Phytomedicine Plus 3 (2023) 100406

Contents lists available at ScienceDirect

Phytomedicine Plus

journal homepage: www.sciencedirect.com/journal/phytomedicine-plus

Bioassay-guided isolation of antimycobacterial compounds from *Aphloia theiformis* (Vahl) Benn root ethanolic extract

Alphonce Ignace Marealle [a,c,*], Michael Qwarse [b], Ester Innocent [c], Ramadhani S.O. Nondo [c], Francis Machumi [b], Kerstin Andrae-Marobela [d], Matthias Heydenreich [e], Mainen Julius Moshi [c]

[a] *Department of Clinical Pharmacy & Pharmacology, School of Pharmacy, Muhimbili University of Health & Allied Sciences, Dar es Salaam, Tanzania*
[b] *Department of Natural Products Development and Formulation, Institute of Traditional Medicine, Muhimbili University of Health & Allied Sciences, Dar es Salaam, Tanzania*
[c] *Department of Biological and Pre-clinical Studies, Institute of Traditional Medicine, Muhimbili University of Health & Allied Sciences, Dar es Salaam, Tanzania*
[d] *Department of Biological Sciences, University of Botswana, Garborone, Botswana*
[e] *Institute of Chemistry, University of Potsdam, Potsdam, Germany*

ARTICLE INFO

Keywords:
Aphloia theiformis
HIV and AIDS-related conditions
Antimycobacterial
Pentacyclic triterpenoid
Proanthocyanidin A_2

ABSTRACT

Background: Aphloia theiformis is used in Makete district, Tanzania, and other areas by HIV and AIDS patients as a weight loss remedy and for treatment of tuberculosis. However, there is no literature information on its antimycobacterial activity.
Purpose: To evaluate antimycobacterial activity of *A. theiformis* root ethanolic extract and isolated compounds.
Methods: The broth microdilution method was used to test the crude root ethanolic extract for activity against different non-pathogenic mycobacteria. Bioautography was used to identify the active constituents. Isolation of the active compounds was carried out using bioassay-guided fractionation. Chemical structures of compounds were established by comparison of their spectra with literature spectral data. The isolated compounds and some fractions were screened for activity against *M. tuberculosis* (MTB) subtype $H_{37}Rv$ and clinical isolates of MTB including strains resistant to rifampicin.
Results: The 80% ethanolic extract of *A. theiformis* displayed activity against all non-pathogenic mycobacteria. The most active fraction was the ethyl acetate fraction from which two compounds were isolated; an epicatechin dimer; proanthocyanidin A_2 (1) and tormentic acid (2) belonging to ursane pentacyclic triterpenoid. Compound (1) had MICs of 60.7 μM against *Mycobacterium madagascariense* and *Mycobacterium indicus pranii* and 255 μM against both standard MTB ($H_{37}Rv$) and clinical isolate of rifampicin-resistant MTB.
Conclusion: This study provides evidence in support of the use of *A. theiformis* extracts by traditional health practitioners for the management of tuberculosis. We recommend more studies to further assess efficacy and safety of the plant constituents using different models.

Abbreviations

AIDS, Acquired immunodeficiency syndrome;
CEF, Chloroform: ethyl acetate: formic acid;
CTRL, Central tuberculosis reference laboratory;
EMW, Ethyl acetate: methanol: water;
HIV, Human immunodeficiency virus;
ITM, Institute of Traditional Medicine;
INT, Iodonitrotetrazolium chloride salt;
MA, Mycobacterium aurum;
MIC, Minimum inhibitory concentration;
MIP, Mycobacterium indicus pranii;
MM, Mycobacterium madagascariense;
MTB, *Mycobacterium tuberculosis*;
MTT, 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyltetrazolium bromide
MUHAS, Muhimbili University of Health and Allied Sciences;
NMR, Nuclear magnetic resonance;
TEA, Toluene: ethanol: ammonia;
TLC, Thin layer chromatography;
XDR, Extensively drug-resistant tuberculosis

* Corresponding author at: P. O. Box 65013, Dar es Salaam, Tanzania.
E-mail address: marealle2010@gmail.com (A.I. Marealle).

https://doi.org/10.1016/j.phyplu.2023.100406

Available online 5 January 2023
2667-0313/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Despite all the available control measures, tuberculosis (TB) is still a major worldwide health problem. It is the leading opportunistic infection causing death among HIV patients (UNAIDS, 2020). While most TB infections are treatable and curable with the available first-line drugs, such as isoniazid, rifampicin, ethambutol and pyrazinamide in some cases, *Mycobacterium tuberculosis* (MTB) can be resistant to at least one of first-line drugs. Multi-drug resistant (MDR) TB which is caused by MTB strains resistant to at least both isoniazid and rifampicin, while the strains causing extensively drug-resistant (XDR) TB are resistant to isoniazid, rifampicin, any fluoroquinolone and at least one of the injectable anti-TB drugs. In XDR-TB the treatment could be complicated given the much fewer treatment options available (Ventola, 2015). Currently, the only most reliable and active drugs for XDR-TB are the two newly introduced drugs delamanid and bedaquiline which were approved for use by the FDA in 2012 and 2013, respectively. Therefore, there is a pressing need to look for new anti-TB drugs with different mechanisms of action, without cross-resistance with the available anti-TB drugs and with low toxicity.

Natural products have contributed widely to the number of conventional medicines available in the market today with more than 25% being derived from secondary metabolites of plants directly or indirectly (Ganesan, 2008; Yuan et al., 2016). After about forty years of searching for new anti-TB drugs with a novel mechanism of action, bedaquiline was indirectly discovered from a natural quinolone. Bedaquiline works by a novel mechanism of action through inhibition of ATP synthase and it is active against all MTB strains including XDR strains (Diacon et al., 2009). Due to the high diversity of secondary metabolites in medicinal plants, they offer hope to overcome the anti-TB needs either directly or indirectly because they have been extensively used as crude extracts for treatment of TB and TB-related symptoms.

Despite government efforts to provide antiretroviral and anti-TB medicines for free to patients in Tanzania, still, some studies have reported traditional medicines to be used in the management of HIV and TB (Kibonde et al., 2018; Kisangau et al., 2007; Marealle et al., 2020, 2021). There is therefore an urgent need to study these plants to determine whether or not they might offer a positive effect on these patients. Our recent ethnomedical study documented different plants in Makete, Njombe Region, Tanzania whereby, *Aphloia theiformis* (Vahl) Benn. (Aphloiacease) which is locally known as litekenyelela in Kikinga language was among the documented plants (Marealle et al., 2020). Its root decoction is used in the management of weight loss. The plant is also extensively reported to be used as traditional medicine in many other countries including Comoros Island, Madagascar, Seychelles Archipelago, Mascarenes Islands and other tropical African countries for the management of several diseases (Picot-Allain, 2018). In Madagascar, the leaves and stem barks are used in treatment of tuberculosis, malaria, sore throat and heartburn (Razafindraibe et al., 2013). The plant is also used in the treatment of abscess, fever, convulsion, eye disease, fatigue, emaciation, postpartum infections, toothache and wounds (Rakotondrafara et al., 2018). A tea prepared from the aerial parts is taken for the management of fever, dysentery and jaundice (Jelager et al., 1998). *A. theiformis* methanol stem bark extract has been reported to exhibit antiplasmodial activity against *Plasmodium falciparum* (Bero et al., 2009; Jonville et al., 2008; 2011). There are also reports on antibacterial activity against several bacteria including *Pseudomona aeruginosa* and *Escherichia coli* (Jelager et al., 1998).

Despite all the reported ethnopharmacological uses and bioassays of the plant still, there is no literature information on antimycobacterial activity of *A. theiformis* extracts and its isolated compounds. This study seeks to address this gap and to generate data for informing policy.

Methods

Collection of study materials

A. theiformis roots were collected in April 2019, in Makete, Tanzania, GPS: 36 L 0,629,275, UTM: 8,953,436 and altitude: 1847 m. The plant was identified by a botanist from the University of Dar-Es-Salaam, Mr. Haji. O. Selemani. Voucher (number: 067) is kept at the Institute of Traditional Medicine, MUHAS herbarium.

Processing of the plant materials

The powdered materials were extracted by maceration at room temperature, with 80% ethanol for 3 days at room temperature with occasional agitation. The extract was dried at a low temperature by a rotary evaporator followed by freeze-drying and then stored −20 °C until the day of experiment.

Screening for activity against selected mycobacteria species

Screening of the 80% ethanolic extract and some fractions for antimycoabacterial activity was carried out using three different non-pathogenic mycobacteria species; *Mycobacterium aurum* (MA), *Mycobacterium madagascariense* (MM) and *Mycobacterium indicus pranii* (MIP). Of these species used in this study, *M. aurum* is a well characterized non-pathogenic, rapidly growing mycobacterium which appears to be closely related to *M. tuberculosis* in terms of mycolate components due to the presence of ketomycolic acid and a cyclopropane ring in both apolar α-mycolic acids and oxygenated mycolic acids. It is recommended as one among the most suitable mycobacteria to identify new potential therapeutics against *M. tuberculosis*, especially cell wall inhibitors because of the structural similarities of mycolates (Chung et al., 1995; Namouchi et al., 2017). The complete genome sequence of *M. aurum* has been described and it is declared a surrogate model for anti-TB drug discovery (Namouchi et al., 2017).

Selected fractions and isolated compounds in addition to the non-pathogenic mycobacterium species they were tested against standard MTB ($H_{37}R$-v) and some clinical isolates of MTB including rifampicin-resistant MTB. Activity screening against all mycobacteria was carried out using broth microdilution method as described in previous studies (Eloff et al., 1998; Marealle et al., 2022). Broth microdilution technique with colourimetric method is among the best methods for slow growing microorganisms like MTB (Bento et al., 2021). Briefly, serial dilution started by putting 50 μl of extracts/compound into the first well of 96 wells microplate containing 50 μl of Middlebrook 7H9 broth. After mixing, 50 μl of the mixture was transferred from the first well to the next well and subsequent wells of each row and at the last row 50 μl was discarded. Then 50 μl of inoculum of mycobacteria equivalent to about 1.2×10^5CFU/ml were added to each well. The plates were incubated for 24 h at 31 °C and 37 °C for MM and MIP, respectively. MTB plates were incubated at 37 °C. In each experiment, a positive control was included (isoniazid/ciprofloxacin) and iodonitrotetrazolium chloride salt (INT) used as a growth indicator. To ensure better estimation of MIC, the growth of mycobacteria in the negative control plates was monitored weekly until when optimal color change was observed (Eloff, 2019).

Identification of antimycoabacterial active compounds using bioautography

Identification of the chemical constituents in the root ethanolic extract of *A. theiformis* was carried out using a bioautographic technique (Choma and Jesionek, 2015). The chemical constituents were separated on aluminum silica gel 60 F_{254} TLC plates using three eluent systems: Toluene/ethanol/ammonia (18:2:0.2) [TEA] (non-polar/basic); chloroform/ethyl acetate/formic acid (6:4:1) [CEF] (intermediate polarity/acidic) and Ethyl acetate/methanol/water (40:5.4:5): [EMW]

(polar/neutral). From each eluent system, some TLC plates were taken and the separated chemical constituents were visualized with ultraviolet light at 264 and 365 nm and by acidified vanillin. The other plates were left open for 3 days for the solvent to dry completely before they were dipped in MA culture suspension followed by incubation for 24 h at 37 °C in a wet chamber for microorganisms to grow. On day 3, a solution of 0.2 mg/ml of MTT (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was sprayed on the bioautograms. The bioautograms were then incubated for 1 h. Inhibition of mycobacterial growth was signified by formation of clear zones against a blue background.

Fractionation of the plant extract

About 170 g of *A. theiformis* ethanolic root extract was fractionated using vacuum liquid:liquid partitioning with different organic solvents. Each time equal volumes of an aqueous layer and an organic layer were thoroughly mixed and left to settle and this was repeated until when a clear organic layer was obtained. The fractionation started by using petroleum-ether followed by dichloromethane and then ethyl acetate solvents. Removal of solvents was done using rotary evaporator followed by freeze-drying to obtain fractions of petroleum-ether, dichloromethane, ethyl acetate and finally water.

Isolation of compounds

The highest antimycobacterial activity was found in the ethyl acetate fraction and therefore, it was selected for isolation of active compounds. Around 7.0 g sample of the dried ethyl acetate fraction solubilized in methanol was mixed with silica gel and then dried using rotary evaporator. Silica gel size 63–200 μm was used in the column chromatography and was packed with petroleum ether. Solvents of different polarity were used to elute the column starting with low polarity. A total of 207 sub-fractions of about 200 ml each were collected. Silica gel 60 F_{254} TLC plates were used to monitor the collected sub-fractions and those with similar profiles were combined. The compounds on the plates were visualized using ultraviolet light at 254 nm and 366 nm, iodine and acidified vanillin. The obtained combinations include fraction 1 – 91 collected at ⟨ 2% of methanol:dichloromethane, fractions 92 – 114 collected at 2 – 2.5% methanol:dichloromethane, fractions 115 – 198 collected at 2.5 – 15% methanol:dichloromethane and fractions 199 – 207 collected at 20% methanol:dichloromethane. The last combination was obtained from fractions collected at ⟩ 20% methanol.

1H and 13C NMR spectra of the compounds isolated were obtained using a Bruker AVANCE NEO 500 MHz. The compounds were dissolved in deuterated solvents. Topspin software was used to process the obtained spectra.

Results

The 80% ethanolic root extract of *A. theiformis* displayed antimycobacterial activity against all mycobacteria species used (Table 1). Before carrying out isolation of active compounds, bioautography technique was applied and indicated that *A. theiformis* root ethanolic extract contained different chemical constituents with antimycobacterial activity against MA (Fig. 1). The active compounds were relatively more polar since TEA could not properly elute them. It was also noted that for better separation of the active compounds in *A. theiformis* root ethanolic extracts the best solvent should be one with an intermediate polarity like CEF.

During isolation of compounds, the antimycobacterial activity of fractions was monitored by checking their activity against MIP and MM whereby the ethyl acetate fraction was the most active; MIC 78 μg/ml and 155 μg/ml on MIP and MM, respectively (Table 1). Two compounds were isolated from this fraction (Fig. 2). The combination of fractions 199 – 207 yielded brown crystals due to compound **1** following separation using Sephadex (LH 20) at 50% methanol:dichloromethane. The amount yielded was 42 mg. It was visible under UV light and iodine and gave red color with acidified vanillin. Based on spectroscopic analysis data, Compound **1** was identified as proanthocyanidin A_2 (Lou et al., 1999; Sun et al., 2007). Compound **1** had MIC of 60.5 μM against both MIP and MM. The MICs of this compound against both $H_{37}Rv$ and rifampicin resistant MTB strains was 255 μM.

Table 1
Antimycobacterial activity results.

Tested material	MIC μg/ml MIP	MM	MA	MTB ($H_{37}Rv$)	RRMTB
80% Ethanolic root extract	313	313	156	X	X
Petroleum ether fraction	1250	1250	X	X	X
Dichloromethane fraction	625	1250	X	X	X
Ethyl acetate fraction	78	155	X	X	X
Aqueous fraction	313	313	X	X	X
>20% Methanol	130	130	X	294	147
Negative control	NA	NA	NA	NA	NA
Positive control	< 19*	< 19*	< 17$	0.065#	0.26#

MTB: *Mycobacterium tuberculosis*, MIP: *Mycobacterium indicus pranii*, MM: *Mycobacterium madagascariense*: MA: *Mycobacterium aurum*, MIC: Minimum inhibitory concentration RRMTB = rifampicin resistant *M. tuberculosis*, NI: Not active, X: Not tested, Positive control = #Isoniazid/$Ethambutol/*Ciprofloxacin.

The mixture of fractions 92 - 144 led to isolation of compound **2** after separation using sephadex (LH 20) at 50% methanol:dichloromethane. The yielded amount was ~2 mg. The compound appeared as white crystals. It was visible under UV and iodine and under acidified vanillin it gave purple color. Based on spectroscopic analysis data, Compound **2** was identified as tormentic acid; (2α,3β)−2,3,19-trihydroxyurs-12-en-28-oic acid, an ursane pentacyclic triterpenoid (Reher and Buděšínský, 1992; Yang et al., 1992).

With regard to antimycobacterial activity; proanthocyanidin A_2 had MIC of 35 μg/ml against MM and MIP and 147 μg/ml against $H_{37}Rv$ and the clinical isolate of rifampicin-resistant MTB. The combination of fractions that were obtained at the polarity of 20% methanol and more and the mixture had MICs of 147 μg/ml against MTB subtype $H_{37}Rv$ and 294 μg/ml against the clinical isolate of rifampicin-resistant MTB (Table 1).

Discussion

The ethanolic extract of *A. theiformis* had activity against the different non-pathogenic mycobacteria used in this study. Compound **1** isolated from this plant displayed MIC of 255 μM against both MTB subtype $H_{37}Rv$ and clinical isolate of rifampicin resistant MTB. This is the first report of isolation of proanthocyanidin A_2 from *A. theiformis* roots and the genus Aphloiaceae. Proanthocyanidin A_2 isolated from *Crataegus sinaica* leaves has been reported to have 50% cytotoxic concentration (CC_{50}) of 156.7 μg/ml against MT_4 cells and anti-HIV-1 activity with 50% effective concentration (EC_{50}) of 21.1 μg/ml suggesting a good selectivity index (Shahat et al., 1998). In the same study other related compounds were found to be active against HIV-1 including proanthocyanidin B_2 and a trimeric proanthocyanidin C_1 with EC_{50} of 21.2 and 8.9 μg/ml, respectively. However, the monomeric epicatechin recorded relatively lower activity at 34.6 μg/ml. Proanthocyanidin A_2 isolated from leaves of *Pometia pinnata* has been reported to have satisfactory anti-HIV-1 integrase activity with 50% inhibitory concentration (IC_{50}) value of 30.1 μM (Suedee et al., 2013). The compound has also been reported to exert in vitro anti-HIV-1 activity through the reduction of viral RNA synthesis (Gallina et al., 2011). Generally, proanthocyanidins have many reports related to antiviral activity in the literature (Brinkworth et al., 1992; Iwasawa et al., 2009).

Low oral bioavailability has been one of the major challenges facing some compounds isolated from natural products. When taken orally, majority of proanthocyanidins reach the colon intact and are broken

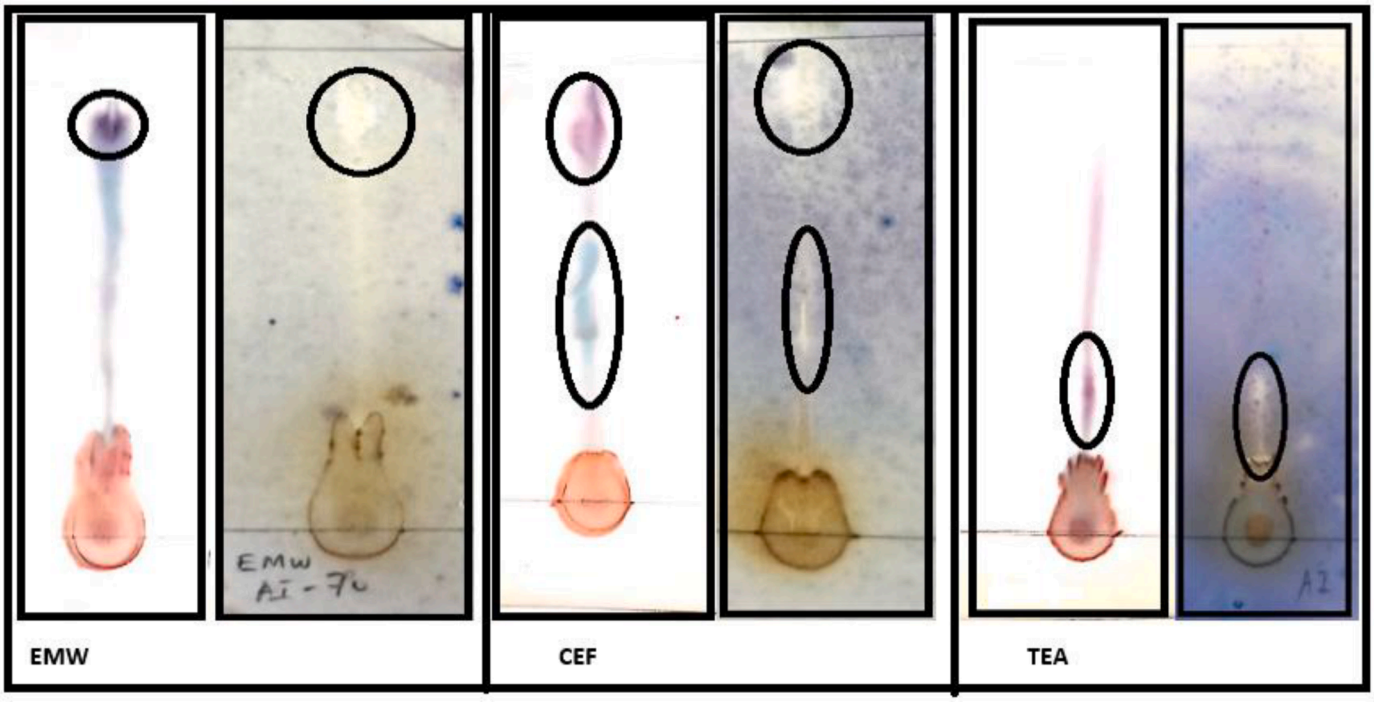

Fig. 1. Bioautogram showing the effect of the chemical constituents of *A. theiformis* on Mycobacterium aurum separated by ethyl acetate:methanol:water (40:5.4:5): [EMW] (polar, neutral); chloroform:ethyl acetate:formic acid (6:4:1): [CEF] (intermediate polarity, acidic); Toluene:ethanol:ammonia (18:2:0.2): [TEA] (non-polar, basic) eluent systems and visualized by acidified vanillin. The clear zones in the blue background indicate inhibition of MA growth.

down into phenylvalerolactones and phenolic acids by microbiota in the colon. The microbial metabolites formed may back up the health benefits of proanthocyanins (Ou and Gu, 2014). Proanthocyanidins when absorbed as intact dimers, they are known to undergo limited phase II metabolism in the intestine and liver in rats compared with its monomer (-)-epicatechin. Previous studies have reported proanthocyanidin A_2 to be bioavailable when it was taken orally. The time for peak plasma concentration (T_{max}) was found to be around 11 hrs in healthy older adults after consuming cranberry juice (McKay et al., 2015). The compound has also been reported to be found in urine (McKay et al., 2015; Ohnishi et al., 2006). The presence of proanthocyanidins in urine could also support their reported usefulness in the management of urinary tract infection, a condition which is also common among HIV-infected patients (Cimolai and Cimolai, 2007).

A. theiformis leaves extract has been reported to have high antioxidant activity, with Ferric Reducing Antioxidant Power (FRAP) mean value of 327.65 ± 2.94 mmol Fe2+/Kg DW (Donno et al., 2021). Therefore, the presence of proanthocyanidin A_2 in the root extract could suggest presence of antioxidant activity (Oldoni et al., 2016). Agents with antioxidant activity are relevant in TB because oxidative stress has been highly reported in TB and HIV-TB co-infection populations (Rajopadhye et al., 2017).

Compound (2) was identified as tormentic acid (Reher and Budĕšínský, 1992; Yang et al., 1992), has been previously reported to be isolated from methanol and 70% ethanol leaf extracts of *A. theiformis* (Olech et al., 2021). The amount of compound (2) obtained was not enough to test for antimycobacterial activity, however it is previously reported to be active against the standard *M. tuberculosis* $H_{37}Rv$ with MIC of 32 μM (Wächter et al., 1999). The compound has also been reported to exhibit antioxidative, antimicrobial, anti-inflammatory, anti-cancer, antidiabetic, antihyperlipidemic, hepatoprotective, anti-osteoarthritic, cardioprotective, neuroprotective, antinociceptive, anti-melanogenic, cytotoxic, and antiparasitic activity (Olech et al., 2021).

Interestingly, both compounds i.e. tormentic acid and proanthocyanidin A_2 have been previously reported to have activity against *C. albicans*. This is particularly important because candidiasis, a mycosis caused by *C. albicans* is among the most common HIV/AIDS opportunistic infections.

The observed effects of the crude extracts and isolated compounds strongly support the traditional use of *A. theiformis* in the management of HIV and AIDS patients and support the claim for the use of the plant in patients with weight loss and tuberculosis. However, given the fact that in Tanzania antiretrovirals are provided by the government and available in all regions of the country it is necessary to investigate the effect of combinations of *A. theiformis* extracts or its active components with antiretrovirals. This is an important policy issue necessitating research to collect data that will inform policy accordingly.

Conclusion

This study provides scientific evidence of the in vitro effects of *A. theiformis* extracts on *M. tuberculosis* subtype $H_{37}Rv$ and rifampicin resistant clinical isolates. More studies are needed to further explore the efficacy and safety of the extract and the isolated compounds using different models.

Author agreement statement

We the undersigned declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us. We understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

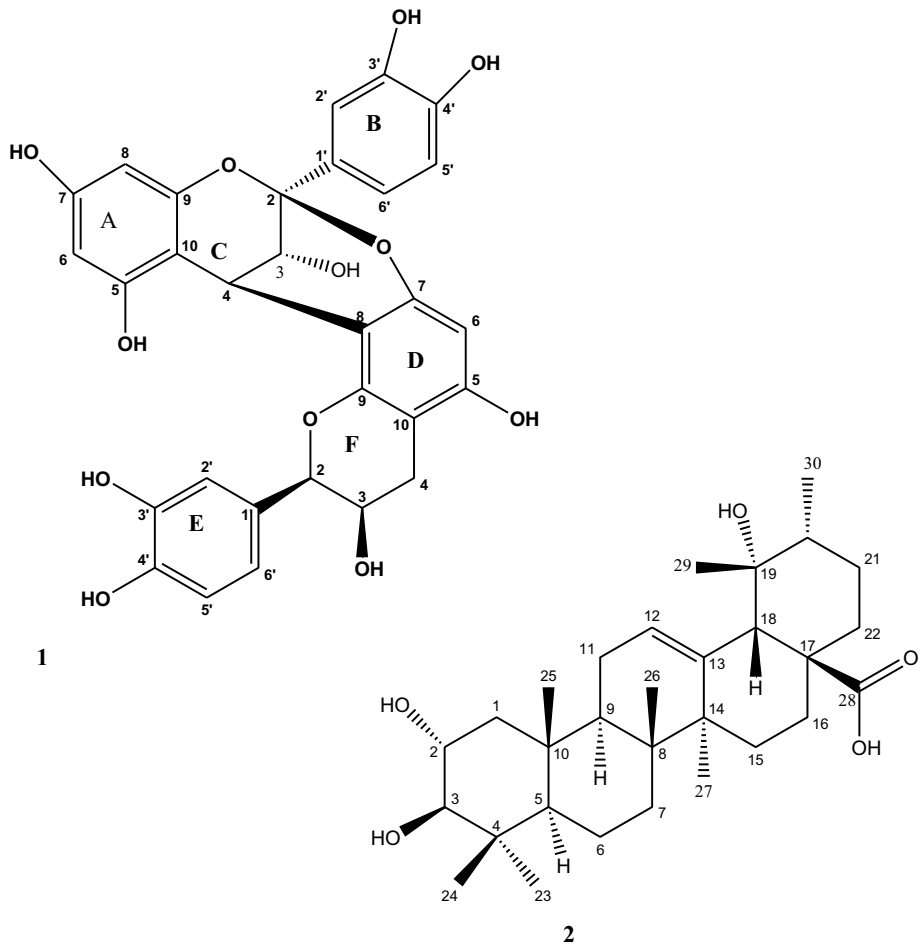

Fig. 2. Structure of proanthocyanidin A_2 (compound **1**) and tormentic acid (compound **2**) isolated from *Aphloia theiformis* ethanolic root extract.

CRediT authorship contribution statement

Alphonce Ignace Marealle: Conceptualization, Methodology, Investigation, Data curation, Writing – original draft. **Michael Qwarse:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Ester Innocent:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Ramadhani S.O. Nondo:** Methodology, Writing – review & editing. **Francis Machumi:** Data curation, Writing – review & editing. **Kerstin Andrae-Marobela:** Conceptualization, Methodology, Investigation, Writing – review & editing. **Matthias Heydenreich:** Data curation, Writing – review & editing. **Mainen Julius Moshi:** Conceptualization, Methodology, Investigation, Writing – review & editing.

Declaration of Competing Interest

No conflicts of interest to declare.

Acknowledgements

We acknowledge the Germany DAAD 57369155 and the BMBF, 01DG17008B for funding this study through TRI-SUSTAIN project. We are grateful to Mr. Abdul Kidukuli, Mr. Michael Clovis from MUHAS for their assistance in antimycobacterial activity screening and isolation of compounds at MUHAS. We also thank Ms. Katlego Makale for assisting the laboratory work at the University of Botswana using *M. aurum*. We acknowledge the management of CTRL for permitting us to use their laboratory.

References

Bento, C.M., Gomes, M.S., Silva, T., 2021. Evolution of antibacterial drug screening methods: current prospects for mycobacteria. Microorganisms. 9, 2562.
Bero, J., Ganfon, H., Jonville, M.-.C., et al., 2009. In vitro antiplasmodial activity of plants used in Benin in traditional medicine to treat malaria. J. Ethnopharmacol. 122, 439–444.
Brinkworth, R.I., Stoermer, M.J., Fairlie, D.P., 1992. Flavones are inhibitors of HIV-1 proteinase. Biochem. Biophys. Res. Commun. 188, 631–637.
Choma, I.M., Jesionek, W., 2015. TLC-direct bioautography as a high throughput method for detection of antimicrobials in plants. Chromatography. 2, 225–238.
Chung, G., Aktar, Z., Jackson, S., Duncan, K., 1995. High-throughput screen for detecting antimycobacterial agents. Antimicrob. Agents Chemother. 39, 2235–2238.
Cimolai, N., Cimolai, T., 2007. The cranberry and the urinary tract. Eur. J. Clin. Microbiol. 26, 767–776.
Diacon, A.H., Pym, A., Grobusch, M., et al., 2009. The diarylquinoline TMC207 for multidrug-resistant tuberculosis. N. Engl. J. Med 360, 2397–2405.
Donno, D., Hassani, S., Sofoini, T., et al., 2021. Traditional foods and sustainable rural development: exploiting the case of the Comoros tea as a potential source of bioactive compounds. Sustainability 13, 5815.
Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Med. 64, 711–713.
Eloff, J.N., 2019. Avoiding pitfalls in determining antimicrobial activity of plant extracts and publishing the results. BMC Complement. Altern. Med. 19, 1–8.
Gallina, L., Dal Pozzo, F., Galligioni, V., et al., 2011. Inhibition of viral RNA synthesis in canine distemper virus infection by proanthocyanidin A2. Antivir. Res 92, 447–452.
Ganesan, A., 2008. The impact of natural products upon modern drug discovery. Curr. Opin. Chem. Biol. 12, 306–317.
Iwasawa, A., Niwano, Y., Mokudai, T., et al., 2009. Antiviral activity of proanthocyanidin against feline calicivirus used as a surrogate for noroviruses, and coxsackievirus used as a representative enteric virus. Biocontrol Sci. 14, 107–111.
Jelager, L., Gurib-Fakim, A., Adsersen, A., 1998. Antibacterial and antifungal activity of medicinal plants of Mauritius. Pharm. Biol 36, 153–161.
Jonville, M., Kodja, H., Humeau, L., et al., 2008. Screening of medicinal plants from Reunion Island for antimalarial and cytotoxic activity. J. Ethnopharmacol. 120, 382–386.

Jonville, M., Kodja, H., Strasberg, D., et al., 2011. Antiplasmodial, anti-inflammatory and cytotoxic activities of various plant extracts from the Mascarene Archipelago. J. Ethnopharmacol. 136, 525–531.
Kibonde, S.F., Augustino, S., Mabiki, F.P., et al., 2018. Ethnobotanical study of medicinal plants used to manage HIV/AIDS opportunistic infections in Rungwe, Mbeya Region, Tanzania. J. Med. Plant Res. 12, 32–41.
Kisangau, D.P., Lyaruu, H.V., Hosea, K.M., et al., 2007. Use of traditional medicines in the management of HIV/AIDS opportunistic infections in Tanzania: a case in the Bukoba rural district. J. Ethnobiol. Ethnomed. 3, 1–8.
Lou, H., Yamazaki, Y., Sasaki, T., et al., 1999. A-type proanthocyanidins from peanut skins. Phytochemistry 51, 297–308.
Marealle, A.I., Innocent, E., Andrae-Marobela, K., et al., 2020. Ethnopharmacological survey of medicinal plants used in the management of HIV and AIDS-related conditions in Makete District, Southern Highlands of Tanzania. Eur. J. Med. Plants 56–83.
Marealle, A.I., Innocent, E., Andrae-Marobela, K., et al., 2022. Safety evaluation and bioassay-guided isolation of antimycobacterial compounds from *Morella salicifolia* root ethanolic extract. J. Ethnopharmacol., 115501
Marealle, A.I., Moshi, M., Innocent, E., et al., 2021. Ethnomedical survey of plants used for the management of HIV and AIDS-related conditions in Mbulu District, Tanzania. J. Med. Plant Res. 15, 1–21.
McKay, D.L., Chen, C.-Y.O., Zampariello, C.A., et al., 2015. Flavonoids and phenolic acids from cranberry juice are bioavailable and bioactive in healthy older adults. Food Chem. 168, 233–240.
Namouchi, A., Cimino, M., Favre-Rochex, S., Charles, P., Gicquel, B., 2017. Phenotypic and genomic comparison of *Mycobacterium aurum* and surrogate model species to *Mycobacterium tuberculosis*: implications for drug discovery. BMC Genom. 18, 1–9.
Ohnishi, R., Ito, H., Kasajima, N., et al., 2006. Urinary excretion of anthocyanins in humans after cranberry juice ingestion. Biosci. Biotechnol. Biochem. 70, 1681–1687.
Oldoni, T.L., Melo, P.S., Massarioli, A.P., et al., 2016. Bioassay-guided isolation of proanthocyanidins with antioxidant activity from peanut (Arachis hypogaea) skin by combination of chromatography techniques. Food Chem. 192, 306–312.
Olech, M., Ziemichód, W., Nowacka-Jechalke, N., 2021. The occurrence and biological activity of tormentic acid—a review. Molecules 26, 3797.
Ou, K., Gu, L., 2014. Absorption and metabolism of proanthocyanidins. J. Funct. Foods 7, 43–53.
Picot-Allain, M., 2018. *Aphloia theiformis* (Vahl.) Benn. A plant with various therapeutic properties. J. Soil Plant Biol. 1, 16–20.
Rajopadhye, S.H., Mukherjee, S.R., Chowdhary, A.S., et al., 2017. Oxidative stress markers in tuberculosis and HIV/TB co-infection. J. Clin. Diagn. Res. 11, BC24.
Rakotondrafara, A., Rakotondrajaona, R., Rakotoarisoa, M., et al., 2018. Ethnobotany of medicinal plants used by the Zafimaniry clan in Madagascar. J. Phytopharmacol. 7, 483–494.
Razafindraibe, M., Kuhlman, A.R., Rabarison, H., et al., 2013. Medicinal plants used by women from Agnalazaha littoral forest (Southeastern Madagascar). J. Ethnobiol. Ethnomedicine 9, 1–13.
Reher, G., Buděšínský, M., 1992. Triterpenoids from plants of the Sanguisorbeae. Phytochemistry 31, 3909–3914.
Shahat, A., Ismail, S., Hammouda, F., et al., 1998. Anti-HIV activity of flavonoids and proanthocyanidins from *Crataegus sinaica*. Phytomedicine 5, 133–136.
Suedee, A., Tewtrakul, S., Panichayupakaranant, P., 2013. Anti-HIV-1 integrase compound from *Pometia pinnata* leaves. Pharm. Biol. 51, 1256–1261.
Sun, J., Jiang, Y., Wei, X., et al., 2007. Identification of procyanidin A2 as polyphenol oxidase substrate in pericarp tissues of litchi fruit. J. Food Biochem. 31, 300–313.
UNAIDS, 2020. UNAIDS Data. WHO.
Ventola, C.L., 2015. The antibiotic resistance crisis: part 1: causes and threats. Pharm. Ther. 40, 277.
Wächter, G., Valcic, S., Flagg, M.L., et al., 1999. Antitubercular activity of pentacyclic triterpenoids from plants of Argentina and Chile. Phytomedicine 6, 341–345.
Yang, M.-.H., Blunden, G., O'neill, M., et al., 1992. Tormentic acid and 2α-hydroxyursolic acid from *Arnebia euchroma*. Planta Med 58, 227. -227.
Yuan, H., Ma, Q., Ye, L., et al., 2016. The traditional medicine and modern medicine from natural products. Molecules 21, 559.