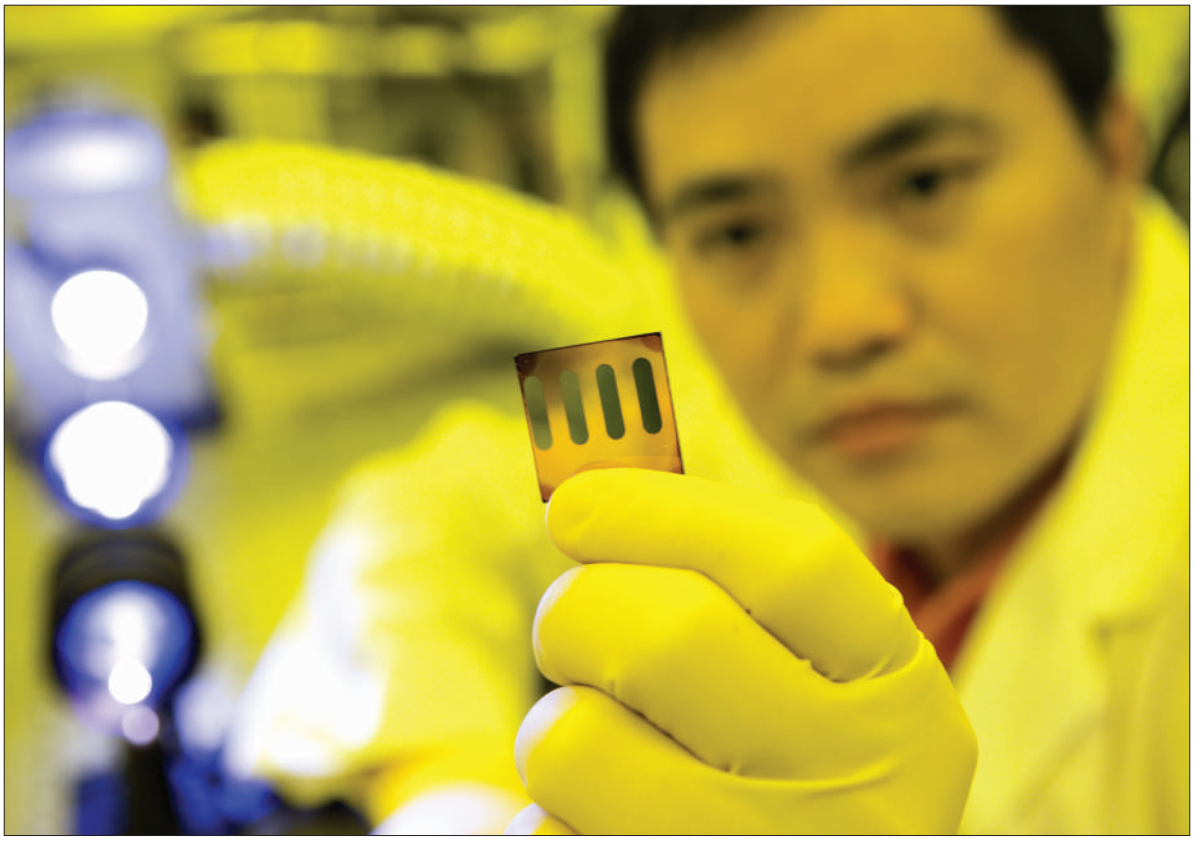

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New Constituents of *Kigelia pinnata* Leaves

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ABSTRACT

Kigelia pinnata (Lam) Benth is renowned for its traditional applications as anti-inflammatory, antioxidant, anti-microbial and anti-cancer effects. The leaves of *Kigelia pinnata* were extracted successively with hexane and ethylacetate at room temperature and the extracts fractionated on an open silica gel column using hexane, ethylacetate and methanol gradient of increasing polarity. Preparative thin layer chromatography was used for further purification and isolation of various compounds. Spectroscopic analyses from UV/VIS, IR, GC/GCMS, ^{1}HNMR, ^{13}CNMR, COSY and HETCOR techniques were used for the characterization of the compounds isolated from the leaves of *Kigelia pinnata*. The compound identification was based on spectral data comparison with literature data. Based on the data available to us, we report for the first time isolation of Hentriacontane, β-tocopherol, 3-hydro-4,8-phytene, trans-phytol, (9Z,12Z)-methyl octadeca-9, 12-dienoate and 1,3,3,5,6,6-hexamethylcyclohexa-1,4-diene from *Kigelia pinnata* leave.

Key words: *Kigelia pinnata*, hentriacontane, trans-phytol, phytene, elaidic acid

INTRODUCTION

Kigelia pinnata (Lam) Benth a species in the Bignoniaceae family commonly known as *Kigelia africana* is locally known as pandoro, uturubien and some other names based on their location in Nigeria and entire Africa (Olatunji and Atolani, 2009; Oladele *et al*., 2011). Wherever, the tree grows it is usually evergreen especially in places where rainfall occurs throughout the year, but deciduous in places of long dry season. The plant is endemic in the south, central and West Africa (Burkill, 1985; Olatunji and Atolani, 2009).

Literature survey on the plant revealed that the aqueous leaves extract of *K. pinnata* has been confirmed to possess antidiarrhoeal activity (Akah, 1996) and antileprotic potential (Lal and Yadar, 1983). The ethanolic stem bark extract showed strong analgesic and anti-inflammatory activities in a study. It was suggested that the ability of the extract components to inhibit the synthesis of prostaglandins and other inflammatory mediators was responsible for its analgesic and anti-inflammatory activities (Owolabi and Omogbai, 2007). The long-term treatment of male Sprague-Dawley rats with *Kigelia pinnata* fruit extract was observed to ameliorates the testicular toxicity after the administration cisplatin (a cytotoxic drug) (Azu *et al*., 2011). The root bark is used for the treatment of cancer of the uterus (Msouthi and Mangombo, 1983). The extract was found

effective when tested against melanoma cells (a tumour of pigmented skin cells) by inhibiting the growth of cultured melanoma cells significantly (Houghton *et al.*, 1994). Chemical analysis of the polar extract of fruit indicated the presence of an anticancer agent, verminoside (Picerno *et al.*, 2005). Recent isolation from the fruits afforded a new phenylpropanoid derivative known as 6-p-coumaroyl-sucrose together with other known phenylpropanoid derivatives and flavonoid glycosides (Gouda *et al.*, 2006). To the best of our knowledge, very scanty scientific works have been reported on the bioactives from the leaves of *Kigelia pinnata*. Therefore, the objective of the study was to identify phytochemicals that are present in the leave of *Kigelia pinnata*.

MATERIALS AND METHODS

Instruments: ^{1}H and ^{13}C NMR spectral were obtained on a Mercury 200 BB spectrometer operating at a basic frequency of 200 MHz. A Gas Chromatography-Mass Spectroscopy, GC-MS system; GCMS-QP 2010 PLUS (Shimadzu Japan) interfaced with a finigan MAT ion trap detector ion source Temp., was used with the following settings; 200°C, interfaced temp., 250°C, solvent cut time; 2.50 min; relative detector mode, ACQ mode; Scan; start time-end time; 3.00-46.00 min, event time, 0.50 sec; Scan speed, 1428. Identification of each component was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS, their spectral were compared with those of NIST library mass spectra. The infra red spectra were recorded on a Shimadzu (8400 s) Fourier Transform-Infrared Spectrocopy (FTIR) Spectrum spectrophotometer using KBr pellet; UV Spectrum was recorded using Schimadzu (1600 sec) and melting points were determined on Barhoworld Scientific world, UK, melting point apparatus. Thin layer chromatography, TLC plate were examined with a UV lamp operating at a wavelength of 366 and 254 nm.

Chemicals: Hexane, petroleum spirit, dichloromethane, ethyl acetate, methanol and ethanol used were analytical, while silica gel F_{254} and vanillin spray reagent were obtained from the chemical store of the Department of Chemistry, University of Ilorin, Nigeria.

Samples and sample preparation: The leaves of *K. pinnata* were collected from a fruiting tree in a farmland in Ado-Ekiti, Nigeria during the summer and taxonomically authenticated by a taxonomist in the herbarium of the Department of Botany, University of Lagos, Lagos, Nigeria. A voucher specimen number LUT/3525 was obtained. The leaves were dried at room temperature and blended into powder.

Extraction and isolation: The powdered plant material (240 g) was exhaustively extracted with n-hexane at room temperature for six days to afford 3.06 g of brownish green syrup after concentration. The wax and volatile were analyzed as previously reported (Atolani *et al.*, 2009). The residual plant material was further extracted with ethyl acetate to afford 22.1 g crude extract after *in vacuo* concentration. The n-hexane residue was fractionated in a silica gel open column, using n-hexane and ethyl acetate as well as ethyl acetate and methanol in increasing in an increasing varying proportion to give forty six fractions of about 10 to 15 mL each. Fractions with similar TLC profile were combined and concentrated using the rotary evaporator. Combined fractions 7 to 17 was subjected to another silica gel column chromatography to obtain waxy constituent which was re-crystallized to give hentriacontane I. Combined fraction 25 and 26

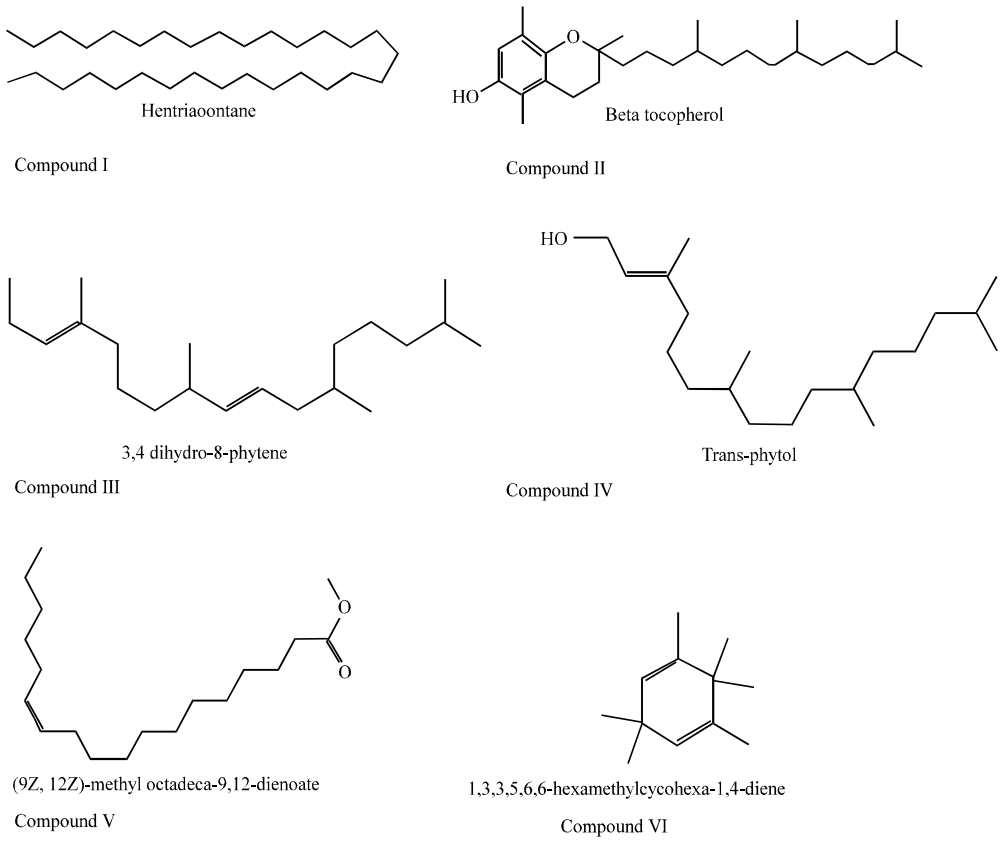

Fig. 1: Compounds isolated from *K. pinnata* leaves

Table 1: Oil profile of fraction 37-46 obtained from the GC/GCMS

Peak no.	Compounds	RT (min)	Yield (%)	A/H	Base peak	KI
1	4,4-dimethyl undecane, $C_{13}H_{28}$	3.86	27.08	22.62	85	1217
2	Methyl nonadecanoate, $C_{20}H_{40}O_2$	14.58	21.20	28.29	74	2214
3	Emery oleic acid ester , $C_{19}H_{36}O_2$	16.38	40.62	39.27	55	2085
4	Methyl n-butyrate, $C_5H_{10}O_2$	16.74	5.08	4.74	74	686
5	2,3-hexanediol , $C_6H_{14}O_2$	22.54	6.02	1.41	73	942

KI: Kovats indices, RT: Retention time, A/H: Area to height ratio

indicated the beta-tocopherol (II). The final isolation process involved a preparative TLC separation technique. Combined fraction 27 to 31 was eluted with hexane/dichloromethane/ethanol; 2:1:1 to afford 1-(decahydro-1,1,4a-trimethyl-6-methylenenaphthalen-5-yl)-3-methylpentan-3-ol (III) with R_f of 0.6. Fraction 37 to 46 yielded yellow oil which was analyzed by GCMS (Table 1). The ethyl acetate residue was also eluted in a silica gel CC as for the hexane residue. Forty three fractions were obtained and combined into eight groups after appropriate comparison of the TLC profile. Fractions 5 to 8 yielded a golden yellow oil, trans-phytol (IV) while fraction 9 afforded a bright yellow oil (9Z,12Z)-methyl octadeca-9,12-dienoate (V). The combined fractions 10 to 13 was chromatographed on a preparative silica gel TLC plates eluted with a 5:1:1 mixture of petroleum spirit/dichloromethane/ethanol. The major pot afforded 1,3,3,5,6,6-hexamethylcyclohexa-1,4-diene (VI) with R_f value; 0.5. The structures of the Compounds isolated from *K. pinnata* leaves has been presented in the Fig. 1.

Compound I: Hentriacontane $C_{31}H_{64}$, (150 mg), MW 436; White glistening plates; R_f TLC (pet. spirit): 0.5; m.pt. 68°C; IR (KBr): 2955, 2850, 1462, 1377, 721 cm^{-1}; MS m/z: 43, 57, 71, 85, 99, 196, 280, 392. The MS, IR and melting point data obtained are in agreement with literature data (Atolani *et al.*, 2009; Kovats, 1958; Khatri and Kazi, 1981; Dauri and Dianese, 2002).

Compound II: beta-tocopherol $C_{28}H_{48}O_2$ (45 mg); MW 416; IR (KBr): 3400, 3030, 2929, 2856, 1739, 1637, 1030, 675 cm^{-1}; MS m/z: 43, 55, 71, 151, 207, 281, 416. The MS data obtained is consistent with literature (Snyder *et al.*, 1993).

Compound III: 3,4-dihydro-8-phytene: $C_{21}H_{40}$, colorless oil (40 mg), MW 292; R_f 0.6 (hexane/dichloromethane/ethanol; 2:1:1) IR (KBr), 2980, 2920, 2849, 1697, 1545 (C = C), 1465, 1091, 1031, 719 cm^{-1}; MS m/z: 193, 292. ^1H NMR (deuterated acetone) ppm δ 5.15 (t, 2H, C = CH), 5.35 (t, 2H CH), 2.2-1.9 (qtm, 1H, 4H, 1H CH, CH_2, CH), 1.5-1.2 (m, 15H, CH, CH_2, CH_3), 1.2-0.8 (td, 3H, 9H, CH_3, CH_3); ^{13}C NMR (deutero acetone): ppm. δ 134.8 (C-4, C-9), 125.0 (C-3, C10), 33.3 (C-11), 32.1 (C-5, C-8, C-15), 31.7 (C-13), 30.1 (C-12), 28.9 (C-16), 28.5 (C-6), 28.1 (C-14), 27.8 (C-17), 26.9 (C-18), 26.3 (C-19), 24.7 (C-20), 23.0 (C-2), 22.4 (C-7), 22.0 (C-21), 13.5 (C-1).

Compound IV: Trans-phytol $C_{20}H_{40}$, hight yellow oil (110 mg), MW 296, IR (KBr): 3465 (OH), 2956, 1462, 1097 cm^{-1}; MS m/z: 43, 68, 95, 123, 249, 296. The MS data is in agreement with literature data (Heinrich *et al.*, 2002; Skaltsa *et al.*, 2003).

Compound V: (9Z, 12Z)-methyl octadeca-9, 12-dienoate or methyl linoleate, $C_{19}H_{34}O_2$ (120 mg) bright yellow oil, MW 294: IR (KBr): 2955, 2854, 1738, 1461, 1165 cm^{-1}; ^1H NMR (dimethysulphoxide): ppm δ 5.2 (m, 4H, CH), 3.2 (s, 1H, OH), 2.3 (t, 2H, CH_2), 2.0 (t, 2H, CH_2), 1.2-1.0 (m, 16H, CH_2), 0.8 (t, 3H, CH_3). The MS data are in agreement with literature data (Golovnya *et al.*, 1976).

Compound VI: 1,3,3,5,6,6-hexamethylcyclohexa-1,4-diene $C_{12}H_{20}$ (90 mg) white powder. R_f 0.5, (pet. Spirit/dichloromethane/Ethanol; 5:1:1) ^1H NMR (deutero chloroform): ppm, δ 5.2 (s, 2H, CH), 1.7 (s, 6H, CH_3), 1.3 (s, 12H, CH_3); ^{13}C NMR (deutero chloroform): ppm, δ 135.4 (C-1, C-5), 125.2 (C-2, C-4), 39.9 (C-6), 37.5 (C-3), 33.0 (C-11, C-12), 29.6 (C-11, C-12), 19.7 (C-9, C-10).

RESULTS AND DISCUSSION

The extraction and isolation procedure scheme followed the process shown in Fig. 2.

Analysis of oil fraction: Fractions 37-46 of the hexane extract obtained as oil was analyzed by GC/MS and the components were identified by using their retention time and comparison of the spectrum with the equipment MS library. The relative percentages of the identified compounds are listed in Table 1. From the result presented in Table 1, the entire composition of the oil fraction indicated compounds falling into different classes. Emery oleic acid (40.62%) an important free fatty acid ester was obtained as the main compound. The compound was later obtained as a white precipitate from the oil mixture. It is the only unsaturated free fatty acid with a 19:1 degree of unsaturation and also odd number of carbon. 2,3-hexanediol is the only alcohol accounting for 6% of the oil but 8.2% of the polar lipid component. 4,4-dimethylundecane (27.08%) a saturated alkane is the only non polar component. The entire oil composition can be summarised as 66.9% free fatty ester, 27% alkane hydrocarbon and 6.02% alcohol.

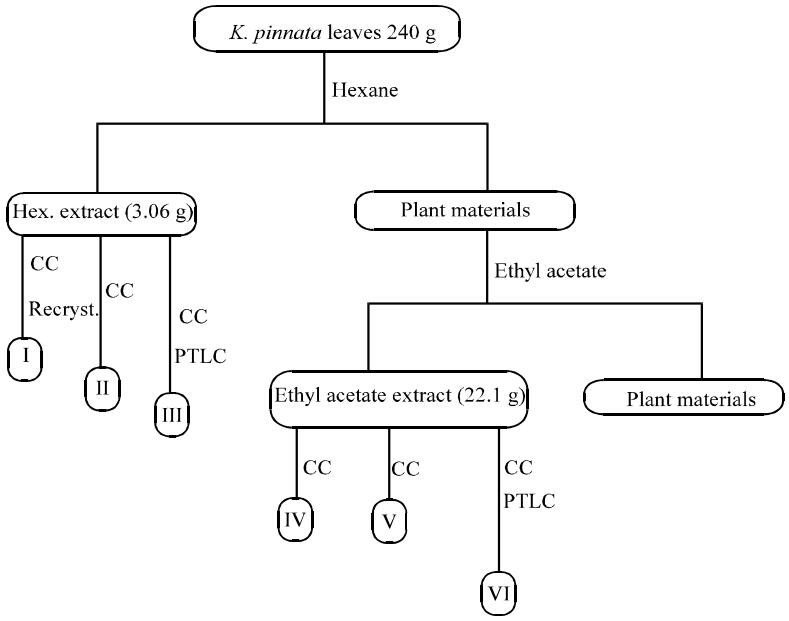

Fig. 2: Fractionation scheme for *K. pinnata* leaves extracts, CC: Column chromatography, Recryst: Recrystalisation, PTLC: Preparative thin layer chromatography

On the basis of comparison with reported data (see experimental section) the identification of compound 1, hentriacontane and compound II, **β**-tocopherol were unambiguously attained. It is noteworthy that they were isolated from this source for the first time. Compound III, 3-hydro-4,8-phytene is isolated new from plant source. Being a close relative of phytol, with relatively simple structure, its characterization was based on IR, MS, ^{1}H NMR and ^{13}C NMR. The allylic protons were clearly depicted at 5.1 and 5.35 ppm. The ^{13}C NMR indicated seventeen carbon signals with two equivalent allylic carbons each at 134.8 and 125 ppm. The spectrum was carefully compare to phytol ^{1}H NMR spectrum with the main difference in the presence of extra allylic proton shift at 5.15 ppm and the absence of the O-H proton at 3.8 ppm in compound III (Aires-de-Sousa *et al.*, 2002).

Compound IV is a well known natural product and was easily identified by comparing its physical properties, infrared spectrum and mass spectrum with data from literature. Phytol is an essential precursor to the synthesis of chlorophyll in plants (De Souza and Nes, 1969). It is predicted that compound IV may be a precursor to the biosynthesis of phytol. To the best of our knowledge, compound V is a free fatty acid ester isolated for the first time in this plant. It is a 19:2 fatty acid ester. It could serve as intermediates in the manufacture of a variety of food ingredients. Interestingly, methyl linoleate, has been used to reduce soft scald of apple (Wills *et al.*, 1980) and also as contact sex pheromones in hermaphroditic shrimp (Zhang *et al.*, 2011). Compound VI, a cylodiene was characterized by ^{1}H NMR, ^{13}C NMR and 2D NMR namely; correlated spectroscopy COSY and heteronuclear correlated spectroscopy, HETCOR. Amazingly, the three proton chemical shifts appeared as singlet each apparently due to the fact that each proton has no immediate neighbor for coupling. The ^{13}C NMR indicated seven equivalent carbon chemical environments. The COSY indicated a secondary coupling interaction between the H at 5.2 ppm and H at 1.7 ppm which corresponds to the coupling of the CH of C-4 and the CH_3 of C-10. A similar interaction is observed between C-2 and C-9. Also, there is a secondary correlation

between the CH_3 of C-7 and the CH_3 of C-9 as well as CH_3 of C-8 and C-10. HETCOR indicated a peak at 5.2 ppm attached to the carbon at 125 ppm which correspond to -CH group of C-2 and C-4 while the singlet of CH_3 (δ 1.3) at C-8 is attached to the C-6 (δ 39.9). The same procedure has been followed for C-6 and C-7 carbons.

CONCLUSION

The leave of *Kigelia pinnata,* a highly medicinal plant have shown the presence of secondary metabolites which include hentriacontane, β-tocopherol, 3-hydro-4,8-phytene, trans-phytol, (9Z,12Z)-methyl octadeca-9,12-dienoate and 1,3,3,5,6,6-hexamethylcyclohexa-1,4-diene which are probably beneficial to the plant' for defense and survival in the environment. The phytochemicals may be partly responsible for the biological activities observed for the leave of the plant when used in folkloric medicine.

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