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Epicuticular Wax and Volatiles of *Kigelia pinnata* Leaf Extract

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Abstract

The fractions of volatile compounds in plants are essential in defining the sensory impact of an aromatic smell, as well as being of some medicinal importance. Epicuticular wax in plants also determine its susceptibility to disease, and herbivore, desiccation and ultraviolet radiation damage. The epicuticular wax consisting of hydrocarbons and some volatile compounds of the leaves of *Kigelia pinnata (Kigelia africana*; family Bignoniaceae), a multimedicinal plant, was extracted, isolated and analyzed qualitatively and quantitatively for the various chemical compositions using GC (equipped with a flame thermionic detector -GC/FTD) and GC-MS ("GCMS-QP2010 Plus, which employs a dual inlet Turbo Molecular Pump (TMP) that provides increased sensitivity and column flow capacity for improved performance and confident results. The evaluation is useful for the future comprehensive analysis of the constituents responsible for the various documented medicinal uses of the leaf. The extracted constituent revealed twelve compounds with the major ones identified as n-hentriacontane (55.40%): a probable anti-tumour compound; 1-tricosene, (18.45%); 11- (2,2-dimethylpropyl) heneicosane, (9.66%); 2,6,10-trimethyldodecane, (4.43%); pentafluoroheptadecyl ester, (4.40%); 2-ethylhexyloctadecyl sulfurous acid ester, (3.05%); heneicosane, (1.61%); and hexyloctyl sulphurous acid ester, (1.42%). Other compounds are recorded in minute quantities. The major component, identified as hentriacontane, a C-31 saturated hydrocarbon apparently is responsible for the plant susceptibility to diseases, protection against UV radiation and for the antitumour and antioxidative potential of the plant. This will give credence to the traditional use of the plant as an anti-inflammatory and anticancer. The ability of the plant to act as anti-sun burn may be due to the presence of the wax.

Key words; *Kigelia pinnata, Kigelia africana*, Epicuticular wax, Bignoniaceae, volatiles, GC-MS, hentriacotane.

Introduction

The pharmaceutical, cosmetic and food industries are constantly being faced with the challenge of identification, isolation and characterization of volatile compounds of medicinal importance in plant materials. *Kigelia pinnnata* (Jacq) DC, also known as *Kigelia africana* (*Lam*) *Benth* and sausage tree on account of its

large fruits, is a member of the family Bignoniaceae. It is a multipurpose medicinal plant with great potentials to be developed as drug by pharmaceutical industries (Sangita *et al*., 2009, Olatunji and Atolani, 2009). The traditional uses of the stem, bark, root, leaves and fruit are enormous. Studies that have been reported on the leaf of this plant are rather limited. The plant's crude extract is used as antiageing and antiburn in cosmetic such as cream. The plant is rich in volatile constituents. Phytochemical studies on the stem bark revealed the presence of Kigelinone and isopinnatal (Akunyili and Houghton, 1993), specioside, verminoside and minecoside (Picerno *et al*., 2005; Neelam *et al*., 2006) and phenylpropanoid (Gauda *et al*., 2006). The root has been reported to possess isopinnatal, kigelinol and isokigelinol (Moideen *et al.,* 1999) and *p*-coumaric acid (Binutu *et al*., 1997). Flavonoids and irridoids have been isolated from its leaves (Gouda *et al*., 2003).

The anti-diarrhoea activity of the aqueous leaves extract has been confirmed (Akah, 1996). The ethanolic extract of the stem bark was examined to show strong analgesic and anti-inflammatory activities by inhibiting the synthesis of prostaglandins and other inflammatory mediators, (Owolabi and Omogbai, 2007). The stem bark extract have been reported for their cytotoxicity activities (Houghton *et al.,* 1994), Central Nervous System Stimulation (Owolabi *et al,* 2008), antifungal activity (Jain and Belsare, 2009) and anti-trypanosomal activity against *trypanosome brucel brucei* in vitro (Moideen *et al*., 1999), while the root extract have been reported for its positive anti-uterus cancer against malignant melanoma (Skin cancer) (Houghton *et al*., 1994 and Msouthi and Mangombo, 1983).

Epicuticular waxes are partially saturated lipid compounds located on the above-ground portions of land plant (Post-Beittenmiller, 1996). The chemical composition of waxes varies by species: in maize, the epicuticular wax is composed of fatty acyl chains that are later converted to alcohols, esters and aldehydes and a small proportion of alkanes (Bianchi *et al*., 1985 and 1989) Flavonoids, fatty acid esters could also be found in epicuticular waxes (Schmutz *et al.,* 1994; Whitaker *et al*., 2001). Epicuticular wax have been extracted, purified by column and thin layer chromatography (Kolattukudy, 1965 and 1966) and reported to determine plant susceptibility to diseases, herbivore, desiccation (Juliana and Silvana, 2006) and ultraviolent radiation protectant (Lacy *et al.,* 2003)

To the best of our knowledge, very little has been reported on the phytochemistry and medicinal use of the leaves of *K. pinnata*, despite its many traditional applications. Hence it is important to demystify the compositions of the components of the leave. This constitutes the main objective of this study.

Materials and Method

All the solvents used were obtained from the Department of Chemistry, University of Ilorin, Ilorin, Nigeria and were pre-distilled before use. Leaves of *Kigelia pinnata* were collected from a fruiting tree in Ado Ekiti, Ekiti

State, Nigeria during the summer time. Prof. Oladele in the Department of Plant Biology, Faculty of Science of the University of Ilorin, Ilorin Nigeria, carried out taxonomic authentication of the plant.

Extraction

Kigelia pinnata leaves were dried at room temperature. The air-dried plant material was blended into powder at a low temperature. 240g of the powdered plant material was extracted exhaustively with cold hexane for six days. The extract was combined, concentrated in vacuum and 3.06g of brownish-green syrup was obtained.

Isolation of volatiles

The syrup was applied to a column chromatography (CC) packed with Silica gel and eluted with hexane. Eleven fractions were collected, examined (talc) and combined as appropriate. The major combined fraction was subjected to a silica gel flash chromatography. Six yellow fractions with same R_f (silica gel) were obtained, combined and evaporated to dryness. This was coded KPLH/CC/FC/0.5. The chromatoplate was sprayed with vanillin spray reagent for detection of other spots.

Separation of Component

The volatile fraction (KPLH/CC/FC/0.5) was analysed by gas chromatography (GC) using a GC 2010 gas chromatography (Shimadzu, Japan) equipped with a flame thermionic detector -GC/FTD and an electronic high pressure control injector. The flow of the carrier gas (He at 100.2 kpa) 1.61mL/min; linear velocity: 46.3cm/sec; total flow: 6.2mL/min; purge flow: 3.0mL/min. The split ratio is 1:0. The analysis was performed using the following program; oven kept isothermally at 60^{o}C for 6 min, increased from 60^{o}C to 220c at a rate of 25^{o}C/min and kept isothermally at 220^{o}c during 5min. It was increased to 280^{o}C at a rate of 5^{o}C/min for 20min; Equilibrium time, 3min. Total ion chromatogram (TIC), 1.0

GC-MS Analysis

Gas Chromatography-Mass Spectroscopy, GC-MS System; GCMS - QP2010 PLUS (SHIMADZU JAPAN) interfaced with a finigan MAT ion trap detector ion source Temp., 200^{o}C; interface Temp, 250^{o}C; solvent cut time, 2.50min; relative detector gain mode, ACQ mode; Scan: start time - end time; 3.00min - 46.00min; event time, 0.50 sec; Scan Speed, 1428. Identification of the volatile components was carried out using the peak enrichment technique of reference compounds and as final confirmation of the peak identification by GC-MS, comparing their spectra with those of the NIST commercial library mass spectra.

FT-IR analysis

The infrared spectrum of the volatile was recorded on a Shimadzu (8400S) Fourier Transform-Infrared Spectroscopy (FTIR) Spectrum Spectrophotometer using KBr pellet.

Results and Discussion

A total of twelve compounds were detected in the fraction (KPLH/CC/FC/0.5) of *Kigelia pinnata* examined. The compounds identified, the retention times, peak areas, percentage yields and base peaks are shown in Table 1. The IR spectrum of the isolated epicuticular wax showed υ_{max} (cm^{-1}, KBr) 3479 – 3282, (OH); 2955 – 2850, (CH_2, CH_3); 1650, (C=O); 1462 and 1377, (CH_2, CH_3). About 91% of the total isolate is hydrocarbon.

Hentriacontane (55%), a saturated hydrocarbon, the major compound identified has been isolated from *Scabiosa comosa* (Dargaeva and Brutko, 1976). It is reported to be responsible for its uptake in the soil by plant (Éric, 1995) and shown to be involved with stimulation of fungal spore germination (Dauri and José, 1995). Hentriacontane has also been isolated from spinach leaves, and discovered to be unsaponifiable (Edith and Ida 1929) and shown to have possible anti-tumour activity (Takahashi *et al*., 1995). Methyl 12- Methyl tetradecanoate, a lipid of biological significant was also identified in a microscale. It has been identified in trace amount in *Cryobacterium psychrophilum* (Ken-Ichiro *et al*. 1997), *Xylella fastidiosa* plant (Ana Valéria *et al*., 2007), and *Corynebacterium sepedonicum* plant (Paul *et al*., 1988). It was reported for its inhibition capacity on the development of corneal angiogenesis, which is responsible for blindness and other infections (Cole *et al*., 2007). The three main non-hydrocarbons obtained are 2-ethylhexyl-octadecyl ester (3.05%), heptadecyl ester (pentafluoropropionic) (4.40%) and hexyloctyl ester (1.42%). Tetradecanoic acid (12-methyltetradecanoate) was obtained in a low quantity (0.29%). Hexadecanoic acid has earlier been reported as a component in alcohol extract of the leave of *Kigelia pinnata* (Grace *et al*., 2002). The components reported in this work have not been reported in literature before.

In conclusion, twelve volatiles were identified and characterised by the GC/GCMS. The major component, identified was hentriacontane, a C-31 saturated hydrocarbon which might be apparently responsible for the plant susceptibility to diseases, herbivore, desiccation, protection against UV radiation and for the antitumour and antioxidative potentials of the plant. This will give credence to the traditional use of the plant as an anti-inflammatory and anticancer. The ability of the plant to act as anti-sun burn when used in cream may be partly due to the presence of the wax. We hereby report that the major paraffin in the epicuticular wax of *Kigelia*

pinnata leave is *n*-hentriacontane, an *n*-C_{31} hydrocarbon (Kolattukudy, 1965 and 1966).

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Table 1: GC/GCMS data of the components obtained from the isolate.

Peak #	Compounds	Retention Time (min)	% Yield	Peak Area	Base Peak	KRI
1	4,4 – Dimethylundecane ($C_{13}H_{28}$)	03.853	0.20	83342	85	1229
2	Methyl 12-Methyl tetradecanoate ($C_{16}H_{32}O_2$)	14.582	0.29	124810	74	1715
3	1-Iododecane ($C_{10}H_{21}I$)	23.054	0.64	269635	71	1430
4	1-Iodohexadecane ($C_{16}H_{33}I$)	24.647	0.45	190717	85	2026
5	Heneicosane ($C_{21}H_{44}$)	26.184	1.61	683477	71	2109
6	Hexyl octyl-sulfurous ester ($C_{14}H_{30}O_3S$)	27.653	1.42	602261	71	2036

7	11- (2,2-dimethylpropane) heneicosane ($C_{26}H_{54}$)	29.099	9.66	4093298	57	2457
8	Pentafluoroheptadecyl ester ($C_{20}H_{35}F_5O_2$)	30.406	4.40	1865691	97	1872
9	Hentriacontane ($C_{31}H_{64}$)	32.309	55.40	23485610	57	3103
10	2,6,10-Trimethyldodecane ($C_{15}H_{32}$)	34.130	4.43	1878570	57	1320
11	1-Tricosene ($C_{23}H_{46}$)	36.571	18.45	7822828	57	2298
12	2-ethylhexyloctadecyl-sulfurousester ($C_{26}H_{54}O_3S$)	42.760	3.05	1293600	71	3165

KRI: Kovats Retention index