PRELIMINARY PHYTOCHEMICAL SCREENING AND FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS OF THE LEAF AND STEM BARK EXTRACTS OF *Khaya senegalensis*

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Abstract: *Khaya senegalensis*, a member of the family Meliaceae, is a plant commonly used by the local people of Adamawa State Nigeria for the treatment of dysentery, inflammation, malaria, anemia, diabetic, gastrointestinal diseases, mucous diarrhoea and wound infections. The aim of this study was to carry out phytochemical screening and Fourier transform infrared spectroscopy of the stem bark extract of *K. senegalensis*. The Fourier transform infrared (FTIR) spectra of methanol extract revealed the presence of alcohol (OH) stretching for alcohol group, alkanes (C-H), carbonyl (C=O) and amines (N-H) as functional groups. Results of the phytochemical screening showed that saponins, tannins, alkaloids, steroids, terpenoids and flavonoids were the active compounds present in the leaves and bark of the plant. This study demonstrates the potentials of *K. senegalensis* as a source of antimicrobials that could be harness for use in the health care delivery process.

Keywords: *Khaya senegalensis*, phytochemical and fourier transform infrared spectroscopy

Introduction

Medicinal plants are bioresources harnessed by humans to combat diseases and maintain healthy life. Plants have proved to be a source of inspiration for the discovery of novel drug compounds, as plant derived medicines has made large contributions to human health and well-being (Buba, 2016). *K. senegalensis*a tree in the family Meliaceae is a native of West Africa (Senegal) and extends to Sudan and Uganda (Keay, 1989). The tree is commonly called the dry zone mahogany, and it is widely distributed in the Savannah regions (Makut *et al.*, 2008). In Nigeria, the tree is called with several local names in different parts of the country; 'Madachi' in Hausa, 'Dalehi-Kahi' in Fulfulde, 'Oganwa' in Yoruba and 'Ono' in Igbo languages (Makut *et al.*, 2008). The plant is easily recognized by its round evergreen crown of dark shining foliage pinnate leaves and characteristic round capsules. In its natural habitat, the plant is a medium to large sized tree that grows up to 30 m high and 3 m girth, with dense crown and short bole covered with dark drey scaly bark. Slash dark pink, bark bitter yielding gum when wounded. Leaves with 3 - 4 (exceptionally 5) pairs of leaflets, 5 – 10 cm long by 2.5 - 5 cm broad, more or less elliptic, round, obtuse or shortly acuminate at apex; stalks of leaflets 4 mm long (Irvine, 1961; Keay, 1989). It has attracted world-wide attention for its high quality timber production and its high usage in the tropics (Arnold, 2004).

In northern Nigeria, the Hausa and the Fulani tribes utilize *K. senegalensis* traditionally as a remedy for several human and animal ailments (Deeniand and Sadiq, 2002). It is used extensively as a bitter tonic for the treatment of a variety of pro-inflammatory disease. The plant is also commonly used in African traditional medicine for pain and Inflammation (Xiao & Stephanie, 2006). The stem-back and leaves of *K. senegalensis* have been used in Adamawa State in forms of decoction and concoctions for the cure of mucous diarrhea, syphilis, pyrexia, malarial fever, dysentery and wound infections (Olayinka, 1992).

Phytochemicals are naturally occurring in the medicinal plants leaves, vegetables, fruits, bark and roots that have defense mechanism and protect from various diseases (WHO, 2016). Phytochemicals have two categories i.e., primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugar and amino acids. Secondary constituents contain many compounds such as terpenoids and alkaloids. The medicinal value of secondary metabolites is due to the presence of chemical substances that produce a definite physiological action on the human body. The most important of these include: alkaloids, glucosides, glycosides, steroids, flavanoids, fatty oils, phenols, resins, phosphorus and calcium for cell growth, replacement, and body building (Chidambara, 2003). The study was aimed to explore the phytochemical constituents and Fourier transform infrared spectroscopy of the stem bark extract of *K. senegalensis*.

Materials and Methods

Collection and processing of plant materials

Fresh sample of the stem bark and leaves of *K. senegalensis* were collected directly from the farm in July, 2019 from Hong Local Government Area of Adamawa state, Nigeria. The identity of the plant was Expunge and confirmed by a botanist, Iliyasu Idris of Plant Science Department, Modibbo Adama University of Technology Yola, with voucher number were not deposited. The samples of the plant parts were thoroughly washed and air dried at room temperature for period of two weeks before powdering.

Extraction of plant material

Fiftygrams of the powdered material of the leaves and stembark ware soaked separately in methanol and distilled water for 72 h at room temperature of about 33º with intermittent shaking. Extracts were first filtered through Whatman No. 1 filter paper. After filtration, the extracts were concentrated to dryness and kept in a refrigerator until needed.

Phytochemical screening

Qualitative preliminary phytochemical analysis of all the solvent extracts was conducted in accordance with the standard procedure (Harborne, 1992) to detect the phytoconstituents Expunge present in the plant materials. The Tests for saponins, tannins, flavanoids, glycoside, alkaloids and phenols were carried out by standard qualitative methods (Trease and Evans, 2002; Sood *et al.*, 2012). Saponins, flavanoids and glycosides were tested using the methods of Harborne (1992), tannins and alkaloids (Sood and Gupta, 2012), phenol and steroids (Trease and Evans, 2002).

Thin layer chromatography

Freshly coated plates with silica gel G254 was allowed to air dry at room temperature and transferred to oven for activation and maintained at 110ºC for 30 min. Solvent system described in literature for flavonoids were toluene, ethyl

acetate and formic acid in the ratio of 5:3:2 was attempted to achieve better resolution. The plates are placed into the developing chamber and allowed to run until it reaches a height of about 14 cm from the point of spotting. Spraying agent used for detection of spots was 10% vanillin sulphuric acid reagent.

Fourier transform infrared spectrophotometer (ftir)

For the FTIR spectrophotometer analysis, the extract was centrifuged at 3000 rpm for 10 min and was filtered through Whatman No. 1 filter paper by using high pressure vacuum pump. The extract was scanned in the wavelength ranging from 4000-1000 nm. FTIR analysis was conducted using Perkin Elmer Spectrophotometer where the characteristic peak were detected and their functional groups. Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds/functional groups present in the phytochemicals. The wavelength of light absorbed is salient feature of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a compound can be determined. Dried powder of the stem bark extract of *K. senegalensis* was used for FTIR analysis (Geethu *et al.,* 2014).

Results and Discussion

The result of preliminary phytochemical screening of leaves and stem-bark extracts of *K. senegalensis* indicates the presence of Alkaloids, Flavonoids, Saponins, Tannins, Glycosides, Phenols and Steroids (Table 1).

Table1: Result of phytochemical screening of leaves and stem bark extracts of *K. senegalensis*

Parameter	Methanol extract		Aqueous extract	
	Bark	Leaves	Bark	Leaves
Alkaloids	-	+	+	+
Flavonoids	+	+	+	+
Saponins	-	+	+	+
Tannins	+	+	+	+
Glycosides	-	-	-	-
Phenols	+	+	+	+
Steroid	+	+	+	+

– = Absent, + = Present

Result of FTIR analysis

A mahogany stem bark spectrum shows that the double peak at 3394 and 3351 cm^{-1} indicates the presence of N-H stretching. The second double peak which occurs at 2932 and 2873 cm^{-1} identified the occurrence of C-H stretching. The band at 1737 cm^{-1} presume the appearance of C=O stretching. Furthermore, the peak at 1616 and 1519 cm^{-1} affirm the N-H bending of amine and N-O stretching, respectively. The fingerprint region of the spectra account for the presence of C-H bending and C-N stretching at 1448, 1371 and 1247 cm^{-1}, respectively.

Results of phytochemical analysis in the different solvent revealed the presence of saponin, flavonoid, tannin, alkaloid, glycoside and steroid which is in agreements with the work of (Aguoru, 2017; Maku, 2007; Wakirwa, 2013). These observations therefore support the use of *K. senegalensis* in herbal care remedies. Other study approved that the plant *K. senegalensis* contains alkaloids which are ranked the most efficient therapeutically significant plant substance. Pure isolated plant alkaloids and their synthetic derivatives are used as a basic medicinal agent for their analgesic, antispasmodic and bactericidal effects (Marvit *et al*., 2018). Saponins have been reported to have an antimicrobial effect and could serve as precursors of steroidal substances with a wide range of physiological activities (Aguoru, 2017). The biological function of flavonoid includes protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, hepatotoxins and tumors (Okwu, 2004). Tannin is one of the major active ingredients found in plant based medicines (Haslam, 1996). They are used in the dyestuff industry as caustics for cationic dyes (tannin dyes), and also in the production of inks (iron gallate ink), textile dyes, antioxidants in beverages, and coagulant in rubber production as well as possessing antiviral, antibacterial, and antitumor activity (Haslam, 1996; Khanbabaee and Van Ree, 2001). Tannin has been reported to selectively inhibit HIV replication (Kashiwada *et al*., 1992). Alkaloids have a wide range of pharmacological activities including antimalarial (e.g., quinine), anticancer (e.g., homoharringtonine), antibacterial (e.g., chelerythrine) and antihyperglycemic activities (e.g., piperine) (Namadina *et al.,* 2019).

Thin layer chromatography

Thin layer chromatography (TLC) is a technique applied in phytochemical study (of herbal materials) to separate the components of an extract (Sani *et al*., 2012). From the results obtained in this study, toluene, ethyl acetate and formic acid extract separated leaving spots on different areas of the plate. Methanol extract left five distinct spots on the plate before the solvent front. The ultraviolet light view mainly revealed unsaturation and aromaticity of the sample separated (Table 2 and Fig. 1). TLC is also used to determine the degree of purity of a drug or extract. It also indicates the presence of any form of contamination or adulteration. Deposit of a high concentration of the extract on a spot indicates more than one constituent with probably similar molecular weights (Sani *et al.,* 2012).

Table 2: Thin layer chromatography (TLC) profiling of the stem bark

Extract	Solvent System (SS)	DTS (cm)	No. of Spots	Colour	Rf - Values (cm)
Ks	T:E:F (5:3:2 ml)	14.2	5	YG	1.00
				BY	0.73
				BG	0.67
				LG	0.63
				BG	0.57

Ks: *Khaya senegalensis*, DTS: Distance travel by the Spot, Rf: Retention factor, T: E: F: Toluene, Ethyl acetate and Formic acid, YG: Yellowish Green, BY: Brownish Yellow, BG: Bluewish Green, LG: Light Green and GB: Greenish Black

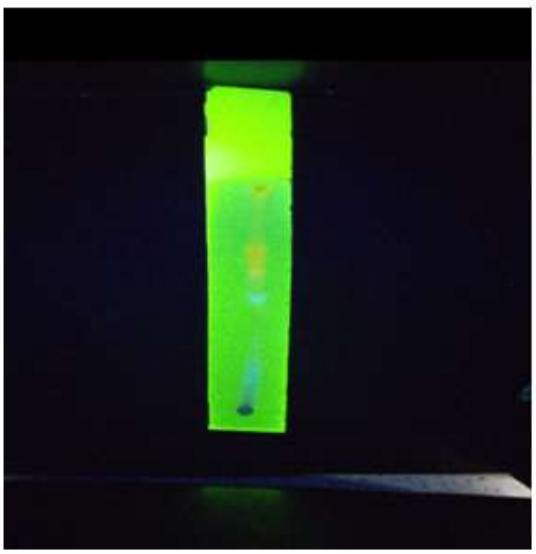

Fig. 1: TLC of methanolic stem bark extract of *K. senegalensis*

Absorption frequencies of FTIR result obtained from stem bark extracts

The FTIR spectrum was used to identify the functional group of the active components based on the peak value in the region of infrared radiation. The results of FTIR peak values and functional groups were presented in (Table 3, Fig. 2). The solvent had its respective functional group like alkanes, amines, carbonyl group and methylene (N-H) group. Hence, the methanol crude extract of *Khaya senegalensis* subjected to FTIR analysis is used for the identification of chemical constituents present in the extract. In addition, FTIR spectroscopy is proved to be a reliable and sensitive method for detection of bio molecular composition (Patil, 2006).

Table 3: FTIR analysis data interpretation of the methanolic stembark extract of *K. senegalensis*

Type of compound	Absorption Frequency (cm^{-1})	Intensity	Remark an assignment
NH group (amine)	3394.15	M	Primary amine stretching
NH group (amine)	3351.29	M	Primary amine stretching
Alkane	2932.66	M,W	C-H stretching
Alkane	2873.74	M,W	C-H stretching
Carbonyl C=O	1737.62	S	C=O stretching
NH group (amine)	1616.01	M	Primary amine stretching
N-O stretching	1519.31	S	Asymmetric stretching
Methylene group	1448.23	M	C-H bending
OH group (alcohol)	1371.98	M	OH- stretching, H bonded
C-N amine	1247.85	M	C-N stretching

S=Strong, M=Medium, W=Weak

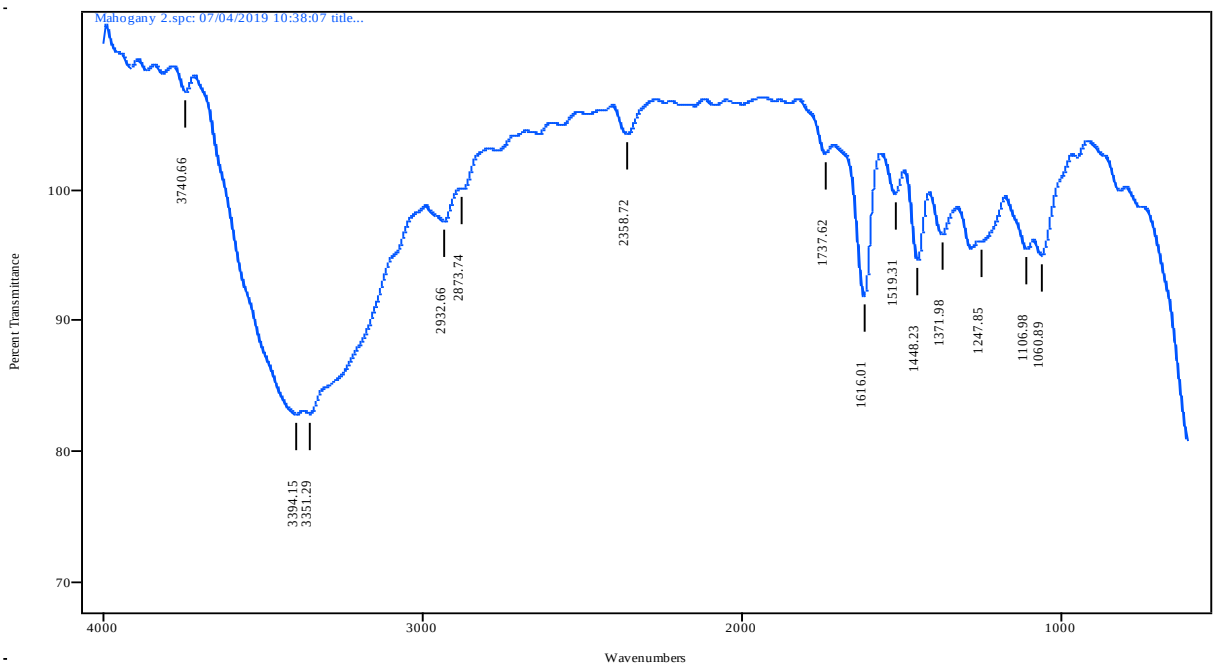

Fig. 2: FTIR spectra of methanolic stem bark extract of *K. senegalensis*

FTIR analysis of the stem bark extracts of *K. senegalensis* has absorption bands and the wave numbers (cm^{-1}) of the prominent peaks obtained were described in Table 3. The peak at a frequency of 2850, 2920 and 1750 cm^{-1} were strong while the others vary from medium to weak corresponding to C=O, C-H, NH, OH/C-N and N-O bending vibrations. The present FTIR results confirmed the presence of alkanes, alkenes, amines, carboxylic acids and alcohols in the methanol extracts of *K. senegalensis* (Tables 3) based on previous studies conducted in other plants (Muruganantham *et al.*, 2009; Meenambal *et al.*, 2012; Vijayalakshmi *et al.*, 2012; Janakiraman *et al.*, 2011).

Conclusion and Recommendation

Khaya senegalensis (African mahogany) is medicinally important and used for treatment of various ailments. Phytochemical screening showed that the leaves and stem-bark extracts were rich in phytoconstituents which validated its application traditionally as promising herbal drug material. The study of chemical composition may pave way for identification and isolation of significant bioactive compounds with pronounced antioxidant and other therapeutic activities for drug development.

Conflict of Interest

Authors declare that there is no conflict of interest reported in this work.

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