analysis, the MS was auto-tuned to perfluorotributylamine (PFTBA) using already established criteria to check the abundance of  $m/z$  69, 219, 502 and other instrument optimal & sensitivity conditions. While analysis validation was conducted by running replicate samples in order to see the consistency of the constituent compound name, respective retention time, molecular weight (amu), Quality ion (Q-Ion) and Percentage Total.

## 3. Results and Discussion

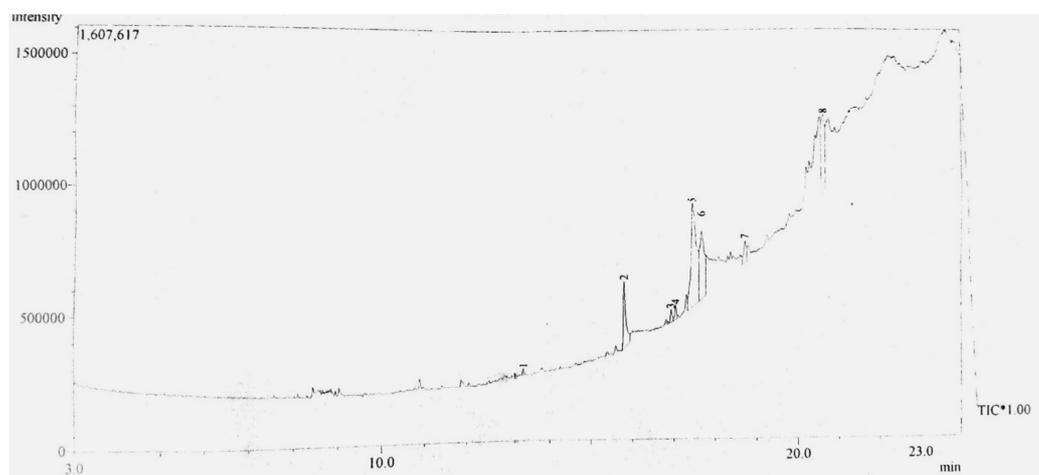


Figure 1. GC chromatogram of *Khaya senegalensis* bark methanol–chloroform extracts.

GC-MS is one of the best techniques to identify the constituents of volatile matter obtained from plant and animal; these may include: long chain, branched chain hydrocarbons, alcohols acids, esters etc. The GC-MS analysis of *Khaya senegalensis* stem bark extract revealed the presence of twelve phytochemical constituents that could contribute to the

medicinal quality of the plant. The identification of the phytochemical compounds was confirmed based on the peak area, retention time and molecular formula. The active principles with their Retention time (RT), Molecular formula, Molecular weight (MW) and peak area in percentage are presented in Figure 1 and Table 1.

**Table 1.** Components detected in the stem bark of chloroform-methanol extract of *Khaya senegalensis*.

No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	13.38	Sulfurous acid, decylpentyl ester	C <sub>15</sub> H <sub>32</sub> O <sub>3</sub> S	292	0.51
2.	15.82	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	12.08
3.	16.96	1-Pentadecanol	C <sub>14</sub> H <sub>30</sub> O	228	1.84
4.	17.07	13,16-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	1.71
5.	17.48	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	39.16
6.	17.69	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	21.90
7.	18.74	Dodecanoyl chloride	C <sub>12</sub> H <sub>23</sub> ClO	218	3.93
8.	20.60	cis-11-hexadecenal	C <sub>14</sub> H <sub>30</sub> O	214	18.88
					100.00

The first compound identified with the least retention time (13.38 min) was Sulfurous acid, decylpentyl ester, whereas Cis-11-hexadecenal was the last compound which took longest retention time (20.60 min) to identify. Other

compounds detected are n-Hexadecanoic acid (15.82 min), 1-Pentadecanol (16.96), 13,16-Octadecadienoic acid, methyl ester (17.07 min), Oleic acid (17.48 min), Octadecanoic acid (17.69 min) and, Dodecanoyl chloride (18.74 min).

**Table 2.** Activity of Phyto-Components identified in the stem bark of chloroform-methanol extract of *Khaya senegalensis*.

No.	Name of the compound	Molecular Formula	Nature of compound	**Activity
1.	Sulfurous acid, decylpentyl ester	C <sub>15</sub> H <sub>32</sub> O <sub>3</sub> S		Increase aromatic amino acid decarboxylase activity, Acidifier, Acidulant, Arachidonic acid-inhibitor, inhibit production of uric acid Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Lubricant, Antiandrogenic, Flavor, Hemolytic 5-Alpha reductase inhibitor,
2.	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	Saturated Fatty acid	Acidifier, Acidulant, Arachidonic acid-inhibitor, inhibit production of uric acid, Anaphylactic, Antitumor (Naopharynx), increase Natural killer cell activity, inhibit production of tumor necrosis factor
3.	1-Pentadecanol	C <sub>14</sub> H <sub>30</sub> O	Alcoholic compound	No activity reported
4.	13,16-Octadecadienoic acid, methyl ester	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	Unsaturated Fatty acid ester	Acidifier, Acidulant, Arachidonic acid-inhibitor, inhibit production of uric acid
5.	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	Unsaturated Fatty acid	Increase aromatic amino acid decarboxylase activity, Acidifier, Acidulant, Arachidonic acid-inhibitor, inhibit production of uric acid
6.	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	Saturated Fatty acid	Acidifier, Acidulant, Arachidonic acid-inhibitor, inhibit production of uric acid
7.	Dodecanoyl chloride	C <sub>12</sub> H <sub>23</sub> ClO	Acid chloride	No activity reported
8.	cis-11-hexadecenal	C <sub>14</sub> H <sub>30</sub> O	Aldehyde	Antimicrobial

The phytochemicals identified through GC-MS analysis showed many biological activities (Table 2) relevant to the use of *Khaya senegalensis* stem bark by traditional practitioners. Traditional healers normally use water as extractants in medicinal plant preparations for the treatment of ailments. However, majority of the antimicrobial and other phytoconstituents of plants that have been identified are not water soluble. Reports have shown that extracts made from organic solvents are more potent and usually exhibit better and consistent antimicrobial activity [24, 25].

It was observed that the 18 carbon fatty acids; Oleic acid and Octadecanoic acid were the most abundant compounds from the oil extracted with percentage quantity of 39.16 and 21.90 % respectively. *K. senegalensis* extract from the work

of Rabadeaux *et al.* [17], reported presence of phenolics, saponins, tannins and triterpenes as the most abundant phytochemicals observed from methanol and chloroform extracts of *K. senegalensis* bark. They also showed potent bacterial growth inhibitory efficacy supporting the efficacy of cis-11-hexadecenal, anti-giardial activity and carcinoma cell anti-proliferative activity.

Secondary metabolites including alkaloids, terpenoids, and flavonoids etc. from plants have been found to possess antimicrobial activity [27, 29]. Plants have been found to synthesize flavonoids in response to microbial infection and these flavonoids have shown in vitro antimicrobial activity against microorganisms [26]. The antimicrobial activity is believed to be due to their effectiveness in inactivating microbial

adhesions and metabolism, and also impeding development and functioning of enzymes, cell envelope and proteins [30-32]. These phytochemical constituents also form complexes with polysaccharides and proteins in the microbes rendering them inactive [33]. Tannins have also shown to be active against bacteria and filamentous fungi including *C. albicans* [34] and possess astringent properties [28]. Olowosulu and Ibrahim [35] reported that flavonoids have been shown to have antimicrobial activity and this activity, according to Tsuchiya *et al.* [33] may be attributed to their ability to complex with extracellular and soluble proteins, and cell walls of the bacteria. Therefore, the antimicrobial activity of the methanol-chloroform extracts may be due to the physiological activities of the phytochemical constituents present in them.

The presence in these plant parts with considerable amounts of saponins which active principles have shown to be poisonous to animal pest at high doses also substantiates on the release of VOCs stored in such plant barks. This implies that some of the active constituents present in the extract, and some as reported by Dr. Duke's Phytochemical and Ethnobotanical Databases could contribute to such medicinal properties of the plant extract. Also, since there is no study that can give a clear idea and be accurate on the mode of action of the essential oils, given the complexity of their chemical composition, every thing suggests that this mode of action is complex, and it is difficult to identify the molecular pathway of action [10]. Hence, it is very likely that each of the constituents of the essential oils has its own mechanism of action [2], or they work in synergy to address the myriad health benefits that has been reported from the use of *K. senegalensis* bark in herbal medicine.

## 4. Conclusion

These investigations provide supporting evidence to the use of the bark of *K. senegalensis* by herbal medicine practitioners in Nigeria, and it also corroborates the findings of previous works on the properties of *K. senegalensis* stem bark. However, further research needs to be done to ascertain the other bioactives these plant part does possess to explore their Pharmacological advantages.

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