

PHYTOCONSTITUENTS AND NUTRITION POTENTIAL OF *Malacantha alnifolia* (SAPOTACEAE)

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Received 16 March 2018; accepted 11 June 2018, published online 23 July 2018

ABSTRACT

Malacantha alnifolia has been widely utilized in traditional medicine, but reports on its phytoconstituents are scarce in literature. The roots were collected, shade dried, extracted with *n*-hexane and chromatographed on silica gel packed column. GC-MS experiment conducted on compounds obtained from *n*-hexane extract of *M. alnifolia* exhibited three known fatty acids: tridecanoic acid (**1**), pentadecanoic acid (**2**) and octadecenoic (oleic) acid (**3**); alongside a cyclic ester (heptadecanolide, **4**) and an unsaturated monoglyceride (Z, Z 9,12-octadecadienoic acid, **5**). Quantitative estimation of the phytochemicals in the pulverized roots of *M. alnifolia* revealed steroids (43.74 ± 1.00 mg/100g), saponins (9.69 ± 1.25 mg/100g) and alkaloids (8.61 ± 1.20 mg/100g). Mineral estimation of the roots sample afforded Ca (144.27 ± 2.50 ppm), Fe (4.23 ± 0.25 ppm), Pb (0.06 ± 0.01 ppm), Zn (0.61 ± 0.05 ppm), Cu (1.00 ± 0.20 ppm), P (7.25 ± 0.70 ppm) and Mg (118.13 ± 3.30 ppm). Proximate estimation revealed that *M. alnifolia* contained crude fibre (6.85 ± 0.30 %), ash content (19.6 ± 1.10 %), dry matter (9.00 ± 0.50 %), moisture content (13.61 ± 0.20 %), crude fat (49.20 ± 1.30 %) and crude protein (5.39 ± 0.40 %). The results obtained in this study confirmed nutrition potentials of *M. alnifolia*. The roots of the plant can also be a good source of fat and the presence of important phytochemicals in the plant provides scientific justification attributable to its folkloric application in traditional medicine in South-Western Nigeria..

Key words: *Malacantha alnifolia* root extracts, tridecanoic acid, Pentadecanoic acid, heptadecanoic acid, proximate analysis, nutrient content

Introduction

Plants served as therapeutic means to diseases ravaging mankind. In pre-historic record, several drugs are gotten from plant source. Plants contain veritable nutrients, edible nuts, mushrooms, herbs, fruits, spices, gum, fibers and fodders used by mankind for

medicinal and other cultural purposes. The use of traditional medicine and medicinal plants in most developing countries, as a basis for the maintenance of good health has been widely observed by UNESCO [1]

Malacantha alnifolia (Sapotaceae) is a perennial plant found in the tropical rain forest zones of West Africa, particularly in Togo Republic, Ghana and Nigeria [2a]. It belongs to the Sapotasceae family of Sapotaceae distribution is pan tropical (2ab). Morphology of *M. alnifolia* has been described [2a], [2b].



Fig. 1: Shoots of *M. alnifolia*

Sapotacea family has been reported for anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal, anti-diabetic, anti-ulcer, analgesic, antimalarial, cytotoxic and anti-pyretic activities [3], [4], [5]. [6], [7], [8]. Folkloric reports of *Malacantha alnifolia*

Material and Methods

General Experiment

Melting points (mp) were obtained on Gallenkamp apparatus and are uncorrected. Column chromatography (CC) was acquired on a silica gel (PF₆₀, 70-230 mesh; Merck, England) loaded column, 5 cm diameter and 2 m long. Thin layer chromatography (TLC) was obtained on precoated silica gel [60, PF₂₅₄₊₃₆₀, 0.3 mm thick, Merck, England) coated aluminum foil plate, visualized under iodine tank. All solvents used in this study

flowering plants and to the order, Ericales. The family contains about 1,100 species of evergreen trees and shrubs in around 53 genera (35-53).

ranges from the cure of arthritis, rheumatism, diarrhea to dysentery. It is also used for the treatment of conjunctivitis of the eyes and stomach ache in traditional medicine [2a], [9], [10].

As part of our searches for useful plants of phytochemical importance and nutrition benefits in tropical rain forest zones of Nigerian, we investigated phytochemical constituents and nutrition potentials of *M. alnifolia*. To our knowledge, a report on phytoconstituents and nutrition potential of *M. alnifolia* is scarce in literature. Herein, we report for the first time, isolation of three known fatty acids (**1-3**), alongside two known esters (**4, 5**) as well as the nutritive values of *M. alnifolia*. Compounds **1-5** though known are reported for the first time from *M. alnifolia*.

were obtained from Sigma representative in Nigeria. Gas Chromatography – Mass Spectrometry (GC-MS) experiments were performed using Hewlett-Packard model 5890 Gas Chromatography coupled with Electron Impact (EI) at 70 eV on a Mass Spectrophotometer. Injector and detector temperature were measured at 230°C and 275°C respectively, using hydrogen as carrier gas (1.0 mL/min).

Collection, Preparation and Extraction of Plant material

The roots of *M. alnifolia* was collected at the University of Ibadan botanical garden, Ibadan, Oyo State, Nigeria and authenticated by Mr. Michael of the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State, Nigeria in January, 2017, where a voucher specimen (FH1 22335) was deposited. The roots of *M. alnifolia* were air

dried for three weeks under shade and pulverized using automated machine. Hot solvent extraction was carried out in an aspirator bottle fitted with extraction gadgets. 2 kg of pulverized sample was packed in the aspirator bottle of 20 L and extracted using soxhlet extraction pattern. Extract was concentrated by distillation.

Isolation and Characterization of compounds in the n-hexane roots extract of *M. alnifolia*

8 g of crude n-hexane extract of *M. alnifolia* was chromatographed in a silica gel (Merck, 70-230 mesh) packed column, . Elution commenced with increasing percentage of ethyl acetate (EtOAc) in *n*-hexane. In all, 40 fractions were collected, containing 100 mL at a time. Fractions were bulked into 8

groups, on the basis of their TLC profiles. *N*-hexane : EtOAc (90 : 10, vol : vol) eluted compound **1** after purification (TLC: $R_f = 0.80$). The *n*-hexane : EtOAc (70 : 30, vol : vol) eluted compound **2** after crystallization (TLC: $R_f = 0.90$). Compound **3** was eluted with *n*-hexane : EtOAc (50 : 50, vol : vol). The *n*-hexane : EtOAc (60 : 40, vol : vol) furnished compound **4** (TLC: $R_f = 0.85$), while the *n*-hexane : EtOAc (30 : 70, vol : vol) yielded compound **5** (TLC: $R_f = 0.75$).

Quantitative determination of the phytochemical constituents of *M. alnifolia* Estimation of alkaloids

Quantitative determination of alkaloid was according to the methodology of Harborne

et al., (11) with slight modification. The residue was dried in an oven and the percentage of alkaloid was deduced following known procedure [23]

Determination of saponnins

Quantitative determination of saponnins was carried out as reported previously [12], [13], [14] with slight modifications.

Quantitative Estimation of Steroids

Quantitative estimation of steroid was as previously described [12], [13], [14]

Proximate analysis

Proximate analysis were carried out on *Malcantha alnifolia* includes moisture

content, dry matter content, ash content protein content, fat content and fibre

content. Moisture content was determined at 105 °C. Ash content was determined at 550 °C. Protein, fat and fibre contents were

according to the procedures of AOAC (1990). Crude nitrogen was determined based on the Kjeldhal procedure.

Ash content determination

1 g of sample was weighed into a weighed crucible and heated in a muffle furnace for about 3 h at 500⁰C. The crucible was removed and cooled in a desiccator and then reweighed. It was heated again in a muffle furnace for 30 min which was then cooled

and reweighed again. This was repeated consequently until a constant weight is obtained (ash becomes white or grayish white). Weight of the ash gave the ash content of the sample.

Estimation of dry matter content

1 g of the sample was weighed into a glass crucible and kept in an oven at 105⁰C, cooled in a desiccator and reweighed. The

weight loss was regarded as a measure of the dried matter.

Digestion of plant sample

1 g of the powdered roots of *M. alnifolia* was placed in a beaker, followed by the addition of mixtures of HNO₃ and HClO₄ in 3:1 (15 mL HNO₃ and 3 mL HClO₄). It was placed on a regulated heating mantle in a fume cupboard for digestion. The sample was

heated and distilled. Water was added intermittently to prevent the sample from charring for about 4 h till the solution becomes clearer. The digested sample was washed with 1% H₂SO₄ and filtered to remove every trace of suspended particles of *M. alnifolia* from the digested sample.

Estimation of crude fibre

Crude fibre analysis was carried out following the procedure of AOAC [22]

Estimation of Crude fat

Crude fat analysis was carried out following the procedure of AOAC [22]

Estimation of carbohydrate

The carbohydrate was estimated as the sum of all proximate component subtracted from 100%

$$\text{Carbohydrate (\%)} = 100 - (\% \text{moisture} + \% \text{crude protein} + \% \text{crude fibre} + \% \text{ash}).$$

Elemental analysis using AAS

The digested root sample of *M. alnifolia* was used to estimate the concentration of elements in the root of *M. alnifolia* by

means of atomic absorption spectrophotometer. Zn, Fe, Cu, Pb, Mg, P and Ca were estimated in the sample.

Results and Discussion

GC-MS data of isolated compounds

Tridecanoic acid (1)

Compound **1** eluted as feathery white powder (200 mg) from the column using 90 % *n*-hexane in EtOAc and recrystallized in methanol yielded white powder (**1**); TLC: $R_f = 0.90$ (*n*-hexane : EtOAc, 3:1; vol:vol); mp 225-226°C. GC-MS (*m/z*, rel int, %): (*m/z* 214, M^+ , (15%); *m/z* 196 ($M^+ - H_2O$, 2%), *m/z* 171 (35%), *m/z* 157 (2%), *m/z* 143 (3%), 129 (50%), *m/z* 73 (100%) - Fig 2

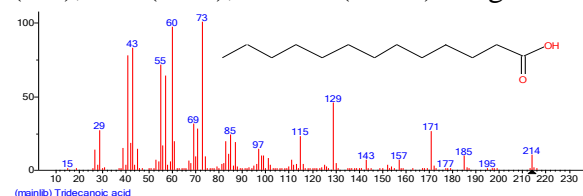


Fig 2- Mass Spectrum of compound 1

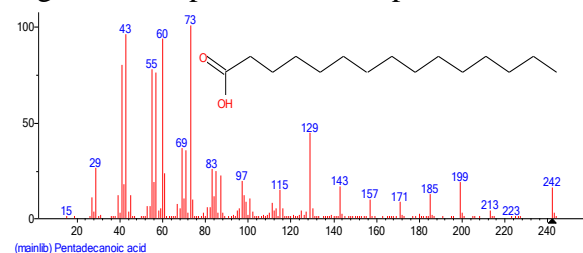


Figure 3- Mass spectrum of compound 2

9-octadecenoic (oleic) acid (3)

50 % *n*-hexane in EtOAc from the silica gel packed column eluted compound **3** as yellow powdery solid. After careful washing in *n*-hexane:EtOAc (3:1; vol:vol), a light-yellow solid emerged (100 mg); TLC: $R_f =$

Fig 2- Mass Spectrum of Compound 1

Pentadecanoic acid (2)

Elution of the column with 70 % *n*-hexane in EtOAc yielded dirty brown solid, washed repeatedly in *n*-hexane and later recrystallized in MeOH to afford white powder, **2** (160 mg); TLC: $R_f = 0.85$ (*n*-hexane : EtOAc, 3:1; vol:vol); mp 200-202°C. GC-MS (*m/z*, rel int, %): (*m/z* 242, M^+ , (10%); *m/z* 222 ($M^+ - H_2O$, 5%), *m/z* 129 (30%), *m/z* 115 (2%), *m/z* 73 (90%), 69 (44 %), *m/z* 43 (100%) - Fig 3.

0.70 (*n*-hexane : EtOAc, 3:1; vol:vol); mp 205-206 °C. GC-MS (*m/z*, rel int, %): (*m/z* 282, M^+ , (30 %); *m/z* 264 ($M^+ - H_2O$, 52%), *m/z* 222 (10 %), *m/z* 111 (24%), *m/z* 97 (60%), 83, (75 %), 69 (80 %), *m/z* 55 (100 %), *m/z* 41 (70%) - Fig 4.

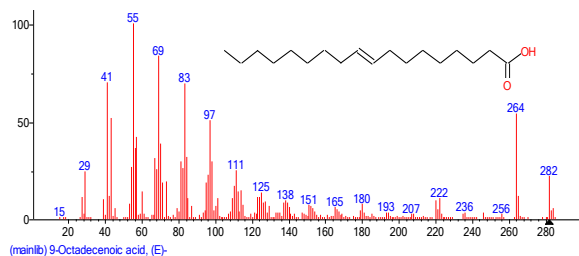


Fig 4- Mass Spectrum of Compound 3

Heptadecanolide (Cyclic ester, **4**)

Compound **4** was eluted with 30 % *n*-hexane in EtOAc, from silica gel packed column as feathery grey powder (155 mg), after recrystallization in MeOH, washing in *n*-hexane/EtOAc (7:3; vol : vol) and filtering. TLC: R_f = 0.50 (*n*-hexane : EtOAc, 2:1; vol : vol); mp 109-110 °C. GC-MS (*m/z*, rel int, %): (*m/z* 268, M^+ , (5 %); *m/z* 250 ($M^+ - H_2O$, 12%), *m/z* 222 (10 %, $M^+ - H_2O - CO$), *m/z* 208 (4 %, $M^+ - H_2O - CH_2$), *m/z* 111 (25 %), *m/z* 97 (55 %), 83 (60 %), *m/z*

69 (55 %), *m/z* 55 (100%), *m/z* 41 (100%) - Fig 5

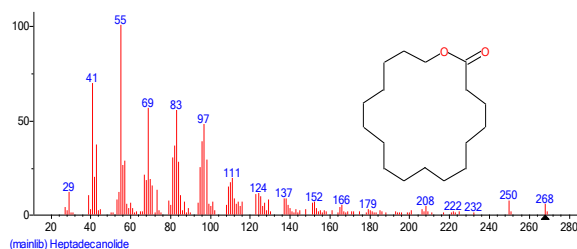


Fig 5- Mass Spectrum of Compound 4

Elution from the column with 100 % EtOAc afforded dirty green solid. After purification, a white-yellow solid (220 mg) emerged. TLC: R_f = 0.80 (*n*-hexane : EtOAc, 1:1; vol : vol); mp 180-182 °C. GC-MS (*m/z*, rel int, %): (*m/z* 294, M^+ (42 %); *m/z* 279 ($M^+ - CH_3$, 2%), *m/z* 263 ($M^+ - CH_3-O$ 20%), *m/z* 245 (2 %, $M^+ - CH_3-O-CO$), *m/z* 111 (25 %), *m/z* 97 (55 %), 83 (60 %), *m/z* 69 (55 %), *m/z* 55 (100%), *m/z* 41 (100%) - Fig 6

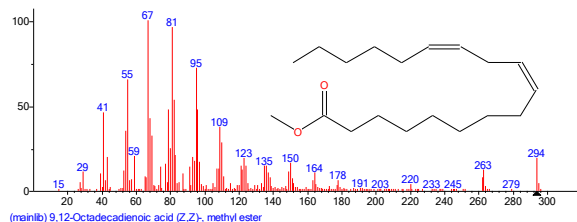


Fig 6- Mass spectrum of compound 5

The GC-MS data of compounds **1**, **2** and **3** are consistent with those reported for tridecanoic, pentadecanoic and 9-octadecenoic (oleic) acid respectively, known fatty acids (15, 16).

Compound **4** was elucidated using the GC-MS data (Fig V) exhibits molecular ion (M^+) peak at *m/z* 268, corresponding to the molecular formula $C_{17}H_{32}O_2$; that of a cyclic ester (heptadecanolide, **4**). Subsequent fragment ions at *m/z* 250 ($M^+ -$ Loss of octyl group (C_8H_{15}) gave rise to *m/z* 111.

H_2O); confirm the M^+ at *m/z* 268. The daughter ion at *m/z* 222 results in further loss of CO from *m/z* 250; consistent with fragmentation pattern of esters. Appearance of fragment ions at *m/z* 210 suggests C1-C2 fission and C17-C16 fission, consistent with the fragmentation pattern of cyclic ester [17]. Several loss of 14 amu, corresponds to loss of $-CH_2$ units, at *m/z*: 41, 55, 69, 83, 97, 111, etc, suggesting the presence of several methylene units in the structure of **4**.

The GC-MS spectrum of compound **5** displays molecular ion (M^+) peak at m/z 294, corresponding to the molecular formula $C_{19}H_{34}O_2$, and consistent to those of 9,12-octadecadenoic acid (unsaturated monoglyceride, **5**). The peak at m/z 279 indicates loss of $-CH_3$ unit from the M^+ , confirming exactly a true M^+ . Compound **5** gave positive test to 2,4-DNP hydrazine, confirming carbonyl functional group; and total decolourisation of bromine water., suggesting vinylic bond. The fragment ion at m/z 263 ($M^+ - CH_3-O$ 20%), m/z 245 (2 %, $M^+ - CH_3-O-CO$) confirms typical pattern for glycerides [18].

Quantitative estimation of phytoconstituents in the roots *n*-hexane extract of *M. alnifolia*

(Table 1) revealed alkaloids (8.61 mg/100 g), steroids (43.74 mg/100 g) and saponnins (9.69 mg/100 g). Presence of these phytochemicals in *M. alnifolia*, indicates its medicinal potential in human and animals, especially in areas of coronary heart disease, ulcers, diabetes, high blood pressure, muscular degeneration, inflammation, infection and psychotic diseases, which have been treated by herbs containing alkaloids, steroids, saponnins, etc [19].

Table 1 Quantitative Phytoconstituents in the root extract of *M. alnifolia*

Sample	Steroids (mg/100g)	Saponnins (mg/100g)	Alkaloids (mg/100g)
<i>M. alnifolia</i> (roots)	43.74 \pm 1.00	9.69 \pm 1.25	8.61 \pm 1.20

Table 2 illustrates proximate analysis carried out on powdered roots of *M. alnifolia*; suggesting ash (19.6 %), moisture (13.61 %), dry matter (9.00 %), crude fibre (6.85 %), crude fat (49.20 %) and protein content (5.39 %). This is comparable to proximate analysis carried out on *Madhuca longifolia* (Sapotaceae) with moisture contents of 14.8

%, ash contents of 19.36 %, however, the crude fats content differs significantly, 6.5 % [20], compared to 49. 2% in *M. alnifolia*. The plant is a very good source of fat, this can be useful for animal breeding. The mineral nutrient content is also confirmed by the moderate ash content of 19.6%.

Table 2 Proximate Analysis in the roots of *M. alnifolia*

Test	Dry matter (%)	Moisture content (%)	Crude Fat (%)	Crude Protein (%)	Crude Fibre (%)	Ash content (%)
Results	9.00 \pm 0.50	13.61 \pm 0.20	49.20 \pm 1.30	5.39 \pm 0.40	6.85 \pm 0.30	19.6 \pm 1.10

Result obtained from mineral content analysis (Table 3) revealed that the root of *M. alnifolia* is moderately rich in Ca (144.27

ppm), Mg (118.13 ppm), P (7.25 ppm), Fe (4.23 ppm), Cu (1.00 ppm), Zn (0.61 ppm), Pb (0.06 ppm). The presence of calcium in

the body helps to build strong bones, magnesium served as food supplements, phosphorus helps to repair body tissues and cells, iron is part of the constituents of hemoglobin which helps muscles to store

and use energy. However, Pb has the least value, not required in the body system. Little amount of zinc was found in the root, although very little amount is needed for the human health [21].

Table 3 Nutrition composition of the powdered root of *M. alnifolia*

Mineral elements	Ca (ppm)	Mg (ppm)	P (ppm)	Fe (ppm)	Cu (ppm)	Zn (ppm)	Pb (ppm)
Results	144.27 ± 2.50	118.13 ± 3.30	7.25 ± 0.70	4.23 ± 0.25	1.00 ± 0.20	0.61 ± 0.05	0.06 ± 0.01

Conclusion

Five compounds (three fatty acids, a cyclic ester and an unsaturated monoglyceride) were isolated and characterized from the n-hexane roots extract of *M. alnifolia*. The compounds though known are reported for

the first time from n-hexane extract of the roots of *M. alnifolia*.

A root of *M. alnifolia* was found to contain vital phytoconstituents and mineral contents that are beneficial to health. This study provides scientific justification for the traditional use of the roots of *M. alnifolia*.

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