Isolation and Characterization of Pyropheophorbide-a from Moringa oleifera Lam

Ukachi E. Igbo1*, John O. Igoli2,4, Samuel O. Onyiriuka3, Cynthia E. Oguke1, Atu A. Ayuk3, Alexander I. Gray2

1Federal Institute of Industrial Research, Oshodi, P.M.B.21023 Ikeja, Lagos, Nigeria.
2Strathclyde Institute of Pharmacy and Biomedical Sciences. University of Strathclyde, 161 Cathedral Street, Glasgow G4 ORE, Scotland.
3Department of Chemistry, Federal University of Technology, Owerri, Nigeria.
4Department of Chemistry, University of Agriculture, Makurdi, Nigeria.

Article history:
Received 20 September 2019
Revised 05 December 2019
Accepted 10 December 2019
Published online 10 December 2019

Copyright: © 2019 Igbo et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

ABSTRACT

Moringa oleifera is a plant rich in pharmacologically active compounds. This study investigated the phytochemical constituents of Moringa oleifera leaf extracts. Dried M. oleifera leaves were ground and extracted successively with hexane, ethyl acetate and methanol using a Soxhlet apparatus. Different chromatographic techniques were used to fractionate the ethyl acetate extract. Thin layer chromatography was used to pool similar fractions together. Fractions obtained were purified using sephadex glass column. The structures of isolated compounds were identified using nuclear magnetic resonance (NMR), electrospray ionization mass spectrometry (ESI-MS) and comparison with published data. Phytochemical screening revealed the presence of flavonoids, terpenoids, carbohydrates and phenols. Ethyl acetate extract which was subjected to column chromatography resulted to the isolation of two compounds: mono acetyl glycerol and pyropheophorbide-a, from the leaves of M. oleifera. This is the first report on the isolation of pyropheophorbide-a from the leaves of M. oleifera.

Keywords: Moringa oleifera. Isolation, Pyropheophorbide-a, Monoacetyl glycerol.

Introduction

Man has always used plants for the treatment and prevention of various disease conditions due to the presence of varied active ingredients in different parts of plants. According to the World Health Organization (WHO), 75% of people rely on plant based traditional medicines for primary health care globally; and about 85% of traditional medicines are based on the use of plant extracts. 1 The demand for natural products from plants is due to special attributes of natural products not present in synthetic drugs. They are said to be “safe” or at least safer than conventional medicine with little or no side effects, eco-friendly. Other attributes include having drug-like properties, low cost and increased evidence for an association between high consumption of fruits/vegetables and reduced risk of diseases. 2 Historically, plants are the major sources of some of the most important drugs based on their use in traditional medicine. For example, Morphine, from opium poppy (Papaver somniferum), which became the first pure substance of natural origin to be commercialized as a drug used as analgesic, 3 Digoxin and other digitalis glycosides, from foxglove (Digitalis spp.), used to treat cardiac failure, 4 Taxol, from the Pacific yew (Taxus brevifolia), and its semisynthetic derivative docetaxel used as anticancer treatment 5 and Quinine, from Cinchona bark (Cinchona spp.), used in the treatment of malaria. 6

Moringa oleifera Lam. (Syn. Moringa pterygosperma Gaerth) belongs to a monogenic family of Moringaceae, 6, 7 and is widely distributed in many tropical and sub-tropical countries. 8 The plant is commonly known as drumstick or horseradish tree. In Nigeria, it is known by various local names such as; Ewe ile (Yoruba), Zogali, or Zogallagandi (Hausa) and Okochieghu (Ibo). Moringa oleifera is a highly valuable plant, with nearly all parts used for medicinal and nutritive purposes. Its medicinal use has long been recognized in the Ayurvedic and Unani systems of medicine. 9 Some of the medicinal effects are antitumor, 10, 11 antipryretic, anti-inflammatory, antilucre, 12, 13 antispasmodic, 14, 15 diuretic, 16, 17 antihypertensive, 17, 18 cholosterol lowering, 19, 20 antioxidant, anti-diabetic, hepa-to-protective, antibacterial and antifungal activities. 21 The potential of M. oleifera in the treatment of typhoid fever in Cameroon 22 and HIV/AIDS in Uganda has also been reported. 22 In Nigeria, M. oleifera is used to treat various diseases such as inflammation, asthma, fever, cough, pains, liver and pancreatic disorders, venereal infections, diarrhea and malaria. 23, 24 In addition, Igbo et al 2015 reported that ethyl acetate extract of M. oleifera leaves exhibited antitrypanosomal activity with minimum inhibitory concentration (MIC) value of 25 µg/mL. 25 Other uses of M. oleifera include animal forage (leaves and treated seed cake), biogas (leaves), domestic cleaning agent (leaves), blue dye (wood), fencing (living trees), fertilizer (seed cake), green manure (leaves), gum (tree trunks) sugar cane, juice-clarifier (powdered seeds), ornamental plantings, bio-pesticide, pulp (wood) and water purification (powdered seeds). 26 Moringa species contain a wide range of fairly unique compounds called glucosinolates and isothiocyanates. 27, 28 Also, the plant family is particularly rich in thiamone glycosides. Some pharmacologically active compounds such as 4-(4′-O-acetyl-α-L-rhamnopyranosyl)benzyl isothiocyanate, 4-(α-L-rhamnopyranosyl) benzyl isothiocyanate, niazimicin, pterygospermin, benzyl isothiocyanate, and 4-(α-L-rhamnopyranosyl) benzyl glucosinolate; also, thiocabamates, carbamates, and nitrile glycosides have been isolated from the fresh

*Corresponding author. E mail: ukachiigbo@yahoo.com
Tel: +234 7064771788

Citation: Igbo UE, Igoli JO, Onyiriuka SO, Oguke CE, Ayuk AA, Gray A
I. Isolation and Characterization of Pyropheophorbide a from Moringa oleifera Lam. Trop J Nat Prod Res. 2019; 3(10): 314 - 318. doi:10.26538/tjnp/v3i10.3

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.
leaves of *M. oleifera*. These compounds are responsible for the hypotensive activity of the leaves. Flavonoid compounds consist of glycosides, rutinosides, malonylglycosides and traces of acetylglycosides of kaempferol, quercetin and caffeoylquinic acid. Moringine and mormoridine have also been isolated from the plant. Plants are source of bioactive compounds which can serve a lead in drug discovery. *M. oleifera* has been widely studied and tested for its biological properties, but it is not completely exhausted in search for more bioactive compounds. Therefore, this report is on isolation of compounds from *M. oleifera* leaf ethyl acetate extract.

**Materials and Methods**

**General Experimental Procedures**

HPLC grade solvents were obtained from Avantor VWR™ and Fisher Scientific UK. NMR experiments were performed on a JEOL (JNM LA 400) or Varian (VXR 400) MM spectrometers coupled to an Ultimate 3000 LC system. Thin Layer Chromatography (TLC) was conducted using commercially available pre-coated Merek F254 silica gel plates. The spots were visualized under UV light at 254 nm and 366 nm, and by spraying with anisaldehyde-sulfuric acid mixture and heated to 110°C.

**Plant Material**

Fresh leaves of *M. oleifera* were collected from Gusau in Zamfara State, Nigeria on February 10, 2012. The plant material was authenticated at the University of Lagos Herbarium. A voucher specimen LUH 6062 was deposited at University of Lagos Herbarium. The leaves were air dried at room temperature for a period of one week. The clean dried leaves were pulverized and stored in a refrigerator until ready for use.

**Extraction**

Powdered *M. oleifera* leaf 650 g was extracted successively with three liters of hexane, ethyl acetate and methanol using Soxhlet apparatus for 72 h. All extracts were filtered to remove any debris and evaporated to dryness using a rotary evaporator at 40°C under reduced pressure; their percentage yields were determined and extracts were stored in a refrigerator until further investigation.

**Phytochemical Screening**

Qualitative phytochemical screening of ethyl acetate and methanol extracts were carried out according to the methods described by Trease and Evans and Sofowora.

**Isolation and Characterization of Pyropheophorbide a**

A sintered glass fitted Buchner funnel with a suction outlet was packed with TLC grade silica gel (60H, Merck, Germany) under vacuum. A non-polar solvent n-Hexane was run through the Buchner funnel under vacuum in order to achieve good packing of the column. Ethyl acetate extract (15.3 g) pre-adsorbed on silica gel was added to the packed vacuum liquid chromatography (VLC) column. The sample was eluted twice each time with 300 mL solvent mixtures of increasing polarity: hexane-ethyl acetate and ethyl acetate-methanol (10% increments of ethyl acetate and methanol, respectively). A total of 28 fractions were collected. Fractions were pooled together based on similarity of TLC profiles, and further evaporated to dryness at 40°C under vacuum using a rotary evaporator. Fractions MOEV-EOAc-1 and MOEV-EOAc-2 (both eluted with 100% ethyl acetate) were combined based on TLC and similarity of their proton nuclear magnetic resonance (1H-NMR). The combined fraction (850 mg) was further purified using Sephadex. A slurry of sephadex LH-20 in methanol was added to a glass column. Fractions eluted with 100% ethyl acetate was dissolved in small quantity of methanol and applied onto the sephadex column. The column was eluted with methanol; 50 fractions (4 mL each) were collected. Compounds 1 and 2 were obtained from Sephadex column fractions MOEVS 36-47 and MOEVS 11-14, respectively. Structures of the two compounds were established using NMR techniques (1H, 13C and 2D) and comparison with published data.

**Results and Discussion**

The percentage yield of *M. oleifera* leaf extracts produced with solvents of increasing polarity gave values for hexane 52.7 g (8.1%), ethyl acetate 22.6 g (3.5%), and methanol 81.9 g (12.6%).

Investigation of the phytochemical constituents of the ethyl acetate and methanol extracts showed the presence of flavonoids, terpenoids, carbohydrates, and phenols. Saponins, alkaloids and anthraquinones were not detected in any of the extracts (Table 1). The presence of these phytochemicals confirms the medicinal importance of *M. oleifera* leaves.

Fractionation of ethyl acetate extract gave compounds 1 (8 mg) and 2 (4.9 mg). A reddish-coloured spot detected by UV (365 nm) was observed in the TLC of fraction MOEVS 36-47. The molecular ion of compound 1 was not observed in its mass spectrum but a quasi-molecular ion at *m/z* = 546.0800 corresponding to [M-CO2H]+ was obtained suggesting a molecular formula C45H45N6O7. The assignments of 1H-NMR and 13C-NMR signals summarized in Table 2 were carried out by two-dimensional (2D) NMR: Correlation Spectroscopy (COSY), Heteronuclear single quantum coherence (HSQC) and Heteronuclear multiple bond coherence (HMBC) experiments.

The structure of compound 1 was established from 1H and 13C correlations observed in its 2D NMR spectra and comparison with published data. The 1H-NMR spectrum showed three well separated singlets (3H) due to methyl groups at δ 3.32, 3.41, and 3.67 ppm, an ethyl group (δ 1.70 (3H, t, J = 7.6 Hz), 3.67 (2H, q, J = 7.8 Hz)) and a vinyl group with protons at δ 7.97 (1H, dd, J = 11.5, 17.8 Hz), 6.29 (1H, dd, J = 1.3, 17.8 Hz) and 6.17 (1H, dd, J = 1.3, 11.5 Hz) were observed.

The 13C-NMR spectrum showed 33 carbon signals and accounted for all the carbon atoms in the structure. Thus compound 1 was identified as pyropheophorbide-a (Figure 1) based on the 1H and two-dimensional (2D) NMR spectra. This result compares with earlier report of pyropheophorbide-a isolated from the leaves of *Atalantia monophylla*. This is the first report on the isolation of pyropheophorbide-a from *M. oleifera* leaves.

The 1H-NMR spectrum of compound 2 gave signals at δ 2.01 (3H, s), 3.49, 3.59 (each 1H, d, J = 4 Hz), 3.85 (1H, m), 4.04 (2H, d, J = 4 Hz). The 13C-NMR gave a signal at 172.0 ppm indicative of a carbonyl group. Table 3 shows the 1H and 13C signals based on correlations observed in its 2D NMR spectrum. The molecular ion was not observed in its mass spectrum. The peak observed at *m/z* = 327 is due to the loss of hydroxyl group ([M-OH]+) and the mass at *m/z* = 313 corresponds to the loss of a CH2: characteristic of long chain aliphatic compounds. Compound 2 was identified as mono acetyl glycerol (Figure 2); possibly produced as a degradation product of glycerol-1-(9-octadecanoate).

**Table 1:** Qualitative phytochemical screening of *M. oleifera* leaf extracts

| Class of compounds | Results |
|--------------------|---------|
| Saponins           | -       |
| Flavonoids         | +       |
| Alkaloids          | -       |
| Terpenoids         | +       |
| Anthraquinones     | -       |
| Carbohydrates      | +       |
| Phenols            | +       |

*; indicate presence of compound
- ; indicate absence of compound
Table 2: $^1$H and $^{13}$C-NMR Chemical shifts (ppm) for compound 1 (CDCl$_3$)

| Position | ppm $^1$H, (J/Hz) | $^{13}$C ppm |
|----------|-------------------|--------------|
| 1        | -                 | 141.5 (C)    |
| 2        | -                 | 131.4 (C)    |
| 2$^1$    | 3.41 (s)          | 12.1 (CH$_3$)|
| 3        | -                 | 135.9 (C)    |
| 3$^1$    | 7.97 (1H, dd, J = 11.5, 17.8) | 129.2 (CH) |
| 3$^{11}$ | 6.17 (1H, dd, J = 1.3, 11.5), 6.29 (d, J =1.3,17.8) | 122.6 (CH$_2$) |
| 4        | -                 | 136.3 (C)    |
| 5        | 9.34 (s)          | 97.3 (CH)    |
| 6        | -                 | 155.4 (C)    |
| 7        | -                 | 136.1 (C)    |
| 7$^1$    | 3.23 (s)          | 11.2 (CH$_3$)|
| 8        | -                 | 145.0 (C)    |
| 8$^1$    | 3.679 (q)         | 19.5 (CH$_2$)|
| 8$^{11}$ | 1.70 (t)          | 17.4 (CH$_3$)|
| 9        | -                 | 150.8 (C)    |
| 10       | 9.46 (s)          | 104.3 (CH)   |
| 11       | -                 | 137.9 (C)    |
| 12       | -                 | 128.3 (CH$_3$)|
| 12$^1$   | 3.65 (s)          | 12.0 (CH$_3$)|
| 13       | -                 | 130.3 (C)    |
| 13$^1$   | -                 | 196.6 (C)    |
| 13$^{11}$| 5.12, 5.27        | 48.0 (CH$_3$)|
| 14       | -                 | 149.0 (C)    |
| 15       | -                 | 106.2 (C)    |
| 16       | -                 | 160.4 (C)    |
| 17       | 4.50 (m)          | 50.1 (CH)    |
| 17$^1$   | 2.29 (m), 2.73 (m) | 29.7 (CH$_2$) |
| 17$^{11}$| 2.38 (m), 2.65 (m) | 30.7 (CH$_2$) |
| 17$^{111}$| 2.69 (m)         | 176.8 (C)    |
| 18       | 4.32 (q, J = 9.3) | 51.6 (CH)    |
| 18$^1$   | 1.83 (d, J = 7.3) | 23.2 (CH$_3$) |
| 19       | -                 | 171.4 (C)    |
| 20       | 8.55 (s)          | 93.1 (CH)    |

$^a$ - Deuterated chloroform

Table 3: $^1$H and $^{13}$C-NMR Chemical shifts (ppm) for Compound 2 (CDCl$_3$)

| Position | ppm $^1$H, (J/Hz) | $^{13}$C ppm |
|----------|-------------------|--------------|
| 1        | 4.04 (2H, d, J = 4.0) | 65.6 (CH$_2$OH) |
| 2        | 3.85 (1H, m)      | 70.6 (CHOH)  |
| 3        | 3.49, 3.59 (1H each, d, J = 4.0Hz), | 63.8 (CH$_2$OH) |
| 1$^1$    | 2.02 (3H s)       | 20.9 (CH$_3$) |
|         | -                 | 172.0 (C = O) |
Conclusions
The result of phytochemical screening revealed the presence of flavonoids, terpenoids, carbohydrates and phenols in ethyl acetate and methanol extracts. Fractionation of *M. oleifera* leaves ethyl acetate extract led to the isolation of pyropheophorbide-a (compound 1) an antioxidant and a photosensitizer used in photo dynamic therapy for the management of cancers. Also isolated was mono acetyl glycerol a compound with wide applications in pharmaceuticals, food and cosmetics industries. The result of this study provides scientific evidence to support the ethnopharmacological use of *M. oleifera* leaves.

Conflict of interest
The authors declare no conflict of interest.

Authors’ Declaration
The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References
1. Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. Medicinal Plants in Therapy. 1985; Bull WHO 63:965–981.
2. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. 2nd Edition. Edinburgh, London, New York: Churchill Livingstone Elsevier. 2012.
3. Goldstein A. Opiate receptors. Life Sci. 1974; 14:615–623.
4. Parker SD, Satyajit D, Latif Z, Gyi A. Natural Products Isolation. 2nd ed. New Jersey: Humana Press Totowa, 2006.
5. Cragg GM and Newman DJ. Plants as a Source of Anticancer Agents. J Ethnopharmacol. 2005; 100 (1–2): 72–79.
6. Nadkarni KM. Indian Material Medica. Bombay Popular Prakashan, 2009; 1: 810–816.
7. Ramachandran C, Peter KV, Gopalakrishnan PK. Drumstick (*Moringa oleifera*): a Multipurpose Indian Vegetable. Econ Bot 1980; 34(3):276–283.
8. Anwar F and Bhaner ML. Analytical Characterization of *Moringa oleifera* Seed oil grown in Temperate Regions of Pakistan. J Agric Food Chem. 2003; 51(22):6558–6563.
9. Mughal MH, Ali G, Srivastava PS, Iqbal M. Improvement of Drumstick (*Moringa pterygosperma* Gaertn.) A Unique Source of Food and Medicine through Tissue Culture. Hamdard Medicus 1999: 42:37–42.
10. Guevara AP, Vargas C, Sakurai H, Fujiwara Y, Hashimoto K, Maoka T, Kozuka M, Ito Y, Tokuda H, Nishino H. An Antitumor Promoter from *Moringa oleifera* Lam. Mutat Res 1999; 440(2):181–188.
11. Bharali R, Tabassum J, Azad MR. Chemomodulatory Effect of *Moringa oleifera*, Lam. on Hepatic Carcinogen Metabolising Enzymes, Antioxidant Parameters and Skin Papillomagenesis in Mice. Asian Pac J Cancer Prev 2003; 4:131-139.
12. Ruckman KS, Davimani BJ, Anandan R. Antiulcer Activity of the Alkaloid Preparation of the Root and Fresh leaf juice of *Moringa oleifera* Lam. Ancient Sci life 1998; 17: 220–223.
13. Devaraj VC, Asad M, Prasad S. Effect of Leaves and Fruits of *Moringa oleifera* on Gastric and Duodenal Ulcers. Pharm Biotechnol. 2007; 45:332–338.
14. Cáceres A, Saravia A, Rizzo S, Zabala D, Le Deon E, Nave F. Pharmacological Properties of *Moringa oleifera* 2. Screening for Antispasmodic, Anti-inflammatory and Diuretic Activity. J Ethnopharmacol. 1992; 36 (3): 233-237.
15. Gilani AH, Aftab K, Suria A, Siddiqui S, Saleem R, Siddiqui BS et al. Pharmacological Studies on Hypotensive and Spasmolytic Activities of Pure Compounds from *Moringa oleifera*. Phytother Res. 1994; 8 (2):87–91.
16. Mazumder UK, Gupta M, Chakrabarti S, Pal D. Evaluation of Haematological and Hepatorenal Functions of Methanolic Extract of *Moringa oleifera* Lam. Root Treated Mice. Indian J Exp Biol. 1999; 37 (6):612–614.
17. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani A. Isolation and Structure Elucidation of Novel Hypotensive Agents, niazinin A, niazinin B, niazicinmic and niazimin A plus B from *Moringa oleifera*: The First Naturally Occurring Thiocarbarnates. J Chem Soc Perkin Trans. 1992; 1:3237-3241.
18. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani A. Novel Hypotensive Agents, niazinin A, niazinin B, Niazicin A and Niazicin B from *Moringa oleifera*: Isolation of First Naturally Occurring Carbarnates, J Chem Soc Perkin Trans 1994; 1:3035-3040.
19. Mehta LK, Balaraman R, Amin AH, Bafna PA, Gulati OD. Effect of Fruits of *Moringa oleifera* on the Lipid Profile of...
Normal and Hypercholesterolaemic Rabbits. J Ethnopharmacol. 2003; 86(2-3):191–195.

20. Nikkon F, Sanda ZA, Rehman MH, Haque ME. In Vitro Antimicrobial Activity of the Compound Isolated from Chloroform Extract of *M. oleifera* Lam. Pak J Biol Sci. 2003; 6 (22): 1888-1890.

21. Roger T, Mapongnetsem PM, Damme PV. Medicinal Plants Used Against Typhoid Fever in Bamboutos Division, Western Cameroon. Ethnobot Res Appl. 2013; 11:163–174.

22. Lamorde M, Tabuti JRS, Obua C, Kukunda BC, Lanyero H, Byakika KP et al. Medicinal Plants used by Traditional Medicine Practitioners for the Treatment of HIV/AIDS and Related Conditions in Uganda. J Ethnopharmacol 2010; 130(1): 43-53.

23. Odugbemisi T. A textbook of Medicinal Plants from Nigeria. Nigeria: University of Lagos Press, 2008; pp. 61, 588.

24. Adebayo JO, Krettli AU. Potential Antimalarials from Nigerian plants: A Review. J Ethnopharmacol. 2011; 133(2):289.

25. Igbo UE, Igoli JO, Onyiriuka SO, Ejele AE, Oguke CE, Ayuk AA. Antitrypanosomal and Antioxidant Activities of *Moringa oleifera* Lam. Leaf Extracts Pharm Chem Biol Sci. 2015; 3(1):17-23.

26. Fuglie LJ. The Miracle tree: *Moringa oleifera*: Natural Nutrition for the Tropics. Church World Service Dakar 1999; pp 68. Revised in 2001 and published as the Miracle Tree: The Multiple Attributes of moringa. 172 p.

27. Fahey JW, Zalcmann AT, Talalay P. The Chemical Diversity and Distribution of Glucosinolates and Isothiocyanates among Plants. Phytochem 2001; 56 (1):50-51.

28. Bennett RN, Mellon FA, Foidl N, Pratt JH, DuPont MS, Perkins L. Profiling Glucosinolates and Phenolics in Vegetative and Reproductive Tissues of the Multi-purpose trees *Moringa oleifera* L. (Horseradish tree) and *Moringa stenopetala* L. J Agric Food Chem 2003; 51(4):3546-3553.

29. Faizi S, Siddiqui BS, Saleem R, Siddiqui S, Aftab K, Gilani AH. Fully Acetylated Carbamate and Hypotensive Thiocarbamate Glycosides from *Moringa oleifera*. Phytochem. 1995; 38(4):957-963.

30. Trease GE and Evans WC. A textbook of Pharmacognosy. 14th ed. Bailliere Tindall Ltd. London, 1996.

31. Sofowora A. Medicinal Plants and Traditional Medicines in Africa. Chichester John Wiley & Sons New York, 1993. 97-145 p.

32. Chansakaow S, Ruangrungsi N, Ishikawa T. Isolation of Pyropheophorbide a from the Leaves of *Atalantia monophylla* (ROXB.) CORR. (Rutaceae) as a Possible Antiviral Active Principle against Herpes Simplex virus type 2. Chem Pharm Bull. 1996; 44:1415-1417.

33. Bauman WJ, Seufert J, Hayes HW, Holman RT. Mass Spectrometric Analysis of Long-Chain Esters of diols. J Lipid Res. 1969; 10(6):703-709.