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Original article

Antimycobacterial Activity and Brine Shrimp Toxicity of Wild Mushrooms Used by Communities in Southern Tanzania

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Abstract

Background and objectives: Several wild mushroom species occur in southern Tanzania and are used as food by the local tribes. Experience shows that some of them could contain phytochemical compounds with therapeutic potential for treating various diseases. This study aimed to evaluate wild mushrooms used by indigenous communities living near the Selous- Niassa corridor in Namtumbo district, in Southern Tanzania for safety and antimycobacterial activity. **Methods**: Wild mushroom samples were collected randomly during the wet season and extracted by cold maceration. Dried extracts were evaluated for safety using the brine shrimp lethality test and for antimycobacterial activity using a twofold microdilution method against non-pathogenic *Mycobacterium madagascariense*, *Mycobacterium indicus pranii*, and *Mycobacterium aurum***. Results**: The mushroom extracts exhibited a good safety profile against brine shrimp larvae with LC_{50} values ranging from 20.28 µg/mL (moderately toxic) to 465.97 µg/mL (nontoxic). The extracts exhibited variable antimycobacterial activity against *M. madagascariense*, *M. indicus pranii*, and *M. aurum* with minimum inhibitory concentrations (MIC) between 0.78 and 12.5 mg/mL against *M. madagascariense*, 0.098 and 6.25 mg/mL against *M. indicus pranii* and 1.25 and 2.5 mg/mL against and *M. aurum.* Nineteen wild mushroom species (59.4%, n = 32) exhibited antimycobacterial activity against all three mycobacterial species used. **Conclusion**: Preliminary investigation has provided evidence that some of the mushrooms locally available are not toxic. Some of these mushrooms have the potential to yield antimicobacterial active compounds. Further studies to determine the therapeutic and nutritional value of these mushrooms are needed.

Keywords: anti-mycobacterial; brine shrimp lethality test; therapeutic potential; wild mushrooms

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Introduction

Tuberculosis is one of the most common opportunistic infections among HIV-infected individuals caused, mainly by *Mycobacterium tuberculosis* (Mtb) [1]. Tuberculosis may also be caused by *M. bovis, M. avium cellulare* and *M. africanum* [2]. The disease is a global health problem that kills and disables millions of people in their early productive age, mainly in developing countries [3]. Tanzania is among the 22 countries with the highest tuberculosis burden globally, and in Africa, it ranks 6th among tuberculosis highest-burden countries. The prevalence of tuberculosis from 2012 to 2020 was 295 per 100,000 population [3-6].

The emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) strains of *M. tuberculosis* against commercially available anti-tuberculosis drugs in the markets is increasing. Also, the resurgence of tuberculosis due to HIV infection and poverty are major problems hindering the elimination of tuberculosis infections and associated deaths [7,8]. Therefore, there is a need to search for alternative ways of dealing with tuberculosis infections and anti-mycobacterial drug resistance. Search for new agents from natural sources including wild mushroom species that can inhibit mycobacteria with different mechanisms of action is one of the promising alternatives [9,10].

Tanzania has a diversity of natural Miombo woodlands and other forest ecosystems that provide potentially edible and non-edible indigenous wild mushrooms [11,12]. Wild edible fungi including mushrooms are a significant source of food, health improvement, income and ecological role for communities living in rural areas where poverty is high. In Tanzania, over 60 edible wild mushroom species have been identified [13-17] and medicinal wild mushrooms are utilized to manage a range of ailments, including those affecting the respiratory and digestive systems, as well as hormonal imbalances. These mushrooms also contribute to improving the health of individuals undergoing prolonged illnesses [16]

Mushrooms contain phytochemicals such as terpenoids, alkaloids, flavonoids, polyketides, amino acids, peptides, coumarins, lignans, phenols, proteins, vitamins, carbohydrates, lipids, as well as nucleic acids. These phytochemicals are responsible for observed activities such as antimycobacterial, antifungal, antibacterial and antiviral activity [18-20]. However, there is little available pharmacological and toxicological information in the literature on these potential wild macrofungi available in Tanzania. Therefore, this study aimed to screen wild mushrooms that are used by communities living near the Selous-Niassa corridor in Namtumbo district, Ruvuma region, Tanzania for safety and potential to yield useful anti-mycobacterial activities.

Material and Methods

Ethical consideration

Ethical clearance was awarded by the Muhimbili University of Health and Allied Sciences (MUHAS) Institutional Review Board (IRB) that endorsed the study with authorization date 2018-08-26, Ref. No. 282/298/01.

Chemicals

Ethanol and methanol were purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Netherlands). Middle brook broth 7H9 bases and Middle brook agar 7H11 agar bases were purchased from HIMEDIA (India), glycerol (AR) was obtained from Lab Equip Ltd (Tanzania), dimethyl sulfoxide (DMSO), Iodonitrotetrazolium (INT) chloride, and ciprofloxacin were purchased from Sigma (UK), microtitre plates (96 wells) were supplied by KAS Medics (Tanzania) and salt was obtained from seawater collected from the Indian Ocean, along the Dar es Salaam shoreline in the United Republic of Tanzania.

Test organisms

Brine shrimp eggs were obtained from Aquaculture Innovations (Grahamstown - South Africa). *Mycobacterium madagascariense* DSM 44641 and *M. indicus pranii* DSM 45239 were supplied by DSMZ, the Germany Resource Centre for Biological Materials, Braunschweig, Germany. *Mycobacterium Aurum* A+ was obtained from the Department of Biological Sciences and Technology, University of Botswana. The three non-pathogenic mycobacteria strains were used as markers for developing a possible anti-TB efficacy of various wild mushroom extracts.

Mushrooms collection

The thirty two fruiting bodies of wild mushrooms were collected from the Likuyuseka village

bordering the Nyerere National Park (formally Selous Game Reserve) in Namtumbo district, Ruvuma region, Southern Tanzania in March 2021 as reported in our previous study [21]. The mushrooms were identified at the Department of Molecular Biology and Biotechnology, University of Dar es Salaam and the voucher specimen are kept at the Herbarium of the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences.

Extraction

Hydroalcoholic extracts were prepared by soaking each of the 32 dried powdered wild mushroom fruiting bodies in 80% ethanol and water (4:1) for 48 hours at room temperature, with occasional agitation. The mixture was filtered, and the remaining residue was re-extracted twice. The extracts of each of the dry mushroom fruiting bodies were combined followed by concentration using a vacuum rotary evaporator (Rotavapor R-205, BÜCHI Labortechnik, Switzerland), at a temperature of 45°C. Complete drying was achieved by freeze-drying (Bench Top Pro®, SP Scientific, England). The dried crude extracts were put into containers and kept in a freezer at 20 °C to prevent degradation of phytochemical compounds until needed for testing.

Rearing the brine shrimps

Artificial seawater was prepared by dissolving 3.8 g/L of dry salt from the ocean in distilled water. The prepared artificial sea water was poured into two compartments of a glass chamber with holes in a partition separating the chamber into two unequal compartments. The brine shrimp eggs were scattered in the large compartment which was covered to prevent light from entering. The small compartment was kept illuminated during incubation at room temperature for 48 hours to allow the larvae to move into it. The larvae were collected for toxicity assays using a glass Pasteur pipette from the illuminated side after 36 to 48 hours of incubation [22-24].

The brine shrimp lethality test (BST)

The determination of toxicity of the 32 wild mushroom fruiting extracts was done according to Meyer *et al*.,1982 [25]. with little modification according to Hamidi *et al.,* (2014) [26]. Serial concentrations of 500, 250, 100, 50, 10, 5 and 1 µg/mL were prepared from the stock solution dissolved in 30% dimethyl sulphoxide (DMSO) in distilled water at a concentration of 40 mg/mL. The different working concentrations were prepared from the stock solution to reduce DMSO concentration to below 10% to minimize toxic effects. The glass vials used for treatment in duplicate were filled with 2 mL of artificial seawater followed by the addition of ten brine shrimp larvae and topped up to 10mL of artificial seawater for each concentration of sample in duplicate. Artificial seawater alone, and artificial seawater containing DMSO (0.6%) were used as negative control while the standard anticancer drug, cyclophosphamide, was used as the positive control. The final concentration of DMSO for preparing the working sample extract was 0.6%. This concentration was not toxic to brine shrimps. The survived larvae were counted after 24 hours of treatment and the percentage mortalities were determined.

Anti-mycobacterial testing

Sub-culturing of *Mycobacterium* species

The *Mycobacterium* strains were sub-cultured in Middlebrook 7H9 broth base containing glycerol during preparation. Middlebrook 7H9 broth base (2.35g) was suspended in a Scotch bottle containing distilled water (450 mL) followed by the addition of glycerol (2 mL) according to the manufacturer's instructions. The mixture was warmed until the broth base dissolved completely and subsequently autoclaved at 121^{o}C for 15 minutes. The mixture was left to cool at temperatures between 31°C and 37°C before being inoculated with *M. madagascariense*, *M. indicus pranii*, and *M. aurum* separately. Thereafter, *M. madagascariense* was incubated at a temperature of 31°C while *M. indicus pranii and M. aurum* were incubated at 37 °C. The optimal growth of the mycobacterial cultures was 24 hours for *M. madagascariense* and *M. indicus pranii* and 48 hours *for M. aurum*.

Determination of minimum inhibitory concentrations (MIC)

The MIC values of wild mushroom extracts against three *Mycobacterium* marker strains were determined by a two-fold microdilution method in sterile flat-bottomed 96-well polystyrene microtiter plates. The turbidity of the prepared mycobacterial inoculums was adjusted to 0.5 McFarland units (approximately 1.2 x10^{8}

CFU/mL). The concentration of stock solutions of the test wild mushroom extracts before serial dilutions was 100 mg/mL. Hence, each hole in the 96 microtitre plate was filled with 50 µL of broth followed by 50 µL of wild mushroom extracts in duplicates. Serial dilution was done such that 50 µL from each of the preceding rows in the 96-well microtiter plate was taken and put into the next row until the last row. Then in the last row, the 50 µL was discarded. The procedure was followed by the inoculation of 50 µL of mycobacteria cultures in each hole. Some wells on the same plate were used as the positive and negative controls. Ethambutol was used as the positive control for *M. aurum* while ciprofloxacin was used as the positive control for for *M. madagascariense*, and *M. indicus pranii*. Broth alone and solution of 10% DMSO in sterilized distilled water was used as the negative control. The 96 well microtitre plates were incubated aerobically for 24 hours at 37°C for *M. indicus pranii* for 48 hours at 37°C for *M. aurum* and 24 hours at 31°C for *M. madagascariense.* Iodonitrotetrazoium chloride (40 µL) was added and incubation continued for one hour to facilitate detection of surviving mycobacteria. Microbial growth was indicated by a colour change from colourless to pinkish [27].

Statistical analysis

The brine shrimp data were used to plot regression equations of percentage mortality converted to probit value against log concentrations. The regression equations obtained were used to determine lethal concentration of sixteen percent (LC_{16}), lethal concentration of fifty percent (LC_{50}) and lethal concentration of eighty-four percent (LC_{84}) values. Confidence intervals (95% CI) were calculated according to Litchfield and Wilcoxon, 1949 [28]. The results were interpreted as follows: LC_{50} above 100µg/mL as non-toxic, LC_{50} between 30 and 100 µg/mL: mildly toxic, LC_{50} between10.0 and 30.0 µg/mL: moderately toxic, LC_{50} between 1.0 and 10.0 µg/mL: toxic and LC_{50} below 1.0 µg/mL as highly toxic [24].

Results and Discussion

The objective of the present study was to evaluate wild mushrooms growing in Namtumbo district, southern Tanzania for safety and potential to exhibit antimycobacterial activity. The results for safety in Table 1 show that 21 out of the 32 mushroom extracts which were tested for safety showed LC_{50} values between 20 and 100 µg/mL and the remaining 11 mushroom extracts had LC_{50} results between 105 and 465.97 µg/mL. There was only one mushroom, *Clavulina wilsoli*, which gave an LC_{50} below 30 µg/mL (20.28 µg/mL) which was slightly higher than that of the positive control cyclophosphamide which had an LC_{50} of 16.78 µg/mL.

The brine shrimp lethality test was used as an initial surrogate assay to determine safety of the mushrooms. The mushrooms reflect three characteristics; mushrooms that are known to be edible and those not known to be edible; mushrooms that are reported to be toxic and mushrooms that are reported to be used for medicinal purposes to protect humans from diseases such as inflammation, rheumatic pain, haemorrhoids, lung and heart conditions, skin infections, stomach pain and cancer, among other conditions.

Table 1. Toxicity evaluation of selected wild mushrooms

Scientific name (family) /voucher number	Local use	LC_{50} (µg/mL)	LC_{50} Extract/ LC_{50} CPMD*	Reference
Afroboletus luteolus (Heinem.) Pegler & T.W.K.Young (Bulelaceae)/M09	Not edible	42.22	2.52	[16,17,21]
Afrocantharellus symoensii Heinem., Bull.Jard.bot.etat.Bruk. (Cantharellaceae)/M06	Edible and highly valued by communities	47.62	2.84	[17,21]
Afrocantharellus platyphyllus Eyssart and Buyck (Cantharellaceae)/M07	Edible and highly valued by communities	61.94	3.69	[16,17,21]
Agaricus spp. (Agaricaeae)/M01	Edible and highly valued by communities	124.64	7.43	[21]
Amanita masasiensis Hark. and Saarim (Amanitaceae)/M32	Edible, the dried mushroom is also used for the wound management	44.19	2.63	[16,17,21]

Table 1. Continued.

Scientific name (family) /voucher number	Local use	LC_{50} (µg/mL)	LC_{50} Extract/ LC_{50} CPMD*	Reference
Auricularia delicate (Mont.) Henn. (Auriculaceae)/M08	Edible and highly valued by communities; used for rheumatic pain, injury, skin inflammation, haemorrhoids, hemoptysis	71.19	4.24	[17,21]
Auricularia polytricha (Mont.) Sacc. (Auriculaceae)/M13	Edible and highly valued by communities; used as therapy for rheumatic pain, skin inflammation and conjunctivitis	103.91	6.19	[16,17,21]
Boletus bicolor Peck. (Boletaceae)/M15	Edible but not highly valued by communities; used to alleviate cold symptoms	222.75	13.27	[17,21]
Boletus pallidissimus Walting (Boletaceae)/M31	Edible but highly valued by communities; skin management	61.01	3.64	
Boletus spectabilisimus Watling (Boletaceae)/M19	Not edible; skin management	347.79	20.73	
Cantharellus cf. floridula Heinem. (Cantharellaceae)/M22	Not edible	125.01	7.45	[16,17,21]
Cantharellus congolensis Beeli (Cantharellaceae)/M03	Edible but not highly valued by communities	77.23	4.6	
Cantharellus isabelinus Heinem (Cantharellaceae) Var. isabellinus/M12	Edible and highly valued by communities; fruiting body is used to cleanse liver, improve vision, regulate breath, nourishe lungs and for diuresis	44.82	2.67	
Chlorophyllum molybdites G. Mey (Agaricaceae)/M10	Poisonous	87.41	5.21	[17,21]
Clavulina spp1(Clavulinaceae)/M16	Edibility not known; the fruiting body is used in improving heartbeats and other heart conditions, also for cancer treatment and reliving stomach pain	31.02	1.85	[21]
Clavulina spp2 (Clavulinaceae)/M17	Edibility not known; used for heart condition, treatment of cancer, relief of stomach pain	105.26	6.27	[21]
Clavulina wilsoli R.H,Peterson (Clavulinaceae)/M05	Edible; the fruiting body is used for improving heartbeats and other heart conditions, relief stomach pain, and skin infection	20.28	1.21	[16,17,21]
Humphreya eminni Henn. (Garnodermataceae)/M21	Not edible	41.34	2.46	[17,21]
Lactarius tanzanicus Karhula and Verbeken (Russulaceae)/M02	Not edible	163.96	9.77	[16,17,21]
Lactarius denigricans Verbeken and Karhula (Russulaceae)/M25	Edible	142.05	8.47	
Lactarius densifolius Verbeken and Karhula (Russulaceae)/M27	Edible and highly valued by communities; fruiting body is used to strengthen weakness of the body, relieve stomach pain, nourish lungs, regulate breath and for the management of cancer	145.27	8.66	
Lactarius edulis Verbeken and Buyck (Russulaceae)/M28	Edible and highly valued by communities	75.88	4.52	
Lactarius heimi Verbeken (Russulaceae)/M20	Not edible	173.06	10.31	
Lactarius kabansus Regler (Russulaceae)/M04	Edible but not highly valued by communities	91.06	5.43	
Lactarius medusae Verbeken (Russulaceae)/M26	Edible but not highly valued by communities	98.51	5.87	
Lactarius pumilus Verbeken (Russulaceae) /M23	Not edible `	60.1	3.58	
Latarius xerampelinus (Russulaceae) Karhula and Verbeken`/M24	Edible, highly valued by communities	182.9	10.89	
Marasmius bekolacongoli Beeli *(*Marasmiusmiaceae)/M29	Not edible	59.02	3.52	
Polyporus molucensis (Mont.) Ryvarden *(Polyporaceae)/M30*	Edible and highly valued by communities; used for the Skin management	465.97	27.77	[16,17,21]
Pseudoboletus paraciticus (Bull.) Sutara (Boletaceae)/M18	Edible, highly valued by communities	35.77	2.13	[21]
Russula affroseovelata Quél. (Russulaceae)/M14	Not edible	72.55	4.32	[16,17,21]
Suillus spp. (Suillaceae)/M11	Not edible	58.01	3.46	[16]
Cyclophosphamide (positive control)	Not applicable	16.78	-	-

*: LC_{50} of extract to LC_{50} of cyclophosphamide (CPMD), positive control; negative control: 0.6% DMSO in artificial sea water

Nine of the wild mushrooms reported in this study, including *Afroboletus luteolus, Boletus spectabilisimus, Cantharellus cf. floridula, Humphreya eminni, Lactarius heimi, Lactarius pumilus, Marasmius bekolacongoli, Russula affroseovelata* and *Suillus* spp. are not considered edible by the local communities. However, according to the brine shrimp results *Boletus spectabilisimus, Cantharellus cf. floridula* and *Lactarius heimi* exhibited very low toxicity and in this respect, they qualify to be classified as non-toxic. It is interesting though to note that *Clavulina wilsoli* with an LC_{50} of 20.28 µg/mL for the brine shrimp results is edible, but is used to treat heart conditions, stomach pain and skin infection [16,17]. The way it is processed for cooking likely removes the possibility of toxicity [28]. It, however, bears the potential to yield useful therapeutic compounds [27]. The LC_{50} results for the remaining mushrooms that are not considered edible by these communities range between 31.34 and 72.55 µg/mL, a range which, according to the set classification, is considered to be mildly toxic [25], that may also suggest the potential to yield compounds with useful biological activities [27]. The mushroom indicated to be *Clavulina* spp*1* (Clavulinaceae) was reported by the local communities to be used to improve heartbeats and other heart conditions, for treating cancer and relieve stomach pain [21]. Its LC_{50} was 31.02 µg/mL indicating that, indeed, it may have the potential to yield anticancer compounds. *Lactarius denigricans* (Russulaceae) is eaten by the local communities and its safety is supported by the brine shrimp results with an LC_{50} value of 142.05 µg/mL, but it is also used to relieve pain, nourish lungs and treat cancer [16,21] which is not surprising because it is not true that all anticancer agents are directly cytotoxic. Their anticancer activities may involve other mechanisms such as modulating the immune system which would fit the nutritional use classification [29].

The brine shrimp results suggest useful information that should be shared with the Namtumbo communities. Some of the other mushrooms that they do not yet eat might be safe. This is important because the types of mushrooms eaten by different communities in Tanzania and elsewhere vary depending on the knowledge existing among the local communities [14,30]. The edible wild mushrooms are highly consumed by the Bena and Hehe tribes in the Njombe and Iringa regions of southern Tanzania [14] as well as the Lamba and Bemba tribes of the Democratic Republic of Congo [31]. *Afroboletus luteolus, Boletus spectabilisimus, Cantharellus cf. floridula, Humphreya eminni, Lactarius Heimi, Lactarius pumilus* and *Russula affroseovelata* which according to the brine shrimp results are mildly toxic are not eaten by the communities living in Namtumbo district but they are mushrooms that are consumed by other communities in Tanzania [14,16,21]. *Lactarius tanzanicus, Lactarius Heimi, Lactarius medusae,* and *Lactarius pumilus* are highly used for food by other communities in Tanzania including Hehe, Yao, Ngido, Nyamwezi, and Bena [14,16,32]. *Russula affroseovelata* is not eaten by communities living in the Namtumbo district in the current study but it is highly valued for food by other communities in Tanzania [14,16]. Burundi and Rwanda [33].

These mushrooms, with their brine shrimp results in parentheses, include *Afrocantherallus symoensii* (47.62 µg/mL), *Amanita masasiensis* (44.19 µg/mL), *Auricularia delicate* (71.19 µg/mL), *Cantheralus congolensis* (77.23 µg/mL), *Cantheralus isabelinus* (44.82 µg/mL), *Clavulina wilsoli* (20.28 µg/mL), *Humphreya eminni* (41.34 µg/mL), *Lactarius tanzanicus* (163.96 µg/mL), *Lactarius kabansus* (91.06 µg/mL), *Lactarius pumilus* (60.10 µg/mL), and *Pseudoboletus parasiticus* (35.77 µg/mL), *Auricularia polytricha* (103.91 µg/mL) and *Lactarius tanzanicus* (163.96 µg/mL) are not, by our classification, toxic to brine shrimp but yet they seem to be viable candidates that may yield active antimycobacterial compounds.

Since the publication of the work by Meyer *et al*., 1982 [25] a lot of information has accumulated which support the utility of the BST test as a predictive test for the toxicity of plant extracts to humans [26]. This has promoted the substitution of animal models with BST in preliminary toxicological tests due to ethical issues in using animal models. Some studies have shown that there is a good positive correlation between LC_{50} for BST and LD_{50} results obtained using animal models including mice and rats in toxicity evaluation. The LD_{50} above 5000 mg/kg body wt for mice and rats and LC_{50} above 100 µg/mL for BST indicate no toxicity in the products [26,34 35,38]. Therefore, BST is a significant model for preliminary toxicity evaluation as an alternative for animal models including mice and rats [36].

The results in Table 2 show the antimycobacterial

activity of the mushroom extracts against the three mycobacterium species. All the 32 mushroom extracts were tested against *M. madagascariense*, *M. aurum* and *M. indicus* pranii. The extracts of *Pseudoboletus parasiticus*, *Afrocantharellus platyphyllus* and *Amanita masasiensis* were the most active against *M. madagascariense* with an MIC of 0.78 mg/mL each followed by *Cantharellus congolensis*, *Auricularia delicate* and *Auricularia polytricha* each with MIC of 3.125 mg/mL.

Table 2. Antimycobacterial activity of the wild mushrooms

Scintific name	MIC (mg/mL)		
	MM	MIP	MA
Afroboletus luteolus	>12.5	>12.5	Nd
Afrocantharellus symoensii	12.5	0.098	>2.5
Afrocantharellus platyphyllus	12.5	0.098	2.5
Agaricus spp	12.5	3.125	Nd
Amanita masasiensis	0.78	0.39	2.5
Auricularia delicate	3.125	1.56	2.5
Auricularia polytricha	3.125	0.78	2.5
Boletus bicolor Massee	12.5	6.25	Nd
Boletus pallidissimus Walting	>12.5	>12.5	Nd
Boletus spectabilisimus	>12.5	>12.5	Nd
Cantharellus cf. floridula	>12.5	>12.5	Nd
Cantharellus congolensis	3.125	0.78	2.5
Cantharellus isabelinus	12.5	0.195	>2.5
Chlorophyllum molybdites	>12.5	>12.5	Nd
Clavulina spp*1*	6.25	3.125	>2.5
Clavulina spp*2*	6.25	1.56	>2.5
Clavulina wilsoli	12.5	0.78	2.5
Humphreya eminni	6.25	1.56	2.5
Lactarius tanzanicus	6.25	1.56	2.5
Lactarius denigricans	>12.5	6.25	Nd
Lactarius densifolius	>12.5	>12.5	Nd
Lactarius edulis	>12.5	>12.5	Nd
Lactarius Heimi	>12.5	>12.5	Nd
Lactarius kabansus	6.25	3.125	2.5
Lactarius medusa	>12.5	>12.5	Nd
Lactarius pumilus	12.5	0.78	2.5
Latarius xerampelinus	>12.5	>12.5	Nd
Marasmius bekolacongoli	>12.5	>12.5	Nd
Polyporus molucensis	6.25	3.125	>2.5
Pseudoboletus parasiticus	0.78	0.195	1.25
Russula aff.roseovelata	6.25	3.125	>2.5
Suillus spp	>12.5	>12.5	Nd

MIC: minimum inhibitory concentration: MM: *Mycobacterium madagascariense*; MIP: *Mycobacterium indicus pranii*; MA: *Mycobacterium aurum*; Nd: not tested; positive control for MIP and MM were ciprofloxacin (MIC <0.004 mg/mL); positive control for MA was ethambutol (MIC <1.56 µg/mL); negative control was broth alone

The extracts of seven mushrooms including *Lactarius tanzanicus*, *Lactarius kabansus*, *Russula aff. Roseovelata*, *Clavulina* spp1, *Clavulina* spp 2, *Humphreya eminni* and *Polyporus molucensis* showed MIC of 6.25 mg/mL and the remaining had MIC values of 12.5 mg/mL and above.

The results in Table 2 show that the mushroom extracts exhibited higher antimycobacterial activity against *Mycobacterium indicus pranii* whereby *Afrocantherallus platyphyllus* and *Afrocantherallus symoensii* gave the lowest MIC of 0.098 mg/mL followed by *Cantharellus isabelinus* (MIC: 0.195 mg/mL), *Amanita masasiensis* (MIC: 0.39 mg/mL). Mushroom extracts of *Cantharellus congolensis*, *Lactarius pumillus*, *Clavulina wilsoli* and *Auricularia polytricha* each had the MIC of 0.78 mg/mL. The MIC results of the extracts against *M. aurum* ranged from 1.25 to 2.5 mg/mL for 11 mushroom extracts and above 2.5 mg/mL for six mushroom extracts which were tested. Fifteen mushroom extracts were not tested against this Mycobacterium species.

The emergence of multi drug resistant (MDR), extremely drug resistant (XDR), and recently reported total drug resistance (TDR) as well as HIV/AIDS and tuberculosis co-infection necessitate an urgent need for new scaffolds from natural products with new mechanisms of action [37,38,39]. One of the objectives of the present study was to investigate the potential of the indigenous mushrooms for antimycobacterial effects. The hydroalcoholic extracts of *Afrocantherallus platyphyllus* had an MIC of 0.098 mg/mL against *M. indicus pranii*. We recently reported isolation of two compounds with good antimycobacterial activitiy from the ethyl acetate extract fraction of a hydroalcoholic extract of the fruiting bodies of *Afrocantherallus platyphyllus* collected from Namtumbo district, Tanzania yielded [40].

Conclusion

Preliminary investigation has provided evidence that some of the mushrooms locally available in Namtumbo district, southern Tanzania, may not be toxic but the local communities are not using them as food. Some of these wild mushrooms have antimicobacterial activity which may yield potential antimycobacterial compounds and other biologically active compounds. Further studies to determine the therapeutic and nutritional value of these mushrooms are needed.

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the antimycobacterial activity screening against *M. aurum.*

Authors contributions

Michael Qwarse conducted the laboratory experiments and drafted the manuscript; Mainen Julius Moshi guided the component on biological testing; Michael Qwarse, Alphonce Ignance Marealle, Ramadhani Sulemani Omari Nondo and Mainen Julius Moshi contributed to data analysis and manuscript development. The manuscript was reviewed by Matobola Joel Mihale and Veronica Mugoyela; Michael Qwarse and Mainen Julius Moshi supported the design of the study, review of the results and edited the draft of the manuscript. The manuscript was approved by all authors before submission for publication.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the accuracy and integrity of the paper content.

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Abbreviations

Anti-TB: anti-tuberculosis; BST: brine shrimp lethality test; CPMD: cyclophosphamide; DAAD: German academic exchange service; DMSO: dimethyl sulfoxide; INT: iodonitrotetrazolium chloride; IRB: institutional review board; LC16: lethal concentration of sixteen percent; LC_{50}: lethal concentration of fifty percent; LC_{84}: lethal concentration of eighty-four percent; MA: *Mycobacterium aurum*; MDR: multi-drug resistant; MIC: minimum inhibitory concentrations; MIP: *Mycobacterium indicus*

pranii; MM: *Mycobacterium madagascariense*; MUHAS: Muhimbili University of Health and Allied Sciences; MTB: *Mycobacterium tuberculosis*; TD: total drug resistance; XDR: extensively drug-resistant