Isolation and Characterization of Secondary Metabolite from the Stem Bark Extract of *Allophylus africanus* Beauv (*Sapindaceae*)

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Received 23 January 2021; accepted 13 March 2021, published online 8 April 2021

Abstract

Isolation of secondary metabolites from the stem bark of *Allophylus africanus* Beauv (*Sapindaceae*) was carried out. The plant has a wide distribution in West Africa, with Nigeria (Mambila Plateau, Calabar) inclusive. It has been used in communities for both its medicinal and economic importance. The stem bark of *A. africanus* was extracted by percolation using chloroform as solvent and concentrated by evaporation at room temperature. Single spot with an R_f value of 0.87 was obtained at a solvent mixture of hexane: chloroform at ratio 2:8 from finger column chromatography. Characterization using spectra analysis which include FT-IR, ¹H-NMR, ¹³C-NMR, H-H COSY, H-C COSY, 135 DEPT and mass spectroscopy. Spectroscopic studies of *A. africanus* stem bark extract led to the elucidation of the structure of the compound. FT-IR spectrum indicates that the compound is an aromatic derivative. Aromatic protons were seen at the ¹H-NMR chemical shift values of 7.25 ppm. Olefinic protons at 5.124, 5.146 and 6.162 ppm appeared as doublets of doublets of doublets. The compound 1-((E)-7(((1E,5E)-6-((2,3-dimethyl-4-((E)-3,4,5,6-tetramethyldeca-7,9-dien-2-yl)oxy)pentyl)oxy)-3,4-dimethylhexa-1,5-dien-1-yl)oxy)hept-4-en-1-yl)-4-propylbenzene was isolated.

Keywords: *Allophylus africanus*, spectroscopy, isolation, characterization, aromatic, chromatography

INTRODUCTION

Allophylus africanus belongs to the *Sapindaceae* family, one of the most important forest species conserved and valued in Africa due to their multiple uses, high nutritional content, and medicinal values. It is commonly known as African false currant, akaito (in Igbo language, Nigeria), ebe/ukpe (esan tribe in Edo state, Nigeria), akanro, akaraesu (in Yoruba, language, Nigeria) and karki (in Hausa, language, Nigeria). It is a shrub with flowers that are small and creamy-yellow [1]. The fruit is fleshy and also red to black in colour when ripe [1]. *A. africanus* mostly grows in riverine areas, open wood land and forest edges, often associated with mounds, at an altitude of 960-1540 m. Its flowering time is usually December to March. It is distributed widely throughout tropical Africa, extending to the Eastern Cape South Africa [1]. *A. africanus* has been reported to be used as not just for medicinal purposes but, also as food and horticulture [1]. In ethno medicine, the leaves are used for the treatment of several ailments such as arthritis, rheumatism, gout, haemorrhoids, dysentery, venereal diseases and malnutrition [1], root and twig are used as chewing stick for dental and oral healthcare and

diarrhoea treatment. *A. africanus* is reported to demonstrate biological activities such as antibacterial, anti-oxidant [2] and anti-malarial activity [3]. Some chemical components reported from *A. africanus* leaves are tannins, saponins, flavonoids and carbohydrates [3]. *A. africanus* is a shrub or small tree with trifoliate leaves, obovate leaflets, variously hairy, margin dentate, serrate or almost entire. Inflorescences axillary, often branched, spike-like. Flowers are creamy-yellow and small [1]. The fruit is near spherical, fleshy, red black when ripe [1].

Figure 1: *Allophylus africanus* stem bark and leaves

The oil of seeds of *Allophylus natalensis* and *Allophylus dregeanus* were reported to both contain type I cyano-lipids, 1-cyano-2-hydroxymethyl prop-2-en-1-ol diesters and also small amounts of type III cyanolipids, 1-cyano-2-hydroxymethyl prop-1-en-3-ol diesters [4].

Allophylus serratus is reported to contain plant constituents like apigenin -4-O- β -D-glucoside, pinitol, quercetin, luteolin-7-O- β -D-glucopyranoside, rutin [5], phenacetamide and β -sitosterol [6]. L-Quebrachitol was reported isolated from *A. edulis* [7].

Reported in preliminary chemical characterization of the aqueous extract of *A. cominia* leaves are protein concentration, fatty acids and carbohydrates [8]. Reported Gas Column-Mass Spectrophotometry (GC-MS) analysis of *A. Serratus* Kurz showed the plant to be rich in phenolics, flavonoids, tanning substances, saponins. Various fatty acids were also identified [9]. Predominance of tannins, saponins, flavonoids and carbohydrates in leaves and other parts of *A. africanus* have been reported [3]. It has been reported that 32 compounds representing 88% of the essential oil from leaves of *A. africanus* were characterized and 35 compounds from oil of flowers of *A. africanus* were identified [10]. In addition, isolation and identification of four new compounds, allotaraxerolide, alloeudesmenol, hanocokinoside and alloaminoacetaldehyde together with 2 compounds known, pinitol and stigmastane-3 β , 4 β -diol were reported in the whole plant of *A. africanus* [11].

Reported isolated compounds from whole plant of *A. africanus* are allotaraxerolide, hanocokinoside, alloeudesmenol, alloaminoacetaldehyde, stigmastane-3 β , 4 β -diol and pinitol. The essential oil from leaves of the plant was reported to be dominated by 2- (1, 1dimethylethoxy)-ethanol, caryophyllene oxide, trimethylbenzene, 1-Ethyl- 21 1,4dimethylbenzene and hexahydrofarnesyl acetone [10]. Phytochemical studies reportedly revealed a predominance of carbohydrates, flavonoids, saponins and tannins [3].

Traditionally, the boiled bark, root and leaves are used in aches, fever and rheumatic pains and economically, the wood is a good source of building materials, and as insecticide. The ethanol extract of *Allophylus serratus* Kurz has been assayed for antiviral activity and was found to show antiviral activity against Ranikhet disease virus. It also showed gross effect on hypothermia and the central nervous system [9]. Antimalarial activity of *A. africanus* extract has been reported following suppression of

parasitaemia in infected mice by 92.82% - 97.81% on day 7 post infection against 96.81% for chloroquine [3].

Allophylus serratus has strong ethnobotanical and ethanopharmacological background. The plant has a distinction of being used in Indian system of medicine (Ayurveda) as an anti-inflammatory, as carminative drug, treatment of elephantiasis, oedema, fractured bones, in bone dislocations, wound, ulcer, several gastrointestinal disorders such as dyspepsia, anorexia and diarrhea [12]. The fruits are sweet, edible and have cooling and nourishing tonic. The flesh of the berry is eaten raw to expel tapeworm. The leaves are crushed into juice and used to reduce fever, to relieve rashes, promote lactation, to treat colic [13, 14]. Grounded leaves with quicklime are applied with heat to relieve stomach aches. Leaves and barks are used as a remedy for elephantiasis; antiulcer and reduce piles [13, 14]. Leaf paste with turmeric is applied for fracture and sprains [15]. The roots of this plant contain tannin and are considered astringent; they are used for treating piles and nose bleeding and to check diarrhea and rheumatic pains [13].

Allophylus serratus contains different bioactive substances which are very important as medicines and other activities. It is rich in phenolic compounds, flavonoids, tanning substances, Steroids, Alkaloids and Saponins [9]. Screened Pharmacognosy and phytochemistry of *A. serratus* (Kurz) have been reported [9]. The presence of various chemicals in different parts was observed [9]. The plant leaves contain β - sitosterol and phenacetamide, Ph-acetamide, alkaloids and Benzylamide. Fruits contain three cyanogenic glycosides (3-O-~-D-glucopyranosyloxy-4- methyl-2 (5H)-furanone, 4-O-D-glucopyranosyloxy-3-hydroxymethyl butyronitrile-2-ene and cardiospermin [17]. There are also alkaloids and various fatty acids in leaves and β -sitosterol in stems [9, 17]. There are reports which indicate that this plant also contains different compounds such as quercetin, pinitol, luteolin-7-O-d-glucopyranoside, rutin and apigenin- 4- O-D-glucoside [18]. Literature reports on isolation and characterization of bioactive compounds from the stem bark of *A. Africanus* is scanty. We hereby present isolation

and characterization of secondary metabolite from chloroformed stem bark extract of *A. africanus*

MATERIALS AND METHODS

Chemicals

All the solvents and reagents used in this work were of analytical grade and were obtained from Merck Chemical Company. Infrared spectrum (KBr pellets) was recorded on a Perkin-Elmer 1605 FT-IR spectrophotometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker AC-400 (400 and 100 MHz) and AC – 500 (500 and 125 MHz) spectrometer using CDCl_3 as solvent and TMS as the internal standard. Mass spectrum was recorded on Shimadzu QP 2010 operating at 70 eV through a direct probe. Columns chromatography was carried out with silica gels (Aldrich 0.05 – 0.20 mm). Silica gel F254G Vetec was used in preparing the TLC plates.

Plant material

The Stem Bark of *A. Africanus* was gotten from Federal Institute of Environment, Forestry Research Institute of Nigeria, in Ahiaeke, Umuahia, Abia State. It was identified by aTaxonomist at National Forestry Institute, Umuahia Nigeria. The stem bark of *A. africanus* was cleaned up and air dried at room temperature for a period of 2 weeks. After this, it was further grounded to powder.

Extraction and isolation of the compound

Reported methods of [19,20] were used with slight modification. Nine hundred (900) g of the mashed stem bark were percolated with 3 litres of chloroform at room temperature for 48 hours (2 days), it was then filtered with Whatman filter paper No 1. The percolated stem bark was re-percolated again with chloroform. The filtrate of the stem bark was concentrated by allowing it to stand at room temperature for 10 days while being covered with a perforated Whatman filter paper. It was allowed to evaporate to dryness till a sticky extract of a constant weight of 3g was obtained. Column chromatography and Thin-Layer Chromatography was carried out on the chloroformed crude extract in order to isolate,

purify and characterise as well as identify its active constituents.

Two grams of the chloroform extract, was dissolved in 20 ml chloroform. Sixty grams of silica gel were added to the mixture and stirred properly until slurry was formed. The slurry was continuously stirred until a dry homogeneous mixture was formed. The slurry was loaded on column and column chromatography was performed using standard procedure where solvents of different polarities like petroleum ether, chloroform and methanol were employed. The process of column chromatography lasted for 3 days, 25 fractions were collected and labelled. The flow rate was monitored.

In thin layer chromatography coated glass slide made of mixing silica gel in distilled water in the ration of 1:3 were used. The surface of the plate (5.0 cm: 20 cm) was wiped with clean cotton wool. The glass plate was coated carefully by smearing the surface with the slurry to form a thin layer plate. This was allowed to stand at room temperature for 3 hours before being activated for 1 hour in the oven for 100 °C. The coated glass plates were now set for spotting. The process of spotting, developing and visualizing of the TLC was done using standard procedure. The retention factor (Rf) of each of the spots was determined using equation 1.

$$R_f = \frac{\text{Dist. moved by spot}}{\text{Dist. moved by solvent}} \text{ (Eqn 1)}$$

This procedure involved using the coated plates as a stationary phase and the solvents of varying polarity as the mobile phase. For spotting, capillary tubes were used and TLC tanks for development. In cases where the visibility of the spot was not clear UV lamps and iodine tank was used to get a visible movement of the spot.

After rigorous TLC attempts no single spot was found, fractions that showed similar and positive spots were pooled together and finger column chromatography was carried out on them. Rf values of the fraction that gave a single spot were calculated. During finger chromatography, solvents of varying polarities like hexane, ethyl acetate, chloroform and methanol were used.

RESULTS AND DISCUSSION

A single spot was obtained with R_f of 0.87 in a solvent mixture of hexane: chloroform, 2:8. The FT-IR spectrum of the isolated chloroformed stem bark extract of *A. africanus* is shown in Figure 2.

Figure 2: FT-IR spectrum of the isolated chloroformed stem bark extract of *A. africanus*

The IR spectrum shows vibrational frequencies at 2927.9 cm^{-1} and 2852.8 cm^{-1} . These vibration frequencies were assigned to aliphatic C-H stretch [21]. The peak at 1162.2 cm^{-1} is a typical peak for C-O stretch. The aromatic C-C stretch was observed at 1463 cm^{-1} . The IR spectrum

indicates that the compound is an aromatic derivative [22].

The Proton NMR of the isolated chloroformed stem bark extract of *A. africanus* is shown in Figure 3

Figure 3: Proton NMR of the isolated chloroformed stem bark extract of *A. africanus*

The chemical shift value at 7.25 ppm is a typical shift of aromatic protons [22]. This is in agreement with the infrared spectrum. The chemical shift values at 5.124, 5.146 and 5.162 ppm are the resonances for olefinic protons. They appeared as doublets of doublets of doublet [22]. Multiplets signals at 4.137, 4.167

and 4.314 ppm corresponded to methyl proton attached to oxygen [22].

The H-H COSY of the isolated chloroformed stem bark extract of *A. africanus* is shown in Figure 4.

Figure 4: Proton-Proton COSY of the isolated chloroformed stem bark extract of *A. africanus*

The signal at 5.35 ppm correlated with the signals at 2.786 and 2.008 ppm. The connectivity between these resonances suggested the presence of $-\text{C}=\text{C}-\text{CH}_2-\text{CH}_2-$ moiety. The signal at 5.162 ppm correlated with the signal at 4.219 and 4.274 ppm. This connectivity between these resonances suggested the presence of $-\text{C}=\text{C}-\text{O}-\text{CH}_2-\text{OCH}_2-$ moiety. The triplet signal at 2.75 ppm correlated with the triplet signal at 2.05 ppm. The connectivity between the resonances suggested the presence of $-\text{CH}_2-\text{CH}_2-$ moiety. The triplet signal at 2.310 ppm correlated with the signal at 1.71 ppm. The connectivity between these

resonances suggested the presence of $-\text{CH}_2-\text{CH}_2-$ moiety. Also, the triplets signal at 2.073 ppm correlated with the signal at 1.3 ppm. This connectivity suggested a $-\text{CH}_2-\text{CH}-$ moiety. The singlet at 1.689 ppm correlated with the doublet signal at 1.3 ppm. This connectivity suggested $-\text{CH}-\text{OH}$ moiety. The doublet signal at 1.3 ppm correlated with the triplets at 0.895 ppm. This correlation suggested the presence $-\text{CH}_2-\text{CH}-$.

The C-H COSY of the isolated chloroformed stem bark extract of *A. Africanus* is shown in Figure 5

Figure 5: Proton-Carbon COSY NMR of the isolated chloroformed stem bark extract of *A. africanus*

The Proton signal at 5.35 ppm correlated with the carbon chemical shifts at 130, 128 and 122 ppm. This shows that the proton is attached to a carbon that bears a double bond [22]. This is in agreement with the suggested moiety in $[-\text{C}=\text{C}-]$ in H-H COSY. The proton signal at 5.126 ppm correlated with the carbon signal at 69 ppm. The connectivity suggested that the proton is attached to $-\text{C}-\text{O}$. This is also in agreement with the suggested moiety in the proton-proton

COSY. The multiplets signal in the proton at 4.274 ppm correlates with the carbon signal at 62 ppm. This correlation indicated the presence of $-\text{CH}_2-\text{O}$ moiety. This is in agreement with the suggested moiety in the H-H COSY. The proton multiplets signal at 4.219 ppm correlated with the signal at 62 ppm. This also was in agreement with the suggested moiety in the proton-proton COSY. The multiplets in the ^1H -NMR at 2.75 ppm correlated with ^{13}C -NMR signal at 27.190 ppm. This connectivity between the resonances

suggested aliphatic functionality, it has the presence of alkanes. This is also in agreement with the proton-proton COSY and FT-IR. The chemical shift values between 2.5 ppm to 0.5 ppm in the proton NMR are all aliphatic ($\text{CH}_3\text{CH}_2\text{CH}$ protons) since they correlate with ^{13}C NMR chemical shift from 10 to 40 ppm.

The Carbon 13 DEPT is shown in Figures 6 and 7.

Figure 6: ^{13}C -DEPT (135) NMR for the isolated chloroformed stem bark extract of *A. africanus*

Figure 7: Expanded ^{13}C -DEPT (135) NMR for the isolated chloroformed stem bark extract of *A. africanus*
chemical shifts 121.703, 127.882, 128.062, 129.265, 130.007, 130.221 and 138.311 ppm are all CH_2 signals.

The CH_3 and CH signals appear at the positive signals and the CH_2 appeared at the negative signals. From Figure 6 the chemical shifts at 19.389, 21.083 and 31.921 ppm are all CH_2 signals. The remaining chemical shifts are all CH_3 or CH signals. Figure 7, which is the expanded ^{13}C NMR (135) DEPT shows the

Figure 8: Mass Spectrum for the isolated chloroformed stem bark extract of *A. africanus*

The mass spectrum for the isolated chloroformed stem bark extract of *A. africanus* is shown in Figure 8. Mass spectroscopy is one of the spectroscopic tools used in the characterization of natural products [23-28]. The isolated compound showed a molecular ion peak at m/z 663. The peak at m/z 663 is the molecular ion. This represents the molecular weight of the isolated compound. The molecular formula of the isolated proposed structure is $[C_{45}H_{74}O_3]^+$. This is a radical cation. The base peak – the peak with the highest intensity- appeared at m/z 413. This has a molecular formula $[C_{28}H_{45}O_2]^+$. Fragmentation patterns of mass spectrum analysis are shown in Figure 9.1 and Figure 9.2. The fragments of m/z 536, corresponds with the

molecular formula $[C_{36}H_{56}O_3]^+$. The fragments at m/z 533 corresponded with molecular formula $[C_{36}H_{53}O_3]^+$, this shows the loss of 3 H atoms from the molecular ion. The fragments at m/z 507 corresponded with the molecular formula $[C_{34}H_{51}O_3]^+$. This has a total loss of 2 C atoms and 2 H atoms. The fragments at m/z 458 has a molecular formula $[C_{30}H_{50}O_3]^+$. The other selected fragment with m/z 457 has a molecular formula $[C_{30}H_{49}O_3]^+$ with a total loss of $C_{15}H_{25}$ from the molecular ion. The fragment at m/z 441 has a molecular formula $[C_{30}H_{48}O_2]^+$ with a total loss of $C_{15}H_{26}O$ from the molecular ion. The fragments at m/z 429 has a molecular formula $C_{29}H_{45}O_2$ with a total loss of $C_{16}H_{29}O$ from the molecular ion.

Figure 9.1: Suggested fragmentation pattern of the mass spectrum of the isolated chloroform stem bark extract of *A. africanus*

Mass spectra fragmentation pattern

Figure 9.2: Continuation of suggested fragmentation patterns of the mass spectrum of the isolated chloroformed stem bark extract of *A. africanus*

Based on the FT-IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$, H-H COSY, H-C COSY, $^{135}\text{DEPT}$ and mass spectra,

the structure in Figure 10 have been proposed as the isolated chloroformed stem bark extract of *A. africanus*

Figure 10: Proposed structure for the isolated chloroformed stem bark extract of *A. africanus*
 1-((*E*)-7-(((1*E*,5*E*)-6-((2,3-dimethyl-4-(((*E*)-3,4,5,6-tetramethyldeca-7,9-dien-2-yl)oxy)pentyl)oxy)-3,4-dimethylhexa-1,5-dien-1-yl)oxy)hept-4-en-1-yl)-4-propylbenzene

CONCLUSION

1-((*E*)-7-(((1*E*,5*E*)-6-((2,3-dimethyl-4-(((*E*)-3,4,5,6-tetramethyldeca-7,9-dien-2-yl)oxy)pentyl)oxy)-3,4-dimethylhexa-1,5-dien-1-yl)oxy)hept-4-en-1-yl)-4-propylbenzene is one the isolated compound from the chloroformed stem bark extract of *A. africanus*. This is the first time this compound is being isolated. The compound is aromatic and may contribute to the anti-inflammatory activities.

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