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Isolation and characterization of bioactive phytochemicals from chloroform extract of *Adenodolichos paniculatus* (hua) Hutch. & Dalz (Fabaceae)

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ABSTRACT: Phytochemicals are the chemicals extracted from plants which make them significant source of drugs with potential for thousands of years. *Adenodolichos paniculatus* have been observed to have some medicinal applications. The aim of this work was to isolate and characterize some bioactive phytochemicals from the chloroform root extract of *A. paniculatus* which may justify its use by traditional healers. *A. paniculatus* roots were collected, identified, dried and pulverized. The pulverized plant material was subjected to serial exhaustive extraction using n-hexane, chloroform, ethyl acetate, methanol and water. Vacuum liquid chromatography of the chloroform root extract led to a number of fractions. TLC analysis was used to study the different fractions. This isolation and purification afforded a dark orange liquid which was subjected to physical spectroscopic identification using IR and GC-MS. The major phytochemicals identified were 9,12-octadecadienoic acid (linoleic acid), undecanoic acid, 10-bromo-(10-bromoudecanoic acid), octadecanoic acid (stearic acid) and n-hexadecanoic acid (palmitic acid). These findings support the traditional use of *Adenodolichos paniculatus* in various diseases treatments specifically sore throat infections.

Keywords: *Adenodolichos paniculatus*, characterization, isolation, phytochemicals, roots.

INTRODUCTION

Phytochemicals are naturally occurring compounds present in varying levels in plants and help significantly in protecting them against pathogens. Due to some observed medicinal applications of *Adenodolichos paniculatus* plant parts, some researches have been carried out to isolate and characterize useful components of this plants.

The plant, *Adenodolichos paniculatus* (Leguminocaea, Fabaceae) is a shrub of 4 to 5 m high found in the savanna, bush and jungle, from Guinea to Northern Nigeria, and across to Sudan. The Hausa name, *wáákén wuta*, means 'fire bean'. This is perhaps because the plant springs up freely after bush burning operation (Burkill,1985a). Different parts of the plant are used for different purposes. For example, the leaves are mostly used as food (for edible caterpillar) and dressing for burns, for toothache, and for heart burn. The root is mostly used to treat liver problem, throat infections, dysentery and also used as a pain-killer, while the stem is used in the treatment of diarrhea and blennorrhoea (Burkill 1985b; Hutchinson and Dalziel, 1958). Sani et al. (2010) reported that the methanolic leaf extract of *A. paniculatus* exhibited significant and dose dependent analgesic and anti-inflammatory effects that were comparable to that of a standard analgesic and anti-inflammatory drug, ketoprofen. They

linked the activity of the leaf extract of the plant to their phytochemical constituents which included flavonoids, tannins, glycosides, anthraquinones and phenols.

Because of some observed medicinal applications of extracts of the plant parts, some researches have been carried out to isolated and characterize useful components of the plant. In his research, four phytochemicals namely 9,12-octadecadienoic acid (linoleic acid), undecanoic acid, 10-bromo-(10-bromoudecanoic acid), octadecanoic acid (stearic acid) and n-hexadecanoic acid (palmitic acid) were isolated from the chloroform root extract. Although, Isyaku (2018) reported that stigmasterol and β-sitosterol were isolated from ethyl acetate leaf extract of *A. paniculatus*. Literature is scanty on isolation and characterization of phytochemicals from chloroform root extract of the plant. Doan et al. (2019) reported the isolation of 9,12-octadecadienal (linoleic acid), octadecanoic acid (stearic acid) and n-hexadecanoic acid from the seed of five varieties Fabaceae species while studying on extraction process, identification of fatty acids, tocopherols, sterols and phenolic constituents and antioxidant evaluation of seed from five varieties of Fabaceae species. Aadesariya et al. (2017) reported the isolation of various fatty acids, 9,12-octadecadienal (linoleic acid), n-hexadecanoic and octadecanoic acid (stearic acid) from hexane extract of *Abutilon pannosum* and *Grewia tenax* while studying on Soxhtherm extraction, isolation and identification of fatty acids present in the hexane extract of *Abutilon pannosum* and *Grewia tenax* using Gas chromatography-Mass spectrometry.

9,12-octadecadienoic acid is an eighteen-carbon fatty acid (also called linoleic acid). It has the following properties: Boiling point (365.4ºC at 760 mmHg), density (0.852 g/cm^3) and flask point (185.8ºC). Undecanoic acid, 10-bromo (10-Bromoundecanoic acid) is a fatty acid with eleven carbon chain. Octadecanoic acid (stearic acid) is a saturated fatty acid with an 18-carbon chain. It has the following properties: Melting point (69.3°C), boiling point (361°C) and density (941 kg/m^3). n-hexadecanoic acid (palmitic acid) is a saturated long-chain fatty acid with a 16-carbon backbone. It has the following properties: Melting point (62.9°C), boiling point (351°C and density (853 kg/m^3).

The antibacterial activity of component B isolated fractions could be majorly attributed to 9,12-octadecadienoic acid (linoleic), octadecanoic and n-hexadecanoic acids identified. The aim of this work was to isolate and characterize some bioactive phytochemicals from the chloroform root extract of the plant which may justify its use by traditional healers. This aim was achieved through the following objectives: Collection and identi-fication of the plant sample, extraction of the pulverized plant using n-Hexane, chloroform, ethyl acetate, methanol and water. Separation, purification and isolation of the bioactive constituents using chromatographic techniques. Characterization and structural elucidation of the isolated compound(s) using spectral techniques (Gas-chromatography-Mass spectrometry (a combination of separation (GC) and identification (MS) techniques) for both quantitative and qualitative analysis and Infrared.

Four compounds were found to dominate the extract accounting for 40.98% of 9,12-octadecadienoic acid, 9.26% of undecanoic acid, 9.26% of 10-bromo-octadecanoic acid and 9.20% of n-hexadecanoic acid.

MATERIALS AND METHODS

Materials

The plant roots were harvested from the wild plant through consulting with the herbalist between the months of October 2018 and March 2019 as his experience was used to find out the right place of the plant within Pushit district of Mangu LGA of Plateau state. The plant identified as *Adenodolichos paniculatus* on voucher number FHJ 205 and deposited at the Herbarium Unit of Federal College of Forestry, Jos. The roots were air dried, powdered, and stored in air tight containers for laboratory analyses.

The serial exhaustive extraction method described by Banu and Catherine (2015) was employed to extract the constituents. One kilogram (1 kg) of powered sample was extracted in flat bottom flask with 2.5 liters hexane by maceration for 24 hours with intermittent shaking with an Orbital flask shaker. The sample mixture was filtered 2 times with muslin cloth, then with vacuum pump filtration and the filtrate collected. The filtrate was concentrated using rotary vacuum evaporator at 40°C. The concentrated/dried extract was collected into a pre-weighed sterile universal bottle and further allowed to dry to constant weight at room temperature (25°C) and weighed. The contents were then weighed for their extract yields and recorded. The bottle was labeled accordingly and stored in a refrigerator for microbial assay. This process was repeated using chloroform, ethyl acetate and methanol after allowing the marc to air dry for 2 to 3 hours. After successive extraction with these solvents, the residue (marc) was collected, air dried (to remove any residue of the solvent) and then macerated with cold distilled water to obtain the aqueous (water) extract.

Isolation

The method described by Okwuchi (2015) was adopted to fractionate the root extract. 28 g of the chloroform root extract was subjected to vacuum liquid chromatography (VLC) on column packed with 120 g of TLC grade silica gel as stationary phase. The elution of components present in the extract were started with hexane and then the polarity of the eluent was gradually increased with addition of chloroform and finally with ethyl acetate as hexane,

hexane/chloroform 80/20, hexane-chloroform 60/40, hexane-chloroform 40/60, hexane-chloroform 20/80, chloroform, chloroform-ethyl acetate 80/20, chloroform-ethyl acetate 60/40, chloroform-ethyl acetate 40/60, chloroform-ethyl acetate 80/20 and ethyl acetate. Silica gel 60 F_{254} (Merck) plate was used for TLC analysis and developed in mixtures of solvents (hexane/chloroform/ethyl acetate 4:4:1). The TLC plates were visualized under normal day light and ultraviolet light (254 and 364 nm) to yield 14 fractions.

The fractions having similar TLC profile were pooled together. Fractions 1 to 3 was combined together as pooled fraction A1, fractions 4 to 5 combined together as pooled fraction A2, fraction 6 to 7 combined together as pooled fraction A3, fraction 8 showed single spot and labelled as fraction A4, fraction 9 to 10 combined together as pooled fraction A5 and fractions 11 to 14 combined together as pooled fraction A6. The six combined fractions were concentrated on rotary vacuum evaporator.

Four compounds 9,12-octadecadienoic acid, undecanoic acid, 10-bromo, octadecanoic acid and n-hexadecanoic acid were obtained from component B of the pooled fraction 5 (9-10)- (eluent of chloroform/ethyl acetate 60:40) upon multiple preparative thin layer chromatography using a solvent mixture of hexane/ethyl acetate, 80:20) (Raju et al. 2014).

Instrumental analysis

The IR spectrum was measured on Cary 630 FT-IR spectrometer (portable)-Agilent Technologies Inc., Santa Clara, CA, USA (2013b) equipped with diamond ATR. The Gas Chromatography-Mass Spectrometry (GC-MS) separation and identification spectral analysis was performed on Gas chromatography-Mass spectrometer (GC-MS) Agilent GC-7890B coupled to an MSD 5977A (Agilent Technologies Inc. Santa Clara, CA, USA, 2013a) at the Multi-User Science Research Laboratory, Ahmadu Bello University, Zaria.

RESULTS AND DISCUSSION

The fractional yields were generally low. The highest yield (33.43%) was obtained from pooled fraction A4, followed by fraction A3 (16.57%), fraction A5 (15.64%), fraction A6 (14.64%), fraction A1 (9.42%) and fraction 2 (8.6%). The result of the pooled fraction yields, characteristics and Rf values is presented in Table 1.

The identification of the phytochemical compounds was based on the peak area, retention time and molecular formula as presented in Table 2 and Figure 1. The bioactive component was found to be a mixture of compounds. The GC-MS analysis/mass spectra revealed a chromatogram showing highest peaks of four major phytochemical compounds which included: 9,12-octadecadienal (linoleic acid) (40.98%), octadecanoic acid (stearic acid) (9.26%), undecanoic acid, 10-bromo- (10-bromoudecanoic acid) (9.26%) and n-hexadecanoic acid (Palmitic acid) (9.2%).

The infra-red spectroscopy spectrum of component B as presented in Table 3 and Figure 2 revealed the presence of six characteristic bands corresponding to these functional groups: C=C stretch bond in alkenes aromatic compounds between 1575 to 1640 cm^{-1}, absorption band at 1710 cm^{-1} confirmed the presence of C=O bond usually found in aldehydes, carboxylic acids, esters, ketones and amines. The absorption bands between 2855 to 2922 cm^{-1} indicated the presence of CH stretch in aliphatic. The absorption band around 3406 cm^{-1} indicated the presence of O-H and N-H stretching bond in alcohol, aldehydes, carboxylic acids and amines.

Based on the analysis of the spectroscopic data and after comparing the results obtained with data available in literature (Mensah-Agyei et al., 2020), the structures of the phytochemicals were proposed to be 9,12-octadecadienal (linoleic acid), octadecanoic acid (stearic acid), undecanoic acid, 10-bromo- (10-bromoudecanoic acid) and n-hexadecanoic acid (palmitic acid) (Figure 1).

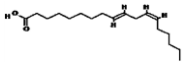
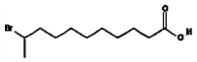
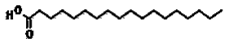
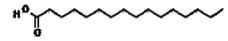
These fatty acid compounds were reported to have been isolated from the dichloromethane extract of leaves of *Helichrysum pedunculatum* and shown to possess antibacterial and a synergistic effect against *Staphylococcus aureus* and *Micrococcus kristinae* (Dilika et al., 2000). Linoleic acid is a polyunsaturated omega-6 fatty acid. Linoleic, stearic and palmitic acids have been found to have antibacterial activity, particularly in inhibiting the growth of Gram-positive bacterial species (Aliyu et al., 2017).

Although Aliyu et al. (2017), stated that fatty acids (linoleic, stearic, palmitic, 10-bromoudecanoic acids) have been isolated from several plants and animals, literature is scanty on isolation and characterization of these compounds from chloroform root extract of *Adenodolichos paniculatus.* To the best of our literature search, this is the first time these compounds will be reported in *A. paniculatus*, as such the compounds identified will add to the database of the plant. Not many studies have been reported on the phytochemicals isolated from this plant. However, many authors have reported the isolation of these phytochemical compounds from plants (Hui et, al., 2015; Koroma et al., 2018; Bulama et al., 2014). Never the less, Isyaku et al. (2020) reported the isolation of stigmasterol and β-sitosterol from methyl acetate leaf extract of *A. paniculatus.* Doan et al. (2019) reported the isolation of 9,12-octadecadienal (linoleic acid), octadecanoic acid (stearic acid) and n-hexadecanoic acid from the seed of five varieties Fabaceae species while studying on extraction process, identification of fatty acids, tocopherols, sterols and phenolic constituents and antioxidant evaluation of seed from five varieties of Fabaceae

Table 1. TLC analysis and percentage yield of pooled column (VCL) fractions from the chloroform root extract of *Adenodolichos paniculatus*

Combinations	Solvent system	Rf value	Observation under naked eye	Percentage yield (%)
A1 (fractions 1-3)	Hex:Chlor:EtAc 4:4:1	0.95, 0.93, 0.94	Light yellow (oily)	9.42
A2 (fractions 4-5)	Hex:Chlor:EtAc 4:4:1	0.8, 0.81	Amber (oily)	8.6
A3 (fractions 6-7)	Hex:Chlor:EtAc 4:4:1	0.73, 0.72	Golden yellow (gummy)	16.57
A4 (fraction 8)	Hex:Chlor:EtAc 4:4:1	0.48	Golden brown (gummy)	33.43
A5 (fractions 9-10)	Hex:Chlor:EtAc 4:4:1	0.41, 0.41	Dark orange (gummy)	15.64
A6 (fractions 11-14)	Hex:Chlor:EtAc 4:4:1	0.6, 0.59, 0.61, 0.60	Brown (oily)	14.64

Table 2. Identified bioactive phytochemicals in the chloroform fraction from the chloroform extract of *Adenodolichos paniculatus*.

RT (min)	Peak area (%)	Name of the compounds	Nature of compound	MW	Molecular formula	Structure
55.79	40.98	9,12-Octadecadienal (Linoleic acid)	Polyunsaturated Fatty acid	280.4	$C_{18}H_{32}O_2$	
56.26	9.26	Undecanoic acid, 10-bromo- (10-bromoudecanoic acid)	Amine and fatty acid	265.19	$C_{11}H_{12}BrO_2$	
56.262	9.26	Octadecanoic acid (Stearic acid)	Fatty acid ester	298	$C_{19}H_{38}O_2$	
50.83	9.2	n-Hexadecanoic acid (Palmitic acid)	Fatty acid	256	$C_{16}H_{32}O_2$	

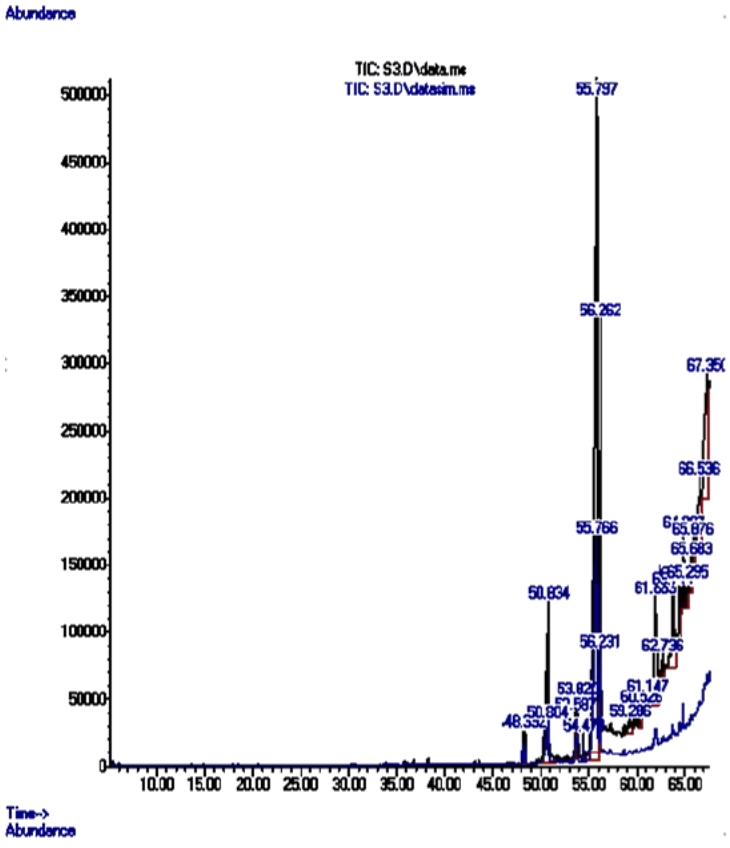


Figure 1. GC-MS chromatogram of bioactive component B.

Table 3. FTIR data of components B bioactive compounds

Peak	Absorption bands (cm^{-1})	Vibration/stretching	Functional group
1	3406.8	O-H group/NH	Alcohol, aldehydes, carboxylic acids and amides
2	2922.2	-C-H stretching	Aliphatic/Alkane
3	2855.1	C-H stretching	Alkane
4	1710.8	C=O	Amides, esters, ketones, carboxylic acid
5	1640	C=C	Alkenes in aromatic compounds
6	1575.4	C=C	Alkenes in aromatic compounds

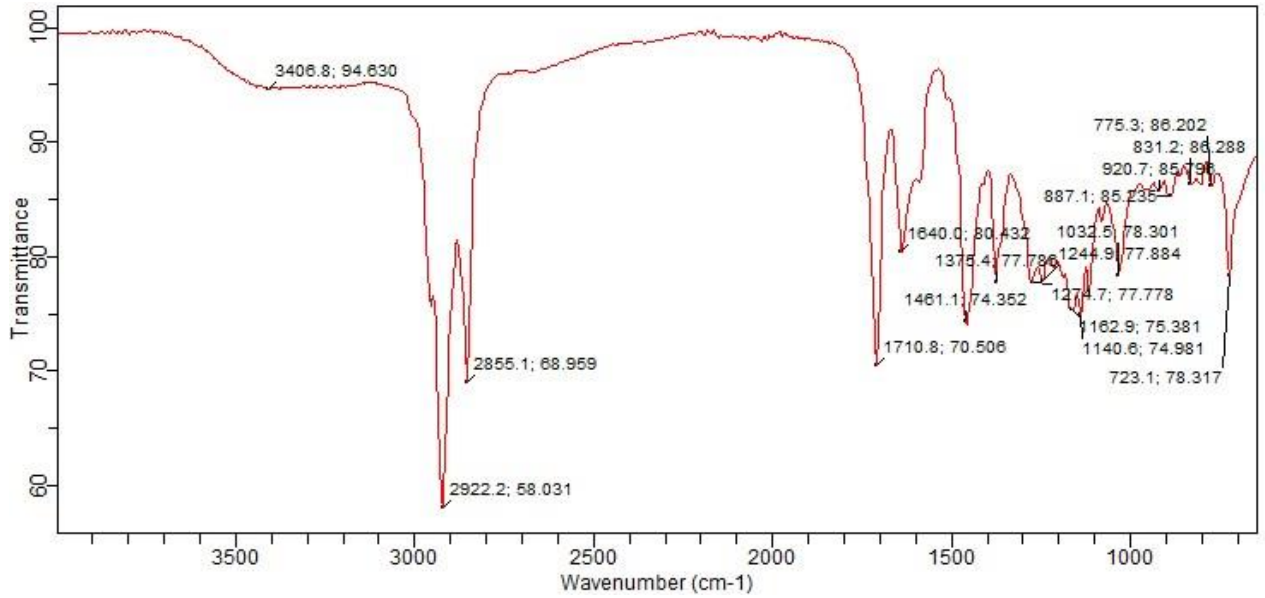


Figure 2. FT-IR spectrum of bioactive component B.

species. Also, Karimi et al. (2015) reported the isolation of fatty acids (palmitic, stearic, oleic and linoleic acids) from aqueous leaves extracts of *Labisia pumila* Benth while studying on fatty acid composition, antioxidant, and antibacterial properties of microwave aqueous extract of three varieties of *Labisia pumila* Benth and demonstrated antibacterial activities on *S. aureus*, *E. coli* and *P. aeruginosa*.

Conclusion

The FTIR analysis of component B proved the presence of aromatic rings, alkenes, alcohols, ethers, carboxylic acid, esters, nitro compounds, hydrogen bonded alcohols and phenols is consistent with the presence of compounds such as: alkaloids, flavonoids, terpenes, saponins and phenols observed in the plant extract.

Although each of the isolated bioactive components confirmed showed a single spot on TLC, however, the GC-MS analysis of bioactive components confirmed that they are mixture of compounds. As such, isolation of pure single compounds could not be achieved in this study with the choice of solvent systems and purification methods.

Further investigation will be required on the choice of solvent systems and purification methods to be able to isolate a single pure compound which will be responsible for the antibacterial activities as this could serve as a lead in discovery of novel drug or lead in phytomedicine development for the treatment of throat related infections.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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