International Journal of Life Science Research Archive

ISSN: 0799-6640 (Online)

Journal homepage: https://sciresjournals.com/ijlsra/

(RESEARCH ARTICLE)

Check for updates

Chromatography fractions from the ethyl acetate fraction of *Harungana madagascariensis* Lam. Ex Poiret fruits (Hypericaceae) inhibited the growth of *Mycobacterium tuberculosis*

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 Unuversity, Igbariam Campus, Nigeria.*
[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.*

International Journal of Life Science Research Archive, 2023, 05(01), 047–051

Publication history: Received on 02 June 2023; revised on 27 July 2023; accepted on 30 July 2023

Article DOI: https://doi.org/10.53771/ijlsra.2023.5.1.0073

Abstract

The economic burden of drug resistant tuberculosis strain is enormous due to its being one of the leading cause of global mortality. New treatments that are faster, simpler and affordable are urgently needed. Thus this research aimed to validate that the ethyl acetate fraction from the Nigeria variety of *Harungana madagascariensis* contain secondary metabolites with anti-*Mycobacterium tuberculosis* activity. The fruit of *H. madagascariensis* was extracted with 70% aqueous ethanol by cold maceration. The crude aqueous ethanol extract was defatted with n-hexane by partitioning and the aqueous portion further partitioned with ethyl acetate to give ethyl acetate portion (EAP) used in this study. Fractionation of EAP was done using chromatographic techniques. Phytochemical screening of the fractions was done using standard methods. Anti- *Mycobacterium tuberculosis* screening was done using Lowestein Jensen(LJ) method (test fractions stock solution 1mg/ml diluted to a 0.01mg/ml in the LJ medium while isoniazid, dihydrostreptomycin, ethambutol and rifampicin at their reported minimum inhibition concentrations(MIC) were used as reference anti-TB drugs). Four (4) chromatography fractions (EAF1 –EAF4) were obtained. All fractions were found to contain phenolics with anthraquinone present in EAF2 and EAF3. Fractions EAF3 and EAF4 inhibited the growth of the *Mycobacteria tuberculosis*. This preliminary screening validates the use of *H. madagascariensis* in traditional medicine and as a potential source of natural products with anti-*Mycobacterium tuberculosis* activity as well as a good source of anthraquinone.

Keywords: *Harungana madagascariensis*; Anti-tuberculosis agents; Phenolics; Anthraquinone

1 Introduction

Tuberculosis (TB), a potentially serious infectious bacterial disease caused by the bacillus *Mycobacterium tuberculosis*, is primarily spread by aerosols from the lungs of patients with active TB through coughing, sneezing or spitting. In a year 2022 joint report of the United Nations programme on the Human Immunodeficiency virus (HIV)/Acquired immune deficiency disease syndrome (AIDS), an estimated 4,100 persons die daily and 30,000 persons fell ill as a result of TB infection[1]. The following eight countries: India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh

* Corresponding author: Ozadheoghene Eriarie Afieroho

and the Democratic Republic of the Congo have been reported to account for well above two thirds of the global TB cases [2]. Despite successful decrease in incidence rate as reported by the World Health Organisation (WHO), reports have shown that there is a rapid decline in treatment success due to increasing drug resistance to commonly prescribed antibiotics. This challenge to treat TB commonly manifests as multidrug resistance tuberculosis (MDR-TB). In a 2018 cohort TB, 54% and 30% developed to MDR-TB and extensive drug resistance tuberculosis (XDR-TB) respectively while in 2019, there were more than half a million cases of drug resistant TB [3]. Because of the invasive increase in MDR-TB, there is an urgent need for new drugs for the treatment. Although international efforts have been in place, it has not been sufficient as only few new drugs; pretomanid, delamanid and bedaquiline have been pushed into clinical use in the last 40 years [4]. This requires more effort to find new treatments that are faster, simpler and affordable.

Natural products have offered chemical diversity for the discovery of drugs and drug lead products as well as inspiration for synthetic chemistry. *Harungana madagascariensis* (Hypericaceae) has been used over many years in African Herbal Medicine (AHM) for the treatment of a wide range of human illnesses such as dysentery, diarrhea, anemia, gastrointestinal diseases [5], typhoid and some heart ailments such as tachycardia, Cough and bronchial distress including blood in sputum [6], gonorrhea, leprosy, hemorrhoids and to facilitate childbirth [7-8]. Recently, the anti-*Mycobacterium tuberculosis* activity of chromatography fractions from the n-hexane fraction of *H. madagascariensis* has been reported [9]. Thus in this follow-up research, the Nigeria chromatography fractions from the defatted ethyl acetate portion of *Harungana madagascariensis* was subjected to *in-vitro* assay to validate it as a potential source of natural product(s) with inhibition effect on the growth of *Mycobacterium tuberculosis* the causative pathogen for tuberculosis.

2 Material and methods

2.1 Reagents and Instruments

All the reagents and solvents were of analytical grade and are products of JHD. Standard control drugs used include ethambutol [99%], rifampicin [99%], isoniazid [99%] and dihydrostreptomycin [99%] which were supplied by the tuberculosis Research laboratory of the Zankli Medical Centre, Abuja, Nigeria, while the pathogenic micro-organisms used(the clinical MTB strains) were cultured at the Tuberculosis Reference Laboratory, Zankli Research Centre, Bingham University, Nassarawa State, Nigeria.

2.2 Sample collection and extraction

The fresh and matured fruits of *H. madagascariensis* were collected from the Medicinal plant Garden of the Department of Pharmacognosy and Phytotherapy and identified in the same Department with Herbarium number: UPHH0585. It was air dried and pulverized. A 600 g of the pulverized sample was extracted with 70% aqueous ethanol by cold maceration for 72hours with filtration and change of solvent done every 24hours. After the extraction, it was concentrated with rotary evaporator at 40 °C to at least one-tenth of its volume. The crude extract was further concentrated by putting it in glass desiccator until all solvents evaporated. The crude aqueous ethanol extract was defatted with n-hexane and further partitioned with ethyl acetate to give the ethyl acetate portion (EAP) used in this study.

2.3 Fractionation of the EAP using column Chromatography

Fractionation of the ethyl acetate portion EAP was done using open column chromatography technique with elution done under gravity. Briefly, 3.78 g of the EAP was pre-adsorbed by mixing in silica gel (2 g) and loaded on a glass column (internal diameter 4 cm), dry packed with silica gel (200–400 mesh, India) 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: ethyl acetate (3:1, 2:2, 1:3, 0:4 v/v); ethyl acetate: 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 thin layer chromatography (TLC). Four different fractions were eluted and were designated EAF1-4 fractions.

2.4 Phytochemical screening

All the fractions were screened for presence of phyto-constituents using standard methods (chemical reagents and on spraying with chromogenic spray reagents on TLC) [10-11].

2.4.1 Anti-Mycobacterium tuberculosis susceptibility test

This was done on the EAF 1-4 using the solid based agar dilution method (Using egg enriched Lowenstein Jensen (LJ) medium) reported by [12]. Briefly, freshly laid eggs (not more than 7 days old) were scrubbed with a brush in a warm water and plain alkaline soap. The washed eggs were then soaked in 70% aqueous ethanol for 15 mins, cracked

thereafter aseptically and homogenized. The LJ stock solution was prepared and 600mL of mineral salt solution was added. The freshly prepared LJ medium was then mixed with the test samples as shown in Table 1. About 7-8 ml of the re-constituted test sample-LJ mixture was then dispensed into sterile bijou tubes and incubated for 45 mins at 85 ℃ in a slanting position. 1.0 and 0.5 McFarland of de-contaminated clinical isolates of *Mycobacterium tuberculosis* (MTB) was prepared in sterile distilled water while 10 µL of each of the inoculum concentrations were inoculated on separate standard drugs, phytodrugs, and negative control reconstituted LJ media slant and incubated for six weeks at 37 ℃. 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. Each sample analysis was done in duplicate. The results are presented in Table 3 for fractions.

Table 1 Preparations of Standard drugs and phytodrug containing LJ slopes

Test fractions/referenc anti-TB drugs	Stock concentration (mg/mL)	Volume of stock solution added to LJ media (mL)	Final volume of dilution with LJ media (mL)	Final test concentration of drug (mg/mL)
Isoniazid	0.02	1	100	0.0002
Dihydrostreptomycin	0.80	1	100	0.0080
Ethambutol	0.20	1	100	0.0020
Rifampicin	8.00	1	100	0.0800
Phytodrug	1.00	1	100	0.0100

3 Results and discussion

Table 2 Class of phytochemicals detected in the fractions

Detected phytochemicals	EAP	EAF1	EAF2	EAF3	EAF4
Phenolics($FeCl_3$ test)	+	+	+	+	+
Anthraquinones (Borntrager's test)	+	-	+	+	-

NB: EAP = Ethyl acetate partitioned fraction, EAF1-4 are chromatography fractions from EAP.

Table 3 Anti-*Mycobacterium tuberculosis* Activity of the Fractions from Ethyl acetate Portions of *H. madagascariensis*

Fractions/drugs	EAF1	EAF2	EAF3	EAF4	Isoniazid	Dihydro-streptomycin	Ethambutol	Rifampicin
Activity	-	-	+	+	+	+	+	+

NB: EAF1-4 are chromatography fractions from the Ethyl acetate partitioned fraction EAP. + = active; - = inactive.

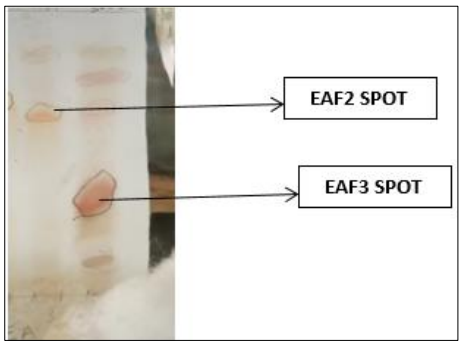

Figure 1 Chromatogram showing the reaction of EAF2 and EAF3 component in ammonia fume

Preliminary phytochemical screening of the EAP showed the presence of phenolic compounds and anthraquinones (see Table 2). The EAP was further fractionated on silica gel column to afford four fractions EAF1-4 eluted with the mobile phase gradients: EAF1[n-hexane: ethyl acetate (3:1 v/v)], EAF2 [n-hexane: ethyl acetate (3:1, 2:2, 1:3 v/v)], EAF3[n-hexane: ethyl acetate (1:3, 0:4 v/v)], and EAF4[ethyl acetate: ethanol (3:1, 2:2 v/v)]. Whereas all fractions contained phenolic compounds, only fractions EAF-2 and EAF-3 were observed to contain anthraquinones (see Table 2) as confirmed from the thin layer chromatogram in Figure 1 on exposing to ammonia fume. These fractions were screened for their anti-*Mycobacterium tuberculosis* activity. Only EAF3 and 4 inhibited the growth of the *Mycobacteria tuberculosis* at the final test concentration as shown in Table 3. In a review, several plant-derived phenolic compounds which included flavonoids and non-flavonoids derivatives have been reported to be potent inhibitors of the growth of *Mycobacterium tuberculosis in vitro* with associated mechanisms of action including one or more of: *Mycobacteria* efflux system inhibition, *Mycobacteria* proteasome Inhibition, mycolic acid biosynthesis inhibition, and nitric oxide inhibition [13]. Phenolic compounds are by far the largest group of phytochemicals and includes: simple phenols and phenolic acids, phenylpropanoids and related coumarins, stilbenes, depsides, flavonoids and tannins [10]. Also closely related to them are the quinone pigments of which the anthraquinones are the largest class of natural quinones. The phenolic compounds and quinone pigments are all aromatic and are known to play ecological role in plants. Anthraquinone derivatives asides their utilization as colorants, have been used since centuries for medical applications, for example, as laxatives, antimicrobial and anti-inflammatory agents. Current therapeutic indications include constipation, arthritis, multiple sclerosis, and cancer [14]. It has also been shown that anthraquinone from medicinal plants have good antitubercular activity [15]. Damnacanthal, an anthraquinone has been reported to possess promising anti-*Mycobacterium tuberculosis* activity with MIC of <20 µg/ml [16].

4 Conclusion

This study validates the medicinal use of *H. madagascariensis* in the treatment of bronchial diseases and cough and highlights the presence of anthraquinone and phenolic compounds containing components of *H. madagascariensis* fruits with anti-*Mycobacterium tuberculosis* activity *in vitro*.

Compliance with ethical standards

Acknowledgments

The authors would like to thank management and staff of the Tuberculosis Reference Laboratory, Zankli Research Centre, Bingham University, Nassarawa State, Nigeria, and the Central Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, University of Port Harcourt, Nigeria

Disclosure of conflict of interest

All authors declare that there is no conflict of interest.

Authors Contribution

OBO, OEA, and KAA design and executed the project while JSB, TTE and AUO did the anti-*Mycobacterium tuberculosis* screening

References

[1] UNAIDS Fact sheet-World tuberculosis day report 2022. Geneva, Switzerland: Joint United Nations programme on HIV/AIDS, March 24, 2022. Available from: https://www.unaids.org/sites/default/files/media_asset/20220324_TB_FactSheet_en.pdf

[2] Global tuberculosis report 2022 factsheet. Geneva, Switzerland: The World Health Organisation April 2023. Available from: https://cdn.who.int/media/docs/default-source/hq-tuberculosis/global-tuberculosis-report-2022/global-tb-report-2022-factsheet.pdf

[3] Tuberculosis (TB) Alliance, 2019. August 4, 2021. Available from: https://www.tballiance.org/

[4] The Working Group for New TB Drugs report 2020. New Drugs for TB Clinical Pipeline, October 19, 2020, Available online at: https://www.newtbdrugs.org/pipeline/clinical 2020).

[5] Mba JR, Weyepe FCL, Mokale ALK, Tchamgoue AD, Tchokouaha LRY, Nole T, Tarkang PA, Nehemie DT, Alembert TT, Mbita M, Dongmo B and Agbor GA . Antidiarrhoeal, Antibacterial and Toxicological Evaluation of *Harungana madagascariensis*. Scholars Academic Journal of Biosciences; 2017;5(3): 230-239.

[6] Bouquet, A (1969). Feticheurs medicine traditionnelles du Congo (Brazzzaville). Memoires O.R.S.T.O.M., No.36, p 282.

[7] Kengni F, Tala DS, Djimeli MN, Fodouop SPC, Kodjio N, Magnifouet HN and 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; 7(3): 1106-1118.

[8] Moulari B, Pellequer Y, Lboutounne H, Girard C, Chaumont JP, Millet J and 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.

[9] Okonkwo OB, Afieroho OE, Bimba JS, Eliya TT, Osuji AU and Abo KA. 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). GSC Biological and Pharmaceutical Sciences. 2022; 18(02), 259–267

[10] Harbourne, J. B. (1973). Phytochemical methods. A Guide to Modern Techniques of Plant Analysis Chapman and Hall Ltd, London

[11] Houghton, P. J., & Raman, A. (1999). Laboratory handbook for the fractionation of natural extracts. Chapman and Hall Ltd, London

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

[13] Mazlun MH, Sabran SF, Mohamed M, Bakar MFA and Abdullah Z. Phenolic Compounds as Promising Drug Candidates in Tuberculosis Therapy. Molecules, 2019; 24(13): 2449

[14] Malik, EM ., Müller C. E. Anthraquinone as pharmacological tools and drugs. Med Res Rev. 2016; 36(4):705-48.

[15] Novie EM, Hendro W, Suharyo HS, Tri NK. Antitubercular activity of Extract and compounds of NONI (Morinda citrifolia Linn). Int J Pharm Sci; 2017; 9(12): 105-109.

[16] Pollo LAE, Martin EF, Machado VR, Cantillon D, Wildner LM, Bazzo ML, Waddell SJ, Biavatti MW and Sandjo LP. Search for Antimicrobial Activity among Fifty-Two Natural and Synthetic Compounds Identifies Anthraquinone and Polyacetylene Classes That Inhibit Mycobacterium tuberculosis. Front. Microbiol. 2021; 11: 622629.