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ISOLATION OF FRIEDELIN FROM THE FRUITS OF THE MEDICINAL PLANT HARUNGANA MADAGASCARIENSIS LAM. EX POIRET (HYPERICACEAE)

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ABSTRACT

In a continued drive to characterized the constituents of the fruits of *Harungana madagascariensis* a plant widely used in ethnomedicine, this study is aimed at a phytochemical screening–guided column chromatography fractionation and isolation of triterpenoids from the non-polar solvent extract/fraction of the fresh and air-dried fruits of *Harungana madagascariensis* Two extraction and isolation protocols: isolation from the dichloromethane fraction from the cold macerated crude absolute ethanol extract of the fresh fruits, and isolation from the n-hexane fraction of the cold percolation crude chloroform extract of the air-dried fruit were employed. The structure of the isolated triterpenoid was elucidated using spectroscopic techniques and by comparing the obtained spectra data with that reported in literature. The known pentacyclic isoprenoid ketone friedelin was isolated and characterized from both the fresh and air-dried fruits of *H. madagascariensis.* The yield obtained with the air-dried fruits was ninety fold higher compared to that with the fresh fruits. This study is reporting for the first time the isolation of friedelin a known plant derived bioactive triterpenoid from the fruits of *H. madagascariensis* a plant widely used in ethno-medicine.

KEYWORDS: *Harungana madagascariensis*, fruits, phytochemistry, friedelin, ethnomedicine.

INTRODUCTION

Harungana madagascariensis (Hypericaceae) is an evergreen bushy tree in the forest of most West African countries. It commonly called dragon blood tree. Its various morphological parts are widely used in Africa traditional medicine practice. It used in the treatment of dysentery, diarrhea, anemia, typhoid and heart ailments are documented.[1-6] There are literatures on the scientific evaluation of the antibacterial,[7-8] antiprotozoan,[9] and antioxidant.[10-12] activities of fruits, stem bark, roots and leaves of *H. madagascariensis*. Extensive report on the phytochemical composition of different morphological parts,[7-9] are documented except fruits and flowers. Recently, a report for the first time on the characterization of the fatty acid constituents of the fruit oil,[13] in addition to earlier reports on the antibacterial.[8] and proximate and antioxidant.[12] properties of the fruits of this plant, are the few apparent scientific documentation available. As a follow-up to these earlier report.[8,12-13] and in a bid to isolate and characterize the constituents of the fruits of this plant, this present study aimed at the isolation of triterpenoids, reports for the first time the isolation and structural elucidation of the known pentacyclic triterpenoid ketone friedelin from the fruits of *Harungana madagascariensis* using chromatography and spectroscopic techniques.

MATERIALS AND METHODS

Sample collection and authentication

Fresh fruits of *Harungana madagascariensis* used for this study were collected from the Medicinal Garden, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Rivers State and authenticated by taxonomist in the herbarium of the Plant Science and Biotechnology Department, University of Port Harcourt with herbarium number: UPH/P/080; UPH/V/1,219. They were air-dried under ambient room condition, pulverized and used for this study.

Reagents, solvents, equipment and apparatus used

All reagents used were of analytical grade and include: chloroform, n-hexane, acetone, methanol, deuteriated DMSO, concentrated sulphuric acid, acetic anhydride. Equipment used include: rotary evaporator, Nuclear Magnetic Resonance spectrometer,(Bruker Avance 500 MHz).

Extraction and isolation protocol for the fresh fruit

Fresh and pulverized fruits (600 g) was extracted exhaustively with absolute ethanol by cold maceration for 8 days with fresh replacement every 48 hours. The absolute ethanol extract obtained was concentrated using the rotary evaporator at temperature of 45^{o}C and partitioned with dichloromethane using a separating flask

to afford the dichloromethane HMD and aqueous HMA soluble fractions. The triterpenoid (C-30 isoprenoid) containing dichloromethane soluble fraction HMD was further subjected to vacuum liquid column chromatography separation using the mobile phase gradient (n-hexane: dichloromethane-1:0, 1:1, 0:1 v/v). At the conclusion of the elution with the stated gradient of mobile phases, a total of twenty-nine eluates were collected which were later pooled into eight fractions (pF1-pF8) based on similarities in colour reaction with chromogenic reagents and retardation factors Rf from thin layer chromatography examination. From the tritepenoid containing pooled fraction pF4 eluted as un-pooled fractions 7 and 8 with n-hexane: dichloromethane (1:1) was isolated a white crystalline solid coded Hf (yield 6 mg = 0.001 %w/w) after recrystalisation with acetone. Purity was ascertain by melting point determination recorded on an electrothermal melting point apparatus and are uncorrected in addition to having a single spot after several conditions of TLC examination.

Extraction and isolation protocol for the air-dried fruit

Air dried and pulverized fruits (138.5 g in duplicate of 70.7 and 67.8 g) were extracted exhaustively with chloroform by cold percolation. Briefly a menstrum of the pulverized samples were transferred into a 250 ml percolation tank and filled to the mark with chloroform. It was then allowed to stand with the tap closed for 24 hours after which the chloroform extract was drained into a clean empty conical flask. The process was repeated two more times for exhaustive extraction. The combined chloroform extract obtained was concentrated using the rotary evaporator at temperature of 45^{o}C and the dried chloroform extract coded HMCE further fractionated by exhaustive cold maceration in n-hexane. The n-hexane extract was then concentrated using the rotary evaporator at temperature of 45^{o}C to obtain a viscous oily mass coded HMHS. The constituents of the n-hexane fraction HMHS were subsequently separated using dried packed normal phase silica gel (200-400 mesh size) column chromatography with n-hexane-chloroform 5 % stepwise gradient following an earlier reported protocol.[13] Briefly, The eluates were collected at intervals of 50 ml and from fractions 7-19 eluted with the mobile phase gradient n-hexane:chloroform (95:5 to 85:15 v/v) was isolated a white crystalline solid Hd (yield 125 mg = 0.09 % w/w) after washing with acetone to remove fatty and phenolic impurities. Purity was ascertain by melting point determination recorded on an electrothermal melting point apparatus and are uncorrected in addition to having a single spot after several conditions of TLC examination.

Phytochemical Screening

Confirmatory triterpenoid phytochemical tests was done using the Liebermann-Buchard and Salkowski's phytochemical screening reagents.[14-15]

Nuclear Magnetic resonance spectroscopy analysis

The ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz (125 MHz for ^{13}C NMR analysis) on a Bruker Avance NMR spectrometer in deuterated $CDCl_3$. Chemical shifts are expressed in parts per million (ppm).

RESULTS AND DISCUSSION

As expected the yield of the compound friedelin was: 125 mg (0.09 % w/w) and 6 mg (0.001 %w/w) for the air-dried and fresh fruits respectively. This showed that prior drying of the fruits before extraction and isolation of friedelin will result in approximately ninety fold higher yield compared to when the fresh fruits is used. The identity of the white crystalline solid compound Hf and Hd isolated from the fresh and dried fruits respectively was confirmed to be the same after TLC examination using TLC technique with several mobile phases with representative specimen as shown in Figure 1. The compound Hd/Hf gave a positive Lieberman test, indicative of a triterpenoidal nucleus.

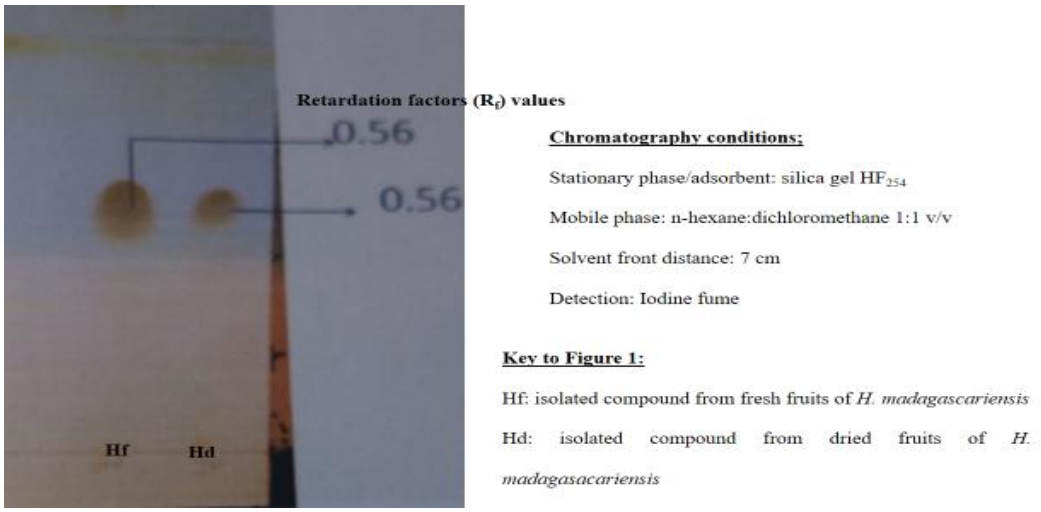
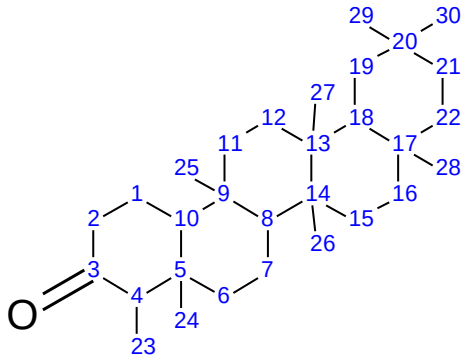


Figure 1: Representative thin layer chromatography profile of compounds Hf and Hd isolated respectively from the fresh and dried fruits of *H. madagascariensis*



Compound Hf/Hd: Friedelin (Synonym friedalan-3-one) isolated from the fruits of *H. madagascariensis*

The NMR (^{1}H and ^{13}C) spectra as rationalized in Table 1 are evident with eight angular methyl protons one of which is a doublet (H23), eleven methylene protons and four methine protons signals from ^{1}H-NMR analysis and from the ^{13}C-NMR/DEPT-135 analysis a total of 30 carbons with corroborating evidence for eight methyl(CH_3), eleven methylene (CH_2), four methine (CH), six quartenary (C) and one ketone (C=O ; δc 213.10ppm; C3) carbon signals from the DEPT-135 analysis.

Table 1: NMR spectral data of the compound Hd/Hf isolated from *H. madagascariensis* fruits, compared with that of friedelin in Literature in $CDCl_3$ (δ in ppm).

Position	δ_H ppm	δ_H ppm literature[16]	δ_C ppm	DEPT-135	δ_C ppm literature[16]
1	1.97, 1.79	1.95, 1.71	22.30	CH_2	22.3
2	2.39, 2.27	2.37, 2.27	41.54	CH_2	41.5
3	-	-	213.10		213.20
4	2.26	2.25	58.23	CH	58.20
5	-	-	42.15		42.10
6	1.72, 1.28	1.73, 1.28	41.29	CH_2	41.50
7	1.48, 1.36	1.49, 1.36	18.24	CH_2	18.20
8	1.38	1.38	53.10	CH	53.10
9	-	-	37.45		37.40
10	1.52	1.53	59.47	CH_2	59.50
11	1.48, 1.27	1.45, 1.26	35.63	CH_2	35.60
12	1.32, 1.30	1.33, 1.33	30.51	CH_2	30.50
13	-	-	39.70		39.70
14	-	-	38.30		38.30
15	1.48, 1.28	1.47, 1.27	32.42	CH_2	32.40
16	1.58, 1.35	1.58, 1.35	36.01	CH_2	36.00
17	-	-	30.00		30.00
18	1.56	1.56	42.79	CH	42.80
19	1.37, 1.20	1.37, 1.21	31.94	CH_2	35.30
20	-	-	28.18		28.20
21	1.50, 1.32	1.50, 1.31	32.77	CH_2	32.80
22	1.50, 0.97	1.50, 0.94	39.26	CH_2	39.20
23	0.90 (3Hd)	0.88	6.84	CH_3	6.80
24	0.74(3Hs)	0.71	14.66	CH_3	14.60
25	0.89(3Hs)	0.86	17.96	CH_3	17.90
26	1.02(3Hs)	1.00	20.27	CH_3	20.20
27	1.07(3Hs)	1.04	18.68	CH_3	18.60
28	1.20(3Hs)	1.17	32.10	CH_3	32.10
29	1.02(3Hs)	0.99	35.04	CH_3	35.00
30	0.97(3Hs)	0.94	31.79	CH_3	31.80

Key: 3Hs singlet methyl protons signal; 3Hd: doublet methyl protons signal

When these observed spectra (^{1}H and ^{13}C the NMR chemical shifts) data for Hd/Hf were compared with literature reports for friedelin,[16] they were observed to be similar. Report on the isolation of friedelin from the stem bark of this plant.[1] have been documented. Friedelin (synonym friedalan-3-one) is an isoprenoid of

the triterpenoid class. It is a triterpene ketone and has been found to be one of the most ubiquitous triterpenoids in nature. It was first isolated in abundant amount from the bark of the cork tree *Quercus suber* L. (Fagaceae). Since then, it has been isolated from several other plants.[17-26] Several biological activities have been reported for friedelin isolated from various medicinal plants some of which include: *in-vivo* analgesic, anti-pyretic and anti-inflammatory activities.[27] Others documented biological activities include: its antibacterial,[24,28] anti-fungal,[29] anti-*Plasmodium* falciparum,[30] antioxidant,[31] pesticidal,[32] and antidiabetic.[33] Considering these diverse biological properties of friedelin, its presence in the fruits could offer an explanation for some of the reported use of *H. madagascariensis* in ethnomedicine.

CONCLUSION

This study is reporting for the first time the isolation of the known pentacycli triterpenoid friedelin from the fruits of *H. madagascariensis* a plant widely used in ethno-medicine. This finding aside offering a rationale for some of the ethnomedicinal uses of this plant as well as being of chemosystemic relevance could be utilized as biomarker for standardization of medicinal herbal preparations containing this plant.

CONFLICT OF INTEREST

There is no conflict of interest as regards the publication of this manuscript.

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REFERENCES

1. Happi GM, Tiani GLM, Gbetnkom BYM, Hussain H, Green IR, Ngadjui BT, Kouam SF. Phytochemistry and Pharmacology of *Harungana madagascariensis Phytochemistry Letters*, 2020; 35(2020): 103-112.
2. Tona, L., Kambu, K., Ngimbi, N., Mesia, K., Penge, O., Lusakibanza, M., Cimanga, K., De Bruyne,T., Apers, S., Totte, J., Pieters, L.,Vlietinck, A.J.. Antiamoebic and spasmolytic activities of extracts from some antidiarrhoeal traditional preparations used in Kinshasa. Congo. *Phytomedicine*, 2000; 7: 31–38.
3. Kengni, F., Fodouop, S.P.C., Tala, D.S., Djimeli, M.N., Fokunang, C., Gatsing, D. Antityphoid properties and toxicity evaluation of *Harungana madagascariensis* Lam (Hypericaceae) aqueous leaf extract. *Journal of Ethnopharmacology*, 2016; 179: 137–145.
4. Irvine,F.R..Woody Plants of Ghana. Oxford University Press, London, 1961.
5. Olagunju, J.A., Oladunni, S.O., Oladimeji, M.S. Status of phosphatase activities in the liver and kidney of rats treated with isosaline leaf and stem-bark extracts of *Harungana madagascariensis* (L). *Cytobios*, 2000; 103: 17–24.
6. Prajapati, N.D., Purohit, S.S., Kumar, T., A Handbook of Medicinal Plants. A Complete Source Book. Agrobios, India, 2003; 262.
7. Okoli, A.S., Okeke, M.I., Iroegbu, C.U., Ebo, P.U. Antibacterial activity of *Harungana madagascariensis* leaf extracts. *Phytotherapy. Research*, 2002; 16, 174–179.
8. Afieroho OE, Izontimi SS. Okoroafor DO, & Caleb B. Antibacterial and phytochemical evaluation of *Harungana madagascariensis* (Hypericaceae) seeds. *International Research Journal of Pharmacy*, 2012; 3(11): 75-77.
9. Iwalewa, E.O., Omisore, N.O., Adewunmi, C.O., Gbolade, A.A., Ademowo, O.G., Nneji, C., Agboola, O.I., Daniyan, O.M. Anti-protozoan activities of *Harungana madagascariensis* stem bark extract on trichomonads and malaria. *Journal of Ethnopharmacology*, 2008; 117: 507–511.
10. Antia BS, Ita BN, & Udo UE. Nutrient Composition and in vitro Antioxidant Properties of *Harungana madagascariensis* Stem bark Extracts. *Journal of Medicinal Food*, 2015; 18(5): 609–614.
11. Afieroho OE & Afieroho MC. Evaluation of the nitric oxide scavenging potential of *Harunguna madagascariensis* Lam ex poir (Hypericaceae) fruits oil. *The Pharma Innovation Journal*, 2017; 6(2): 171-173.
12. Afieroho OE, Ajuzie JI, and Afieroho MC. Proximate composition and evaluation of some antioxidant properties of the fresh fruits of *Harungana madagascariensis* Lam. Ex Poir. (Hypericaceae). *Research Journal of Food Science and Nutrition*, 2019; 4(6): 97-102.
13. Afieroho OE, Okonkwo TJN, Shorinwa OA. Okonkwo CJ, Ajibade S & Afieroho MC. *Harungana madagascariensis* Lam ex poir (Hypericaceae) fruits oil extract: Phytochemistry and acute toxicity evaluation. *International Journal for Pharmaceutical Sciences and Research*, 2017; 8(6): 2539-2544.
14. Harborne JB. Phytochemical methods-a guide to modern techniques of plant analysis. 3rd edition, Chapman and Hall, London, UK, 1998; 302.
15. Houghton PJ, & Raman A. Laboratory handbook for the fractionation of natural extracts. Chapman and Hall, London, UK., 1999.
16. Mahato, S.B.,& Kundo A.P.. ^{13}C-NMR spectra of pentacyclic triterpenoids—a compilation and some salient features. *Phytochemistry*, 1994; 37: 1517–1575.
17. Quintans, JS, Costa, EV, Tavares, JF, Souza, TT, Araújo, SS, Estevam, CS, Barison, A, Cabral, AG, Silva, MS and Serafini, MR. Phytochemical study

and antinociceptive effect of the hexanic extract of leaves from Combretum duarteanum and friedelin, a triterpene isolated from the hexanic extract, in orofacial nociceptive protocols. *Revista Brasileira de Farmacognosia*, 2014; 24(1): 60-66**.**

18. Queiroga, CL, Silva, GF, Dias, PC, Possenti, A and de Carvalho, JE. Evaluation of the antiulcerogenic activity of friedelan-3β-ol and friedelin isolated from Maytenus ilicifolia (Celastraceae). *Journal of Ethnopharmacology*, 2000; 72(3): 465-468
19. Sousa, GFd, Soares, DCF, Mussel, WdN, Pompeu, NFE, Silva, GDdF, Vieira Filho, SA and Duarte, LP. Pentacyclic triterpenes from branches of Maytenus robusta and in vitro cytotoxic property against 4T1 cancer cells. *Journal of the Brazilian Chemical Society*, 2014; 25(8): 1338-1345.
20. Abah, JO, Musa, KY, A, A, M.E, H, Bulama, JS and Abubakar, MS. A Friedelane Type Triterpene From Prosopis africana (Guill. & Perr.) Taub. Stem Bark. *Journal of Natural Sciences Research*, 2014; 4(1): 107-111.
21. Setzer, WN, Shen, X, Bates, RB, Burns, JR, MCClure, KJ, Zhang, P, Moriarity, DM and Lawton, RO. A phytochemical investigation of Alchornea latifolia. *Fitoterapia*, 2000; 71(2): 195-198.
22. Torres-Romero D, King-Diaz B, Strasser RJ, Jimenez IA, Lotina-Hennesen B and Bazzocchi IL. Friedelane tritepenes from *Celastrus vulcanicola* as photosynthetic inhibitors. *Journal of Agricultural and Food Chemistry*, 2010; 58(20): 10847-10854.
23. Siwe-Noundou, X, Krause, R, Van Vuuren, S, Ndinteh, DT and Olivier, D. Antibacterial effects of Alchornea cordifolia (Schumach. and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. *Journal of Ethnopharmacology*, 2016; 179: 76-82.
24. Jiang, R-W, Ma, S-C, He, Z-D, Huang, X-S, But, PP-H, Wang, H, Chan, S-P, Ooi, VE-C, Xu, H-X and Mak, TC. Molecular structures and antiviral activities of naturally occurring and modified cassane furanoditerpenoids and friedelane triterpenoids from *Caesalpinia minax*. *Bioorganic & medicinal chemistry*, 2002; 10(7): 2161-2170.
25. Olmedo, DA, López-Pérez, JL, Del Olmo, E, Vásquez, Y, San Feliciano, A and Gupta, MP. A new cytotoxic friedelane acid–pluricostatic acid–and other compounds from the leaves of Marila pluricostata. *Molecules*, 2008; 13(11): 2915-2924.
26. Mann, A, Ibrahim, K, Oyewale, AO, Amupitan, JO, Fatope, MO and Okogun, JI. Antimycobacterial friedelane-terpenoid from the root bark of Terminalia avicennioides. *American Journal of Chemistry*, 2011; 1(2): 52-55.
27. Antonisamy, P, Duraipandiyan, V and Ignacimuthu, S. Anti-inflammatory, analgesic and antipyretic effects of friedelin isolated from Azima tetracantha Lam. in mouse and rat models. *Journal of pharmacy and pharmacology*, 2011; 63(8): 1070-1077.
28. Kuete, V, Nguemeving, JR, Beng, VP, Azebaze, AGB, Etoa, F-X, Meyer, M, Bodo, B and Nkengfack, AE. Antimicrobial activity of the methanolic extracts and compounds from *Vismia laurentii* De Wild (Guttiferae). *Journal of Ethnopharmacology*, 2007; 109(3): 372-379.
29. Duraipandiyan, V, Gnanasekar, M and Ignacimuthu, S. Antifungal activity of triterpenoid isolated from Azima tetracantha leaves. *Folia histochemica et cytobiologica*, 2010; 48(2): 311-313.
30. Ngouamegne, ET, Fongang, RS, Ngouela, S, Boyom, FF, Rohmer, M, Tsamo, E, Gut, J and Rosenthal, PJ. Endodesmiadiol, a friedelane triterpenoid, and other antiplasmodial compounds from Endodesmia calophylloides. *Chemical and Pharmaceutical Bulletin*, 2008; 56(3): 374-377.
31. Sunil, C, Duraipandiyan, V, Ignacimuthu, S and Al-Dhabi, NA. Antioxidant, free radical scavenging and liver protective effects of friedelin isolated from Azima tetracantha Lam. leaves. *Food chemistry*, 2013; 139(1-4): 860-865.
32. González-Coloma, A, López-Balboa, C, Santana, O, Reina, M and Fraga, BM. Triterpene-based plant defenses. *Phytochemistry Reviews*, 2011; 10(2): 245-260.
33. Ardiles, AE, González-Rodríguez, Á, Núñez, MJ, Perestelo, NR, Pardo, V, Jiménez, IA, Valverde, ÁM and Bazzocchi, IL. Studies of naturally occurring friedelane triterpenoids as insulin sensitizers in the treatment type 2 diabetes mellitus. *Phytochemistry*, 2012; 84: 116-124.