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Original article

Anti-mycobacterial and phytochemical analysis of medicinal plants used in Magu district, Tanzania.

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ABSTRACT

Introduction: Tuberculosis remains a global health threat, further complicated by the rise of multi-drug-resistant of the available antimycobacterial drugs. Some secondary metabolites of plants have demonstrated potential antimycobacterial activity against different mycobacterium stains. This study aimed at investigating antimycobacterial activities and secondary metabolites of medicinal plants claimed to manage tuberculosis in Magu District, Mwanza – Tanzania.

Materials and methods: Medicinal plant were selected from the community. Both 80% ethanol and water extracts (prepared according to traditional methods) were tested against two non-pathogenic mycobacterial strains: *Mycobacterium indicus pranii* and *Mycobacterium madagascariense*, using the broth microdilution method. The Phytochemical screening of the most active extracts was conducted using standard chemical tests.

Results: Among the 17 plants, 11 (64.7%) 80% ethanolic extracts showed antimycobacterial activity, with minimum inhibitory concentrations (MICs) ranging from 19 µg/mL to 1250 µg/mL. Notably, the 80% ethanolic extract of *Zanha africana* (Radlk.) Exell (stem bark and roots) showed the strongest activity, with MICs of 19 µg/mL and 39 µg/mL against *M. indicus pranii* and *M. madagascariense,* respectively. The *Harrisonia abyssinica* Oliv. extracts also displayed good activity with MICs of 39 µg/mL. *Lanna fulva* (Engl.) Engl, and D*ichrostachys cinerea* (L) Wight&Arn. showed average activity at 78 µg/mL. In contrast, decoction extracts prepared using traditional methods showed little to no activity, except for *Z. africana* stem bark, which had weak activity (417±147 µg/mL).

Conclusion: The presence of secondary metabolites in the selected medicinal plants support the reliability of traditional knowledge. This suggests isolation of active antimycobacterial compounds of *Z. africana, H. abyssinica, L. fulva,* and *D. cinerea* warrant.

KEYWORDS: MEDICINAL PLANTS, MINIMUM INHIBITORY CONCENTRATION, SECONDARY METABOLITES, TUBERCULOSIS, *MYCOBACTERIUM INDICUS PRANII* AND *MYCOBACTERIUM MADAGASCARIENSE.*

INTRODUCTION

Tuberculosis is a public concern, especially in developing countries including Tanzania (Migliori and Tiberi, 2022; Tiberi *et al.*, 2022). About 10.6 million cases of TB have been reported globally, and 1.6 million people reported to be associated with HIV and AIDS infections (Begum *et al.*, 2022; Bagcchi, 2023; Marealle, Moyo, *et al.*, 2023).The synergistic effect of HIV and AIDS on the latent state of tuberculosis trends brings in the high prevalence of active tuberculosis (Suma *et al.*, 2018; Mollel *et al.*, 2020).

TB burden has been reported to be associated with a 3% increase in drug resistance (Bagcchi, 2023).The emergence of multi-drug resistance (MDR) and extensive drug resistance (XDR) against first-line conventional drugs like Isoniazid (INH), ethambutol (EMB), rifampicin (RIF) and pyrazinamide (PZA) has resulted to prolonged time of treatment up to 20months (Kostyukova, Pasechnik and Mokrousov, 2023).The use of these drugs has been reported to be associated with loss of income. About 44.9% of people with TB infections faced financial burden challenges due to expenses and some even (53%) sold their assets to afford the TB treatment in Tanzania (Minja *et al.*, 2021).

Medicinal plants have been reported to be used world wide to manage different diseases. Global medicinal plant use for primary health care has been reported to be 60% and 80% in the developing countries (Kisangau *et al.*, 2007).Previously, different medicinal plants have been screened for antimycobacterial activity and showed the promising results.

MATERIALS AND METHODS

MATERIALS

Plant Materials

The selected medicinal plant parts of leaves, roots, stem bark were collected in February 2024 in Magu district with the assistant of Botanist at latitude of S2˚29'1.7232" and longitude of E32˚59'44.80008" and altitude of 1377yd a.s.l. The collected plant parts were, washed, cut into small pieces, and shade-dried for two weeks to preserve the phytochemical ingredients. The dried plant materials were grinded to a coarse powder to facilitate the extraction process (Azwanida, 2015).

Extraction of plant materials

As per traditional use, decoction method was used to prepare the extract of each sample to validate the community knowledge. About 100g of each sample was weighed using a balance and then dissolved into 600 ml of distilled water, heated in a water bath for about one hour. The sample was filtered and allowed to dry in the open plate. For complete water removal, the extracts were freeze-dried at -20°C in the freeze drier to maintain their biological activity.

Again, 100g of powdered materials were kept in contact with 80% ethanol for 48 hours at room temperature with frequent agitation. The extracts were filtered and concentrated using rotary vapour at 50°C and reduced pressure to ensure that labile constituents were not maintained. Frieze-drying was used to remove water content and dry extracts were kept at -20°C to maintain the potency of the phytochemicals for biological activity.

Antimycobacterial assays: Determination of minimum Inhibitory Concentrations (MIC)

The antimycobacterial efficacy of the ethanolic and decoction extracts were conducted at the Institute of Traditional medicine (ITM), Muhimbili University of Health and Allied Sciences, Tanzania. Two non-pathogenic Mycobacterium species, *Mycobacterium madagascariense* (MM), and *Mycobacterium indicus pranii* (MIP) were maintained in Middlebrook 7H9 broth base supplemented with glycerol containing all nutrients. Inoculum was prepared by transferring the stock bacteria to supplement Middlebrook 7H9 broth and grown for 72 hours in the biological and pre-clinical and biological laboratory at the Institute of Traditional Medicine (MUHAS). The Middlebrooks 7H9 broth media base complemented with glycerol was used in growing the Mycobacterium species for the reasons of being nutrient-rich, compatibility and their selectivity mechanisms in growing Mycobacterium madagascariense (MM), and Mycobacterium indicus pranii (MIP). Preparation was made by measuring 2.45 g of 7H9 powder, adding 450 ml of distilled water and 2mls of glycerol according to manufacturer instructions. Autoclaving of the suspensions of broth was made at a temperature of 121˚C within 15 minutes and then allowed to cool to 50 ˚C. The Mycobacterium inoculum suspension was prepared and compared to 0.5 McFarland turbidity in transparent glass vials.

Decoction and 80% ethanolic extracts were used for preparation of stock solution for bioassay.40mg of each selected decoction extract was weighed in the Eppendorf tube followed by the addition of 1000 µL(1ml) of distilled water. Then the extracts were vortexed to make a stock solution of 40 mg/ mL. Again,40 mg of each 80% ethanolic extracts was weighed on the weighing scale into the labelled Eppendorf tube followed by the addition of 200µL (20%) of DMSO followed by the addition of 800 µL (80%) of sterile distilled water. The extracts were vortex mixed to make the concentration of 40 mg/ mL stock solution. Ciprofloxacin was dissolved in sterile water (250ml in 500 mg of Ciprofloxacin) and used as a positive control. Extract-free solution with DMSO (20%) and water (80%) was prepared and negative control to monitor the effect of the solvent. The 7H9 middle brook broth was used as blank and 0.2mg/ml of Iodonitrotetrazolium Chloride Salt (INT) was used as an indicator.

2-fold broth microdilution techniques using 96 well microtiter plates were used to investigate the *in vitro*-antimycobacterial activity of the selected medicinal plant extracts (Marealle, Qwarse, *et al.*, 2023). The 96 microtiter plates were labelled with the name of specific test extract, blank, negative control (extract-free solution with 80%, DMSO and 20%, sterilized distilled water) and positive control (Ciprofloxacin) in the first row, and strains of the organism being tested. A 50 µL of each test extract, blank, negative control and positive control were filled in the first rows of each well of 96-well microtiters plates respectively. Then, 50 µL of the broth media was pre-loaded in each first row of the well. The mixture of the test extracts, blank, negative control and positive control in the first row was mixed well and 50 µL of the mixture was transferred to the second row, and the same volume was transferred to the third row until it reached to eighth row where the last 50 µL of the mixture was discarded. Mycobacterial suspensions of MM and MIP were prepared by diluting the ample Middlebrooks 7H9 broth using a multichannel micropipette to get inoculum under sterile conditions. An addition of 50 µL of mycobacterial suspension was made to make a total of 100µL in each well of the 96-well microtiter plates. The incubation period for MM and MIP plates was done for 24 h at 31 °C and 37 °C respectively.

A 40µL of the indicator (0.2% Iodonitrotetrazolium chloride salt) was prepared and added into each well after the incubation and then 60min of incubation followed. Observations of the colour changes of the Iodonitrotetrazolium chloride salt were the means to determine the presence of antimycobacterial activity in the selected medicinal plants. Conversely, the absence of colour changes indicated the presence of antimycobacterial activity. The minimum inhibitory concentration (MIC) was recorded in µg/mL.

Qualitative Phytochemical Screening

The medicinal plant extracts were exposed to qualitative phytochemical screening to determine the groups of secondary metabolites responsible for the antimycobacterial activity against the non-pathogenic Mycobacterium strains. The selected phytochemical groups which included saponins, tannins, alkaloids, glycosides, terpenoids, flavonoids and phenol were screened using the chemical methods protocols.

Ethical approval for research methods

The ethical clearance for this study was obtained from the Ethical Review Committee **(Ref.No.DA.282/298/01.C/MUHAS-REC-02-2024-2019).** All methods were carried according to the standard operating procedures.

Statistical Analysis

The Antimycobacterial activity data were summarized as the means ± SD using the Microsoft Excel 2017 version. The MIC value for ethanolic extracts and decoction extracts were classified into five different categories; outstanding activity (MIC≤6µg/mL), excellent activity (6<MIC≤16µg/mL), very good (16<MIC≤25µg/mL), good activity (25<MIC≤39µg/mL), average activity(39<MIC≤156µg/mL), weak activity (156<MIC ≤2048µg/mL) and note active (MIC>2048µg/mL) (Kuete, 2023).The qualitative phytochemical data were summarised in the table for presence (+) and absence (-).

RESULTS AND DISCUSSION

Antimycobacterial activity: Minimum inhibitory concentrations (MIC)

The antimycobacterial activity of the decoction extracts was determined using the microdilution method. The extract showed weak activity against MIP and MM strains and some of the extract had MIC above 10,000 µg/mL **(**Table 1). The decoction root extracts of *D. cinerea* and stem back of *Z. africana* showed weak activity with a MIC value of 1667±589 µg/mL, 417±147 µg/ml and 2083±589 µg/ml, 417±147 µg/ml against MIP and MM respectively (Figure 1).

On the other hand, 80% ethanolic extracts of twelve among nineteen (18) of the selected medicinal plants exhibited antimycobacterial activity against *Mycobacterium madagascariense* (MM), and *Mycobacterium indicus pranii* (MIP) with the MIC below 10,000 µg/mL. However, both root and stem bark extracts of *Zanha africana* (Radlk.) Exell exhibited the highest activity with MIC 19±9 µg/ml and 39±19µg/ml for MIP and MM respectively. However, the root extract of *Harrisonia abyssinica* Oliv. Exhibited good antimycobacterial activity with a MIC value of 39±19 µg/ml against MIP and MM. The root extract of *Dichrostachys cinerea* (L.) Wight & Arn. *Lannea fulva, D. ritindula* and *Maerua angolensis* exhibited average antimycobacterial activity with MIC ranging from 78±37µg/ml to 313±156 µg/ml against MIP and MM strains respectively. Root extracts of *Xeroderris stuhlmannii* (Taub.) Mendon¦a & E.C.S, root extract of *Catunaregam spinosa* (Thunb.) Tirveng, *Euphorbia nyikae* whole plant extracts, *Blepharis affinis* whole plant extracts and *Securidaca longipedunculata* Fresen stem extracts exhibited weak antimycobacterial activity with 156 <MIC ≤1250µg/mL against MIP and MM.

Again, six 80% of ethanolic medicinal plant extracts including *Albizia anthlemintica* (stem barks), *Turrarea fischeri* (roots), *Calotropis procera* (roots), *Acacia brevispica* (roots) and *Cenchrus americanus(seeds)* and were observed to have activity above 10000µg/ml (MIC>1000 µg/mL) against MM and MIP (Fig 1, Table 1).

Table 1: Antimycobacterial activity of decoction and ethanol extracts against *Mycobacterium madagascariense* (MM) *and Mycobacterium indicus pranii* (MIP)

S/N	The scientific name of the plant extracts	Plant parts	Decoction extracts MIC in µg/mL		80% ethanolic extracts MIC in µg/mL	
			MIP	MM	MIP	MM
1	*X. stuhlmannii*	Roots	8333±2375	10000±0	182 ± 97	313 ± 180
2	*L. fulva*	Roots	4166±1179	5833±3118	125 ± 18	78 ± 45
3	*D. ritindula*	Roots	4166±1179	5000±3535	156±90	313±156
4	*S. longipedunculata*	Stem Barks	4166±1179	5833±3118	1250±625	1250 ± 722
5	*C. spinosa*	Roots	2083±589	2542±1990	313±156	1250±625
6	*H. abyssinica*	Roots	3333±1179	5417±3584	39±19	39±19
7	*Z. africana*	Roots	2083±589	2917±1559	19±9	39±19
8	*Z. africana*	Stem Barks	417±147	417±147	19±9	39±19
9	*M. angolensis*	Stem Barks	6667±2357	8333±2357	117±55	313±180
10	*Euphorbia nyikae*	Whole	ND	ND	313±156	313±180
11	*D.cinerea*	Roots	1667±589	2083±589	78 ± 37	78 ± 39
12	*Blepharis affinis*	Whole	ND	ND	260±74	520±147
	Ciprofloxacin		<50	<50	<50	<50

Note: ND-Not done, MIC -Minimum Inhibitory Concentrations (µg/ml), Broth alone and Negative control have MIC above 10,000 µg/mL

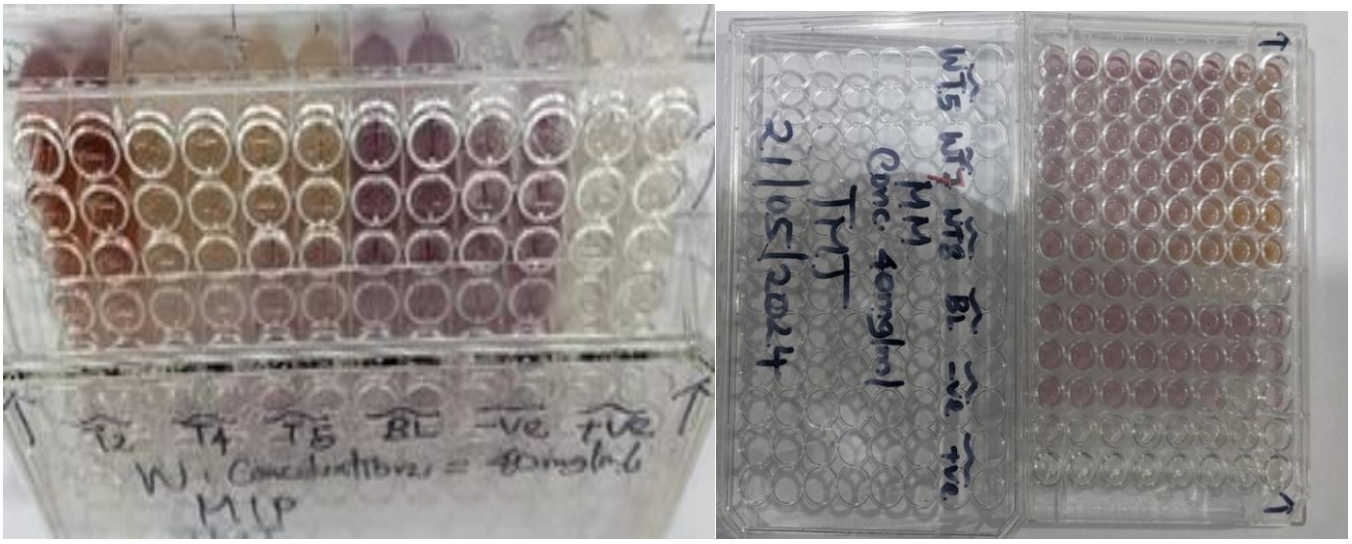

Fig 1: A sample of a 96-well plate depicting colour changes of antimycobacterial activity of the ethanolic and decoction extracts against MIP and MM after the addition of the INT indicator.

Key: T1=Root extract of *X. stuhlmannii*, T2 = *L. fulva*, T4= Root extract of *Dalbegia ritindula*, T5=stem back extract of *S. longipedunculata,* T7=Root extract of *H. abyssinica*, T8=Root extract of *Z. africana*, T9=Root extract of *Z. africana,* T17=Root extract of *D. cinerea*, T16=Root extract of *Euphorbia sp* and T14=Stem back of *M. angolensis* and T10=Root extract of *C. spinosa.* WT=. Decoction Extracts

Qualitative Phytochemical Screening

The selected medicinal plant extracts which were active against two non-pathogenic Mycobacterium species, *Mycobacterium madagascariense* (MM), and *Mycobacterium indicus pranii* (MIP) were investigated for their phytochemical constituents. Qualitative phytochemical screening results shown in **Table 2** represent eight categories of secondary metabolites present in the selected medicinal plants with potential activity against the mycobacterium strains. presented also in some photos below

Table 2: phytochemical constituents present in the selected medicinal plants with potential activity against the mycobacterium strains.

	Phytochemical groups Tested						
Plant species	Alkaloid	Flavonoids	Glycosides	Phenols	Saponin	Tannin	Terpenoids
(T1) *X. stuhlmannii*	+	+	+	+	+	+	_
(T2) *L. fulva*	+	_	+	+	_	+	_
(T4) *B. orientalis,*	+	+	+	+	+	+	_
(T5) *S.longipedunculata*	_	+	_	+	+	+	+
(T7) *H. abyssinica*	_	_	+	+	+	+	+
(T8) *Z. africana*	_	+	+	+	+	+	_
(T9) *Z. africana*	_	+	+	+	+	+	_
(T10) *C. spinosa.*	+	+	+	+	+	+	_
(T14) *M. angolensis*	_	+	_	+	+	+	_
(T16) *E. nyikae*	_	+	_	+	+	+	_
(T17) *D. cinerea*	_	+	+	+	_	+	+
(T6) *B. affinis*	-_	+	_	+	+	+	_

Key:(+)- Present, (-)-Not detected

Discussion

The medicinal plants use is preferred in many communities as a primarily healthcare worldwide. The established cutoff for minimum inhibitory concentrations (MIC) for antimycobacterial activity on analysis of antimycobacterial activity of plant extracts revealed the correlation between the antimycobacterial activity results and the reported ethnomedical use from the community (Kuete, 2023)**.**The root and stem back of 80% ethanolic extract of *Z. africana* were found to have very good antimycobacterial activity (16<MIC≤25µg/mL) against *M. Madagascariense* and *M. indicus pranii* **(Table 1).** This result was in agreement with a previous study within the Sapindaceae family on *D. angustifolia* plant species which showed to be active against pathogenic and non-pathogenic mycobacterium strains (McGaw *et al.*, 2008). These findings correlate with the previous study in the family Sapindaceae that has been reported to manage different infectious diseases due to the presence of phenolic bioactive compounds (Margaret O. Sofidiya, 2012).

The antimycobacterial activity of root extract of *H. abyssinica* against *M. Madagascariense* and *M. indicus pranii* is the first time to be reported. The presence of phenols and terpenoids have been reported to be found in many plants within the Rutaceae family and their presence reveals the activity against MM and MIP (Ramadwa *et al.*, 2019) .

The 80% ethanolic extracts of *D. cinerea* (root extract), *M. angolensis* (stem back*), D. ritindula* (root extract) and *L. fulva* (root extract) exhibited moderate activity against MM and MIP **.**The antimycobacterial study done in South Africa on *M. angolensis* leaf extracts against pathogenic and non-pathogenic mycobacterium strains supports the current study (Madisha, 2014). *D. cinerea* has also been reported to have strong antibacterial activity due to the presence of saponin, alkaloids, flavonoids and tannins secondary metabolites (Neondo *et al.*, 2012). Among the documented medicinal plants, 15 medicinal plants from the family Anacardiaceae family apart from *L. fulva* have been reported to have activity against pathogenic mycobacterium strains (Kabongo-Kayoka *et al.*, 2016).The 80% ethanolic extracts of *C. spinosa*, *E. nyikae*, *S. longipedunculata, B. affinis* and X. *stuhlmannii* exhibited weak activity against the tested non-pathogenic mycobacterium strains. The reported information from the informants revealed that its maximum activity is observed by mixing them with other medicinal plants to enhance activity.

Noteworthy, Decoction extracts displayed low activity against MIP and MM in the laboratory despite being used by the community. The community could administer a high quantity of doses for management of the tuberculosis and its related symptoms. Again, the extract could be proactive outside the body *(in vitro*) and can be pharmacologically active after hydrolysis during metabolism in the body *(in vivo)* (Ferreira *et al.*, 2012). The ethanolic extracts on the other hand scientifically validate the Indigenous knowledge since their activity was high as reported by the informants.

Secondary metabolite groups that are found in plants are major and have the potential for pharmacological functions in the human body (Yadav, Khare and Singhal, 2017). The presence of alkaloids, phenols and flavonoids is associated with the observed activity in these plants (Radji, Kurniati and Kiranasari, 2015).

CONCLUSIONS

The existence of the previous unreported plant species with potential antimycobacterial provide evidence to support the traditional uses. However, the study findings serve as a crucial baseline for further study on safety assessment, pharmacological investigation and new drug development and recommends for the possible conservation strategies the potential antimycobacterial medicinal plants.

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Conflict of interest

The authors have no conflict of interest regarding this article.

Authors contributions

Term	Definition
Conceptualization	Tekla joseph, Michael Qwarse
Methodology	Tekla Joseph, Ramadhani Nondo, Michael Qwarse
Software	Tekla Joseph, Tanga Mafuru
Validation	Michael, Qwarse, Tekla Joseph, Joseph Sempombe, Ramadhani Nondo, Adelina Thomas
Formal analysis	Tekla Joseph, Tanga Mafuru
Investigation	Tekla Joseph, Michael Qwarse
Resources	Michael Qwarse, Tekla Joseph
Data curation	Michael Qwarse, Tanga Mafuru
Writing original draft	Tekla Joseph
Writing -review & editing	Joseph Sempombe, Ramadhan Nondo, Michael Qwarse
Visualization	Tekla Joseph, Tanga Mafuru, Michal Qwarse
Supervision	Joseph Sempombe, Ramadhan Nondo, Michael Qwarse
Project administration	Tekla Joseph
Funding acquisition	Tekla Joseph

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