GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

A triterpene ketone from an anti-mycobacterial tuberculosis chromatography eluate from the n-hexane fraction of the fruits of *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae)

Onyinye Blessing Okonkwo [1, 2], Ozadheoghene Eriarie Afieroho [2, 3, *], John Samson Bimba [4, 5], Timan Taryuta Eliya [5], Augustina Uche Osuji [5] and Kio Anthony Abo [2]

[1] *Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Chukwuemeka Odumegwu Ojukwu University, Igbariam Campus.*
[2] *Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. Port Harcourt, Nigeria.*
[3] *Nuclei for Phytomedicines and Chemical Ecology (NuPaCE), Central Research Laboratory for Phytomedicine, Department of Pharmacognosy and Phytotherapy, University of Port Harcourt. Port Harcourt, Nigeria.*
[4] *Department of Community Medicine, Bingham University, Nassarawa, State, Nigeria.*
[5] *Tuberculosis Reference Laboratory, Zankli Research Centre, Bingham University, Nassarawa State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2022, 18(02), 259–267

Publication history: Received on 07 January 2022; revised on 16 February 2022; accepted on 18 February 2022

Article DOI: https://doi.org/10.30574/gscbps.2022.18.2.0064

Abstract

Clinical cases of drug resistant tuberculosis (TB) are a global threat because of the cost and extended duration of treatment regimen. This underscores the continuous drive to discover new therapeutic agents that are faster, simpler and affordable often by recourse to natural products. This report highlights the potentials of the Nigerian medicinal plant *Harungana madagascariensis* Lam. Ex Poiret (Hypericaceae) as a source of lead agents for the discovery and development of anti-tuberculosis drugs. The fruit of *H. madagascariensis* was extracted with 70% aqueous ethanol by cold maceration. The crude 70 % aqueous ethanol extract was partitioned with n-hexane to give n-hexane (NHF) soluble portions. The NHF was fractionated further on a column packed with normal phase silica gel (200-400 Mesh size) to afford the chromatography fractions used in this study. The structure of isolated compound was elucidated using spectroscopic (NMR, IR, and MS) techniques. Anti-*Mycobacteria tuberculosis* susceptibility screening of the chromatography fractions was done using Lowenstein Jensen method with rifampicin, ethambutol, isoniazid and dihydrostreptomycin as reference anti-TB drugs for comparison. Four fractions NHF1-4 were obtained from the NHF. The chromatography fractions: NHF2 and NHF3 inhibited the growth of the *Mycobacteria tuberculosis.* From the NHF2 was isolated the known bioactive triterpene ketone friedelan-3-one. This preliminary screening underscores the potentials of this ethno medicinal plant as a source of lead compounds for the development of anti-TB drugs.

Keywords: *Harungana madagascariensis*; Hypericaceae; Fruits; Anti-tuberculosis agents; Friedelan-3-one

1. Introduction

Tuberculosis (TB), a chronic and deadly infectious disease caused by the bacillus *Mycobacterium tuberculosis* infects one third of the world population and kills about 15 million people yearly. The global World Health Organization (WHO) reports showed that an estimated 10 million people developed TB with 1.4 million deaths recorded in 2019. Men accounted for 56%, women 32%, and children 12% [1]. Multi-Drug Resistance TB (MDR-TB) is defined by resistance to

*Corresponding author: Afieroho OE
Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt. Port Harcourt, Nigeria.

the two most commonly used drugs (isoniazid and rifampicin) in the current four drug (or first line) regimen, while a TB strain is called Extensively Drug Resistance -TB (XDR-TB) when it becomes resistant to the four first line drugs. This is a huge global threat because of the cost of the medicine and number of years involved for the drug to work. A 2015 United Kingdom parliamentary group report by Astrid [2] stated that MDR-TB could cost the world $16.7 trillion by 2050. Thus, new treatments that are faster, simpler and affordable are urgently needed.

Nature is blessed with plant secondary metabolites with diverse biological activities including anti-microbial such as anti-*Mycobacteria tuberculosis* among others. Research into natural products that has antimicrobial activities have contributed immensely to the discovery and development of drugs for the treatment of infections caused by pathogenic microbes. *Harungana madagascariensis*, a tropical shrub found in Africa has been used in ethnomedicinal practice to treat bacterial infections [3-4], gastrointestinal diseases [5] as well as cough and bronchial distress [6]. This preliminary work reports the anti-*Mycobacteria tuberculosis* potentials of the fruits of *H. madagascariensis.*

2. Material and methods

2.1. Materials

Reagents and solvents used in this study were of analytical grade and are products of JHD Chemicals, China. Standard control drugs used include ethambutol [99%], rifampicin [99%], isoniazid [99%] and dihydrostreptomycin [99%]. The clinical strain of *Mycobacteria tuberculosis* used were supplied and cultured by the Tuberculosis Reference Laboratory, Zankli Research Centre, Bingham University, Nassarawa State, Nigeria.

2.2. Sample collection and extraction

The matured fruits of *H. madagascariensis* were collected from the Medicinal plant Garden of the Department of Pharmacognosy and Phytotherapy, University of Port Harcourt, Nigeria and identified by Taxonomist with voucher specimen deposited at the herbarium of the same department. It was air dried and pulverized. A 600 g quantity of the pulverized sample was extracted with 70% aqueous ethanol by cold maceration for 72 hours with filtration and change of solvent done every 24 hours. After the extraction, it was concentrated with rotary evaporator at 40^0C to at least one-tenth of its volume. The crude aqueous ethanol extract was partitioned exhaustively with n-hexane to obtain the lipid-rich n-hexane fraction (NHF) used for this study.

2.3. Fractionation of N-hexane fraction

Separation of NHF was done using chromatography techniques. Briefly, 6.2 g of NHF was pre-adsorbed by mixing in silica gel (4 g, normal phase, Mesh 200-400) and loaded on a column (internal diameter 4 cm), dry packed with same silica gel as adsorbent to a height of 15 cm. The mobile phase gradient (500 ml of each) used comprised of n-hexane (4:0 v/v); n-hexane: DCM (3:1, 2:2, 1:3, 0:4 v/v); DCM: ethanol (3:1, 2:2 v/v). The eluted fractions were collected at 10 ml intervals and pooled based on observed R_f of resolved spots and color reaction with chromogenic spray reagent from TLC. Four pooled fractions were obtained and were designated NHF 1-4. All the fractions were screened for anti-*Mycobacteria tuberculosis* activity.

2.4. Anti-*Mycobacteria tuberculosis* susceptibility test

This was done using the Lowenstein-Jensen solid based agar dilution method reported by Selvakumar [7]. Briefly, the freshly prepared LJ medium (100 ml) was mixed with the test (chromatography fraction) sample (1 mg/ml, 1 ml). About 7-8 ml of the re-constituted test sample-LJ mixture was then dispensed into sterile bijou tubes and allowed to inspissate for 45 minutes on a water bath at 85^0C in a slanting position. The reference standard drugs: Isoniazid (0.02 mg/ml), dihydrostreptomycin (0.80 mg/ml), ethambutol (0.20 mg/ml) and rifampicin (8.00 mg/ml) were similarly re-constituted as described for the test samples. 1.0 McFarland of de-contaminated clinical isolates of *Mycobacterium tuberculosis* (MTB) was prepared in sterile distilled water while 10 µL of the innoculum concentrations was inoculated on the separate standard drugs, phytodrugs, and negative control reconstituted LJ media slant and incubated for six weeks at 37^0C. Each sample analysis was done in duplicate. Where a test substance showed inhibition with greater than '19'colonies, it is taken as not being active. Where a no colony growth was observed, the test substance is taken as bactericidal.

3. Results

3.1. Bioactivity of fractions from n-Hexane extract

Table 1 Anti-*Mycobacteria tuberculosis* activities of chromatography fractions from n-hexane soluble portion (NHF) of *H. madagascariensis* fruits

Test samples	Eluting mobile phase gradient	Anti-*Mycobacteria tuberculosis* activity
NHF1	n-hexane: Dichloromethane (4:0 v/v)	Inc
NHF2	n-hexane: Dichloromethane (3:1- 1:3 v/v)	+
NHF3	n-hexane: Dichloromethane (0:4 v/v)	+
NHF4	Dichloromethane: Ethanol (3:1-2:2 v/v)	-
Ethambutol	Not applicable	+
Isoniazid	Not applicable	+
Rifampicin	Not applicable	+
Dihydro-streptomycin	Not applicable	+

NHF1-4 are chromatography fractions from NHF; Inc = inconclusive; + = active; - = inactive

3.2. Characterization of isolated compound, friedelan-3-one

Table 2 NMR spectra data of the compound isolated from NHF2 of *H. madagascariensis* fruits compared with that of friedelin in Literature

Position	HSQC (δ_H ppm)	δ_H ppm literature*	δC ppm	δC ppm literature*
1	1.97, 1.79	1.95, 1.71	22.30	22.3
2	2.39, 2.27	2.37, 2.27	41.54	41.5
3	-	-	213.30	213.2
4	2.26	2.25	58.23	58.20
5	-	-	42.16	42.10
6	1.72, 1.28	1.73, 1.28	41.29	41.50
7	1.48, 1.36	1.49, 1.36	18.24	18.20
8	1.38	1.38	53.11	53.10
9	-	-	37.45	37.40
10	1.52	1.53	59.48	59.50
11	1.48, 1.27	1.45, 1.26	35.63	35.60
12	1.32, 1.30	1.33, 1.33	30.51	30.50
13	-	-	39.70	39.70
14	-	-	38.30	38.30
15	1.48, 1.28	1.47, 1.27	32.43	32.40
16	1.58, 1.35	1.58, 1.35	36.01	36.00
17	-	-	30.01	30.00
18	1.56	1.56	42.79	42.80

19	1.37, 1.20	1.37, 1.21	35.35	35.30
20	-	-	28.18	28.20
21	1.50, 1.32	1.50, 1.31	32.77	32.80
22	1.50, 0.97	1.50, 0.94	39.26	39.20
23	0.90 (3Hd)	0.88	6.84	6.80
24	0.74(3Hs)	0.71	14.67	14.60
25	0.89(3Hs)	0.86	17.96	17.90
26	1.02(3Hs)	1.00	20.27	20.20
27	1.07(3Hs)	1.04	18.68	18.60
28	1.20(3Hs)	1.17	32.10	32.10
29	1.02(3Hs)	0.99	35.04	35.00
30	0.97(3Hs)	0.94	31.79	31.80

* Afieroho and Ajuzie [8]

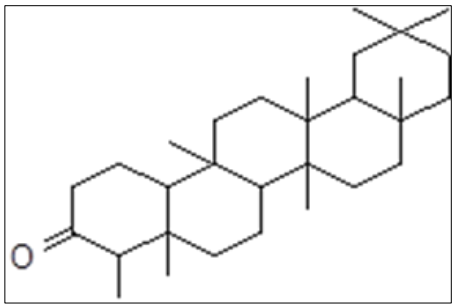

Figure 1 Chemical structure of friedelan-3-one isolated from chromatography fraction NHF2

4. Discussion

The increasing prevalence of drug resistant TB coupled with the no discovery and bench-to market development of newer and more potent anti-TB drugs in the past three decades have made it more imperative to search for new and novel anti-TB lead compounds from nature. In this study, the four chromatography fractions NHF1-4 obtained from the n-hexane soluble portion of the 70 % crude ethanol extract from the fruits of *Harungana madagascariensis* were evaluated for anti-TB activity by agar dilution using the LJ-method. Two fractions NHF2 and NHF3 showed bactericidal activity as shown in Table 1. For these two bioactive fractions NHF1 and NHF2, the observed bacteriocidal effect at the test concentration of 1mg/ml was comparable to that observed for the four standard anti-TB drugs isoniazid, rifampicin, ethambutol and dihydrostreptomycin. This may be due to the bioactive constituents. Further purification of the bioactive fraction NHF2 resulted in the isolation of a creamy to off-white solid. This isolated compound was positive to Liebermann-Burchard test and showed from infra-red (IR) spectroscopy the characteristic aliphatic C-H asymmetric and symmetric stretching vibrations in the region 3002.78 - 2867.64 cm^{-1} (see Figure 2). Also from the IR spectrum is the evidence of the carbonyl (C=O) stretching vibration at 1713.54 giving a hint that the isolated compound is a triterpene ketone. The one dimensional ^{1}H-NMR (500 MHz, $CDCl_3$, δ ppm), ^{13}C-NMR (125 MHz, $CDCl_3$ δ ppm) and DEPT-135 spectra (see Figures 3, 4 and 5) together with the two-dimensional NMR spectra (H-H-COSY, HSQC and HMBC see Figures 6, 7 and 8) are evident for eight angular methyl (CH_3), four ring methine (CH), eleven ring methylene (CH_2), six ring quaternary carbon (>C<) and one carbonyl (C=O) as rationalized in Table 2. These spectra data confirmed the chemical structure to be that of the known bioactive triterpene ketone friedalan-3-one with the observed spectra data being in agreement with previous literature report on the phytochemistry of the fruits [8].

Friedelan-3-one (Synonyms: friedelin) is a saturated pentacyclic triterpene ketone [9]. It has been isolated from several plant parts such as of: *Azima tetracantha* [10], *Quercus species* [11], *Terminalia avicennioides* [12], *Harungana madagascariensis* [8] and *Malus domestica* L [13] among others. Friedelin has been reported to possess many biological

activities such as antifeedant and anti-inflammatory activities [14], hepatoprotective, antimicrobial including antimycobacteria activities [15].

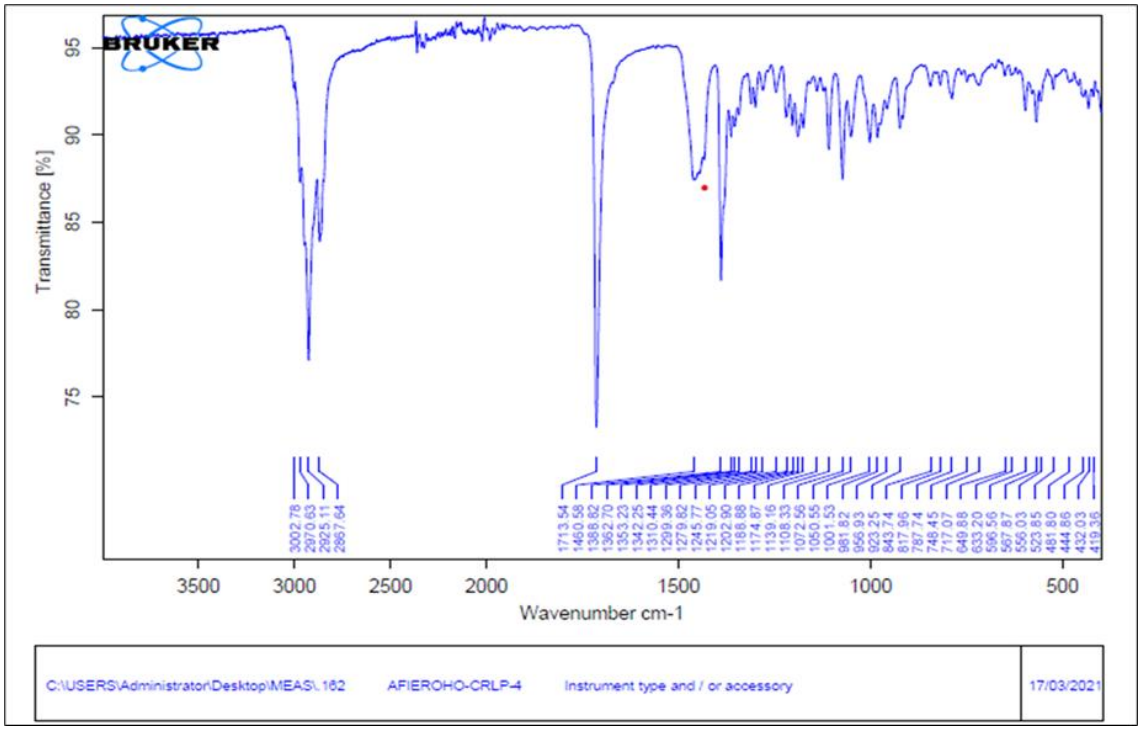

Figure 2 Infra-red spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

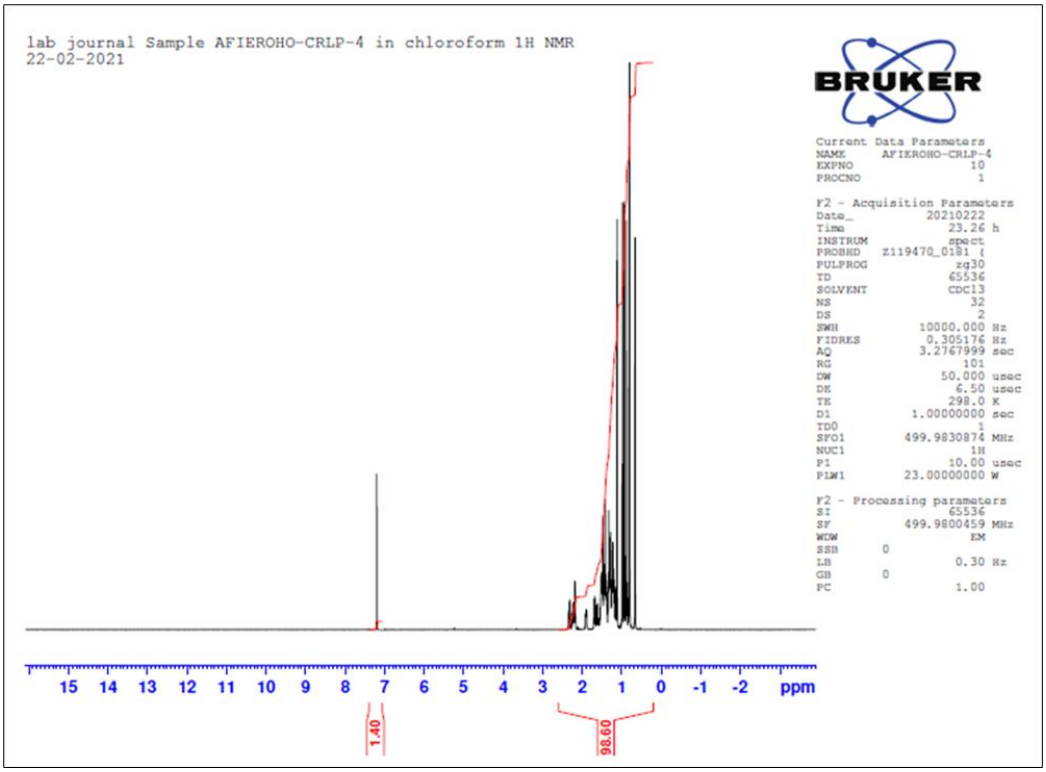

Figure 3 ^{1}H-NMR spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis Lam*. Ex. Poiret

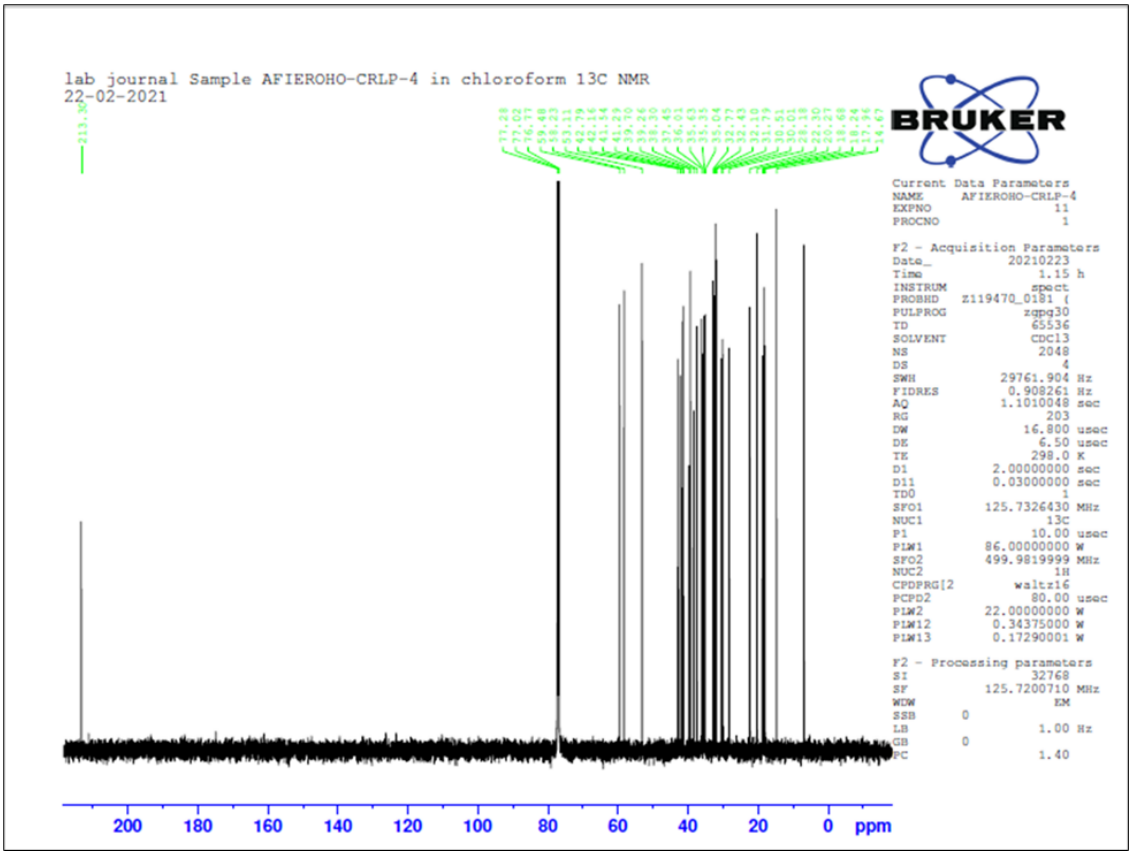

Figure 4 ^{13}C-NMR spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

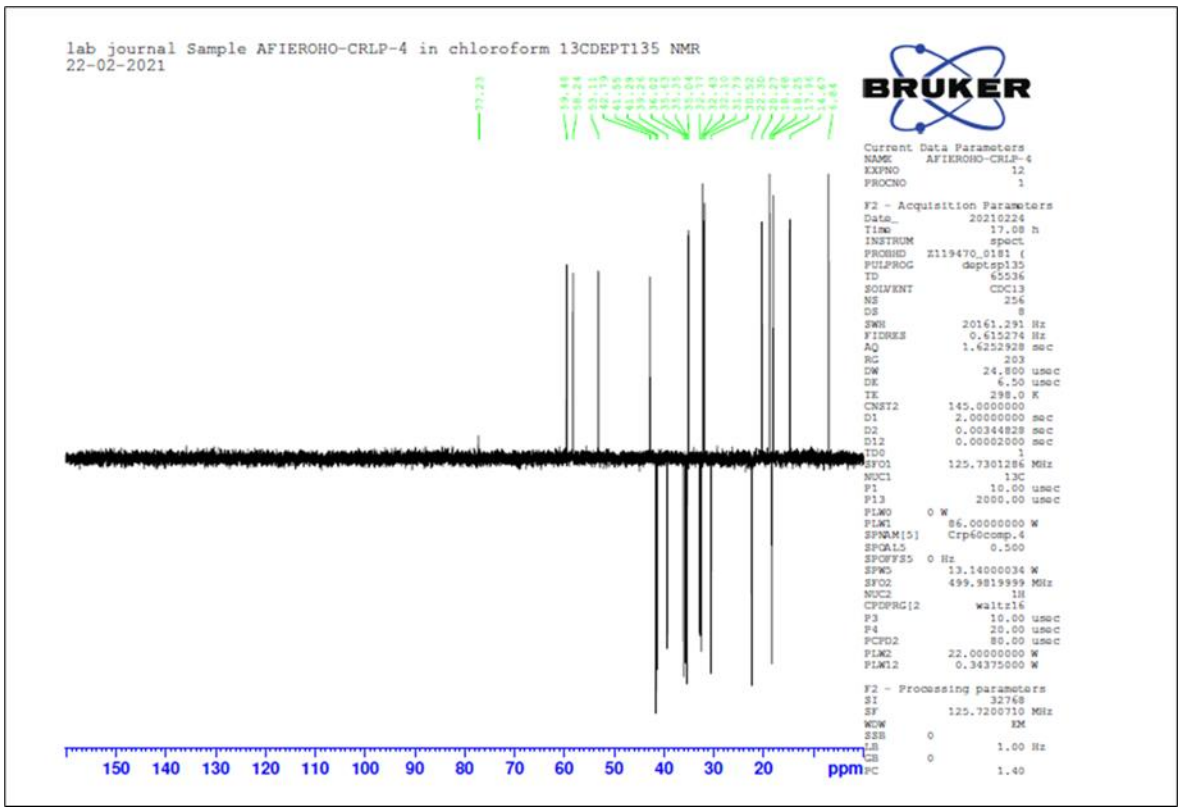

Figure 5 DEPT-135 NMR spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

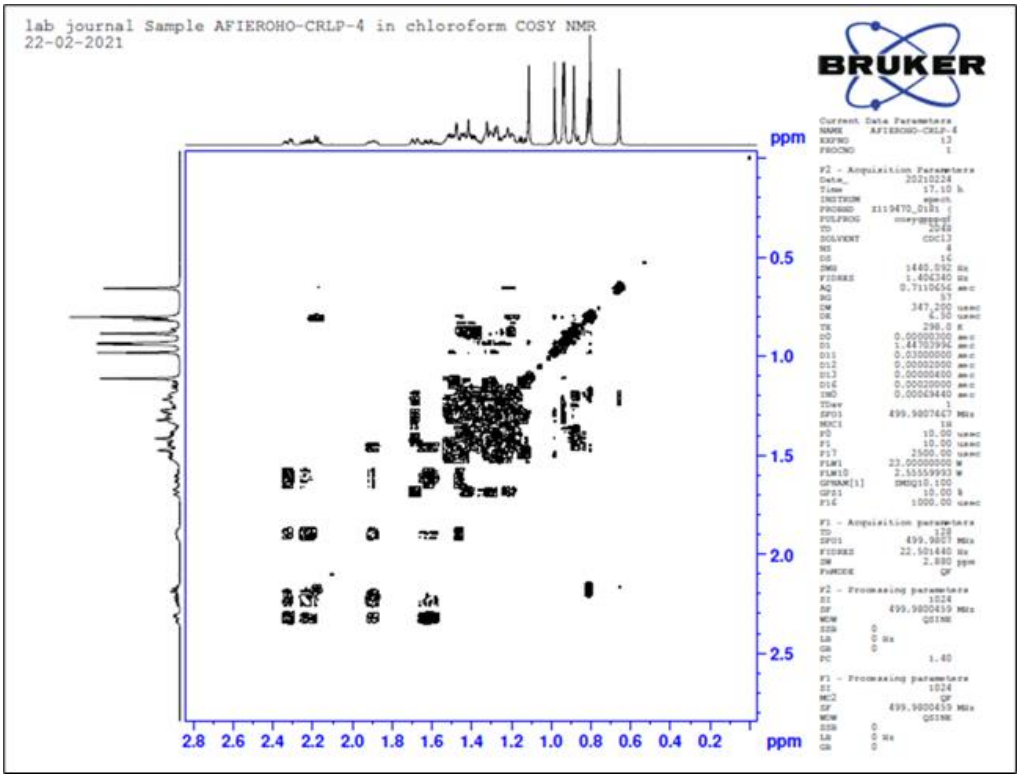

Figure 6 H-H-COSY spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

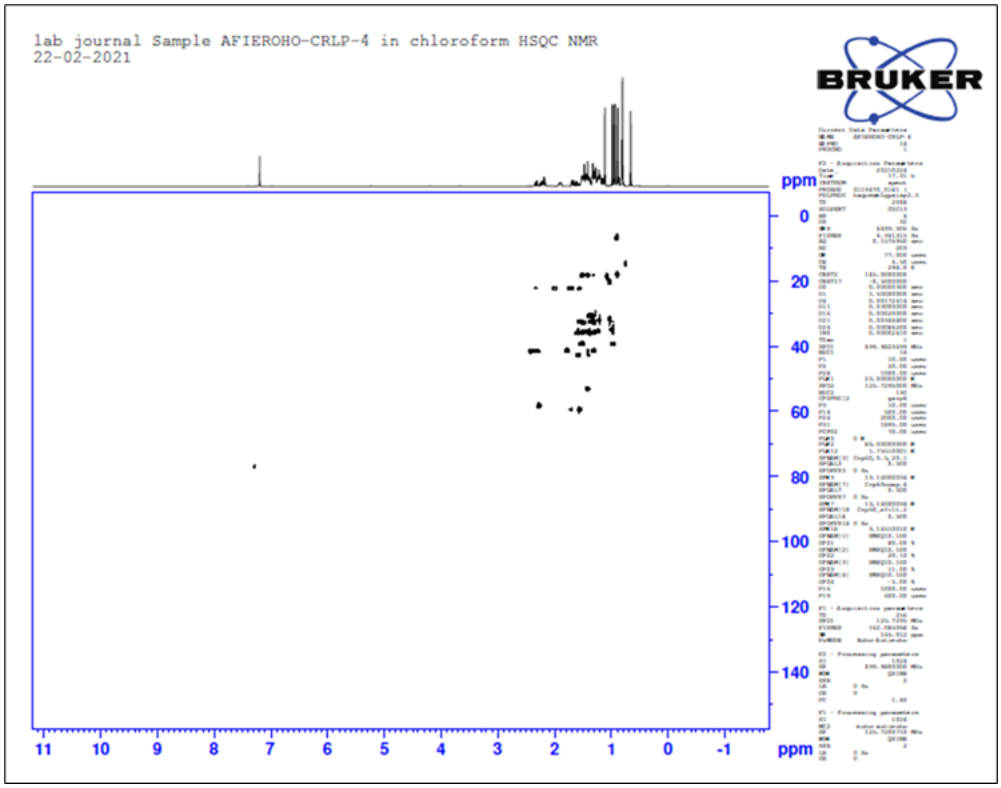

Figure 7 HSQC spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

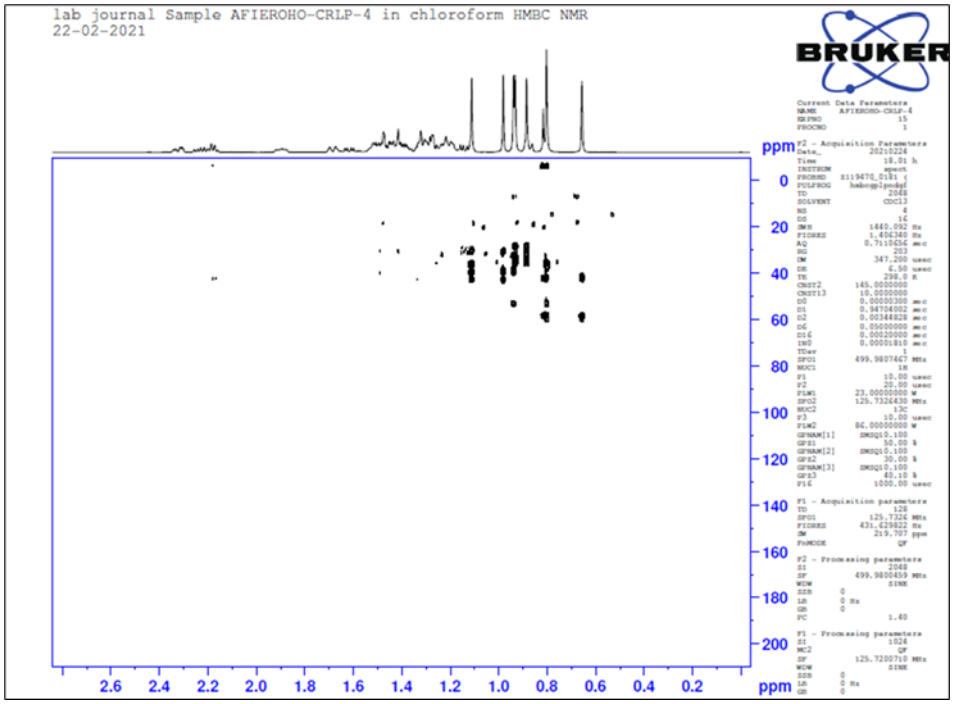

Figure 8 HMBC spectrum of freidelan-3-one isolated from the fruits of *Harungana madagascariensis* Lam. Ex. Poiret

Friedelin isolated from *Maytenus undata* (Celastraceae) was tested against a range of bacteria and fungi implicated in opportunistic and nosocomial infections [13]. Also reported is its antifungal activity against *C. albicans* with a Minimum Inhibition Concentration (MIC) value of 2.44 µg/ml [16]. The antibacterial activity of friedelin against *E. faecalis* with an MIC value of 0.61 µg/ml has been documented [16-17]. Another study by Ichilo *et al*., [18] showed that friedelin isolated from *Pterocarpus santalinoides possess an* antimicrobial activity against many microbes including *S. aureus* and with MIC values of 10 µg/ml for MRSA, *H. pylori*, *E. coli* and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of 40 µg/ml for *E. coli*; 20 µg/ml for MRSA, *H. pylori* and *C. krusei*; and 10 µg/ml for *S. aureus, S. pneumonia* and *C. tropicalis.*

Several reports on the growth inhibition activity of friedelan-3-one against several species of *Mycobacteria* have been reported portraying that friedelin can be optimized as an antimycobacteria drug lead. Friedelin isolated from *Terminalia avicennioides* has been shown to have significant antimycobacterial activity against BCG at 4.9 µg/ml [12] while that from *T. brownii* and *T. laxiflora*gave a MIC of 250 µg/ml against *M. smegmatis* [19]. Friedelin from cannabis demonstrated activity against three non-pathogenic species at a MIC of 800 µg/ml [20].

5. Conclusion

This is the first time that the anti-*Mycobacteria tuberculosis* activity of the fruit of *H. madagascariensis* is being reported thus validating its ethnomedicinal use in the treatment of cough with blood stain sputum [6]. Also, literature reports on the biological activities of the isolated triterpene ketone friedelan-3-one from this plant underscores the potentials of this ethno medicinal plant as a source of lead compounds for the development of anti-TB drugs.

Compliance with ethical standards

Acknowledgments

The authors are grateful to Dr. Edmund Ekuadzi and the entire Scientists at NMR facility of the Central Laboratory, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Disclosure of conflict of interest

The authors declare no conflict of interest.

References

[1] World Health Organisation. Global tuberculosis report 2020. [Internet]. 2020 [Retrieved 28 July 2021]. In https://www.who.int/teams/global-tuberculosis-programme/tb-reports.

[2] Astrid Z. Drug-resistant TB threatens to kill 75million people by 2050, cost $16.7trillion. *Reuters*. (2015, March 23). *Retrieved from* https://www.reuters.com/article/us-health-tuberculosis-economy-idUSKBN0MK00520150324. Retrieved on *28 July 2021.*

[3] Moulari B, Pellequer Y, Lboutounne H, Girard C, Chaumont JP, Millet J, Muyard F. Isolation and *in vitro* antibacterial activity of astilbin, the bioactive flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae). Journal of Ethnopharmacology. 2006; 106: 272–278.

[4] Kengni F, Tala DS, Djimeli MN, Fodouop SPC, Kodjio N, Magnifouet HN, Gatsing D. *In vitro* antimicrobial activity of *Harungana madagascriensis* and *Euphorbia prostrata* extracts against some pathogenic Salmonella sp. International Journal of Biological and Chemical Sciences. 2013; 7(3): 1106-1118.

[5] Mba JR, Weyepe FCL, Mokale ALK, Tchamgoue AD, Tchokouaha LRY, Nole T, Tarkang PA, Nehemie DT, Alembert TT, Mbita M, Dongmo B, Agbor GA.Antidiarrhoeal, Antibacterial and Toxicological Evaluation of *Harungana madagascariensis*. Scholars Academic Journal of Biosciences. 2015; 5(3):230-239.

[6] Burkil HM. The Useful Plants of West Tropical Africa. Kew: Royal Botanic Gardens. 1985-2004.

[7] Selvakumar N, eds. Standard operating protocol for mycobacteriology laboratory. Version1.1. India, Tuberculosis Research Centre. 2010.

[8] Afieroho OE, Ajuzie JI. Isolation of friedelin from the fruits of the medicinal plant *Harungana madagascariensis*. International Journal of Modern Pharmaceutical Research. 2020; 4(2):90-93.

[9] Kohen F, Samson AS, Steveson R. Friedelin and Related Compounds. X. Products from Ultraviolet Irradication of Friedelin. Journal of Organic Chemistry. 1969; 34(5): 1355-1358.

[10] Gayathri G, Nair BR, Babu V. Isolation and characterization of a triterpenoid from the leaves of *Azima tetracantha* Lam. Int J Pharm Sci Res. 2016; 7: 2090-6.

[11] Onishi Y, Hanaoka M. Studies on the chemical components of *Quercus stenophylla* Makino. I. Isolation of friedelin from the leaves of *Quercus stenophylla* Makino. YakugakuZasshi (in Japanese). 1968; 88(9): 1244–1245.

[12] Mann A, Ibrahim K, Oyewale AO, Amupitan JO, Fatope MO, Okogun JI. Antimycobacterial Friedelane-terpenoid from the Root Bark of *Terminalia avicennioides*. American Journal of Chemistry. 2011; 1(2): 52-55.

[13] Selm YA, Litinas KE. Cytotoxic And Antimicrobial Activities of Two New Triterpenoids from the Peels of Local Egyptian *Malus domestica* L. Journal of Chilean Chemical. Society. 2015; 60(2).

[14] Duke JA. A Handbook of Biologically Active Phytochemicals and Their Activities. CRC Press, Boca Raton, FL. 1992. Pp22-25.

[15] Dzubak P, Hajduch M, VydraD, Hustova A, Kvasnica A, David M, Biedermann D, Markova L, Urbanc M, Sarek J. Pharmacological activities of natural triterpenoids and their therapeutic Implications. Natural Products Report. 2006; 23: 394–411.

[16] Kuete V, Komguem J, PenlapBeng V, Meli AL, Tangmouo JG, Etoa FX, Lontsi D. Antimicrobial components of the methanolic extract from the stem bark of *Garcinia smeathmannii* Oliver (Clusiaceae). South African Journal of Botany. 2017; 73: 347–354.

[17] Mokoka TA, McGaw LJ, Mdee LK, Bagla VP, Iwalewa EO, Eloff JN. Antimicrobial activity and cytotoxicity of triterpenes isolated from leaves of *Maytenus undata* (Celastraceae). BMC Complementary and Alternative Medicine. 2013; 13: 111.

[18] Odeh IC, Tor-Anyiin TA, Igoli JO,Anyam JV. *In vitro* antimicrobial properties of friedelan-3-one from *Pterocarpus santalinoides* L'Herit, ex Dc. African Journal of Biotechnology. 2016; 15(14): 531- 538.

[19] Enass YAS, Riitta J-T, Anna-Maija L, Markku K, Olavi L, Marketta S, Mari L, et al. *Terminalia laxiflora* and *Terminalia brownii* contain a broad spectrum of antimycobacterial compounds including ellagitannins, ellagic acid derivatives, triterpenes, fatty acids and fatty alcohols. Journal of Ethnopharmacology. 2018; 227: 82-96.

[20] Chinsembu KC. Tuberculosis and nature's pharmacy of putative anti-tuberculosis agents. Actatropica. 2016; 153: 46–56.