# Antibacterial and antifungal activities of selected wild mushrooms from Southern Highlands of Tanzania

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**Abstract**

The antibacterial and antifungal activities of crude extracts of *Lactarius sp, Russula kivuensis, Amanita muscaria, Amanita phalloides, Lactarius gymnocarpoides* and *Lactarius densifolius* (wild mushrooms) were evaluated against. The results showed that, *A. muscaria* petroleum ether extract (MS1PE) exhibited moderate antimicrobial activity against *Shigella flexneri* and *Klebsiella oxytoca* with MIC of 1.56 mg/mL. Chloroform (MS1C), petroleum ether (MS1PE) and ethanol (MS1E) extracts of *A. muscaria* showed weak activity against *Vibrio cholera* and *Streptococcus pyogens* (3.13 mg/mL). *Mycobacteria* were more resistant to all wild mushroom extracts. On the other hand, *A. muscaria* ethanol extract (MS1E) showed high activity against *Candida albicans* with MIC of 0.78 mg/mL moreover, it was least active against *Cryptococcus neoformans* (MIC = 12.5 mg/mL). The *A. muscaria* chloroform extract (MS1C) showed moderate activity against both *C. albicans* and *C. neoformans* (MIC = 1.56 mg/mL) while its petroleum ether extract (MS1PE) was weakly active against both *C. albicans* and *C. neoformans* (MIC of 6.25 mg/mL). The crude extracts of wild mushroom species tested showed inhibitory activity against some bacteria and fungi species giving insight into development of antimicrobial agent and its application in pharmacological activities.

**Key words:** Wild mushrooms, bioactive compounds, antibacterial, antifungal

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## INTRODUCTION

Mushrooms contain functional or medicinal properties, which may be used as a source of biologically and physiologically active substances where its estimate is about 50% of the annual harvests of 5 million metric tons of mushrooms (Cheung *et al*., 2003; Ferreira *et al*., 2010). According to Mizuno (1995), Wasser (1995), Wasser *et al*. (1999) and Ferreira *et al*. (2010), approximately 700 species of higher Basidiomycetes have been found to possess significant pharmacological activities. Mushrooms have been reported to have great potential as a nutritionally functional food and a source of physiologically valuable and non-toxic medicines (Zaidman *et al*., 2005; Aina *et al*., 2013). Mushrooms have a pronounced potential for the production of useful bioactive metabolites similar to plants and they are prolific resource for drugs with biological activities (Thomas *et al*., 1994; Aina *et al*., 2013). Mushrooms have been found to have potential biological activities such as anti-bacteria, anti-fungi, antitumor, anti-inflammatory, anti-hepatotoxic activity, cardio-tonic activity, cholesterol level lowering activity, antiviral and immune-modulatory activity (Miles and Chang, 1997; Nkunya, 2002; Quang *et al*., 2006; Petrova *et al*., 2007; Mshandete and Cuff, 2008; Salahddin, 2008; Nyigo *et al*., 2009; Ferreira *et al*., 2010 and Saral, 2013). The enormous structural diversity of natural compounds originate from mushrooms offer prospective potential opportunities for discovering new drugs (Russo *et al*., 2007). Therefore, due to the increasing demand for functional foods nutraceuticals and food supplements particularly for individuals with compromised immunity, wild mushroom species that are rich in bioactive compounds, are interesting candidates for evaluation as potential functional food substrates (Baraza *et al*., 2007). Multi-drug resistance from synthetic drugs has prompted the use of natural products such as wild mushrooms as a possible source of bioactive compounds since they have little or no side effects. Most of the synthetic drugs are potentially toxic and not free of side effects on the hosts (Maregesi *et al*., 2008) thus formulation of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for chemical antimicrobial agents is potential and of current concern (Pandian *et al*., 2006; Maregesi *et al*., 2008). Current problem of microbial drug resistance and increased concern on opportunistic infections makes the alternative drugs especially those originating from plants and Basidiomycetes to be prospective. Since few studies have been reported on the antimicrobial activity of different wild mushroom extracts in Tanzania, this study is aimed at screening bioactive compounds from different species of wild mushrooms as antibacterial and antifungal agents.

## MATERIALS AND METHODS

### Materials

The edible (*Lactarius densifolius)* and inedible *(Lactarius sp, Lactarius gymnocarpoides*, *Russula kivuensis*, *Amanita muscaria* and *Amanita phalloides*) wild mushrooms species were collected in Njombe and Iringa regions, Tanzania in January, 2014. The wild mushroom samples were identified by a taxonomist at the University of Dar Es Salaam where voucher specimens were deposited. Gram positive bacteria *Mycobacterium madagascariensis* (DSM 44641) and *Mycobacterium indicuspranii* (DSM 45239) were obtained from the Germany Resource Centre for Biological materials (DSMZ, Braunschweig, Germany). *Streptococcus pyogens* (clinical isolate) were obtained from Muhimbili University of Health and Allied Sciences (MUHAS). Gram negative bacteria, *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 29953), *Salmonella kisarawe* (clinical isolate), *Salmonella typhi* (NCTC 8385), *Vibrio cholerae* (clinical isolate), *Klebsiella pneumoniae* (clinical isolate) *Shigella flexneri* (clinical isolate) and *Klebsiella oxytoca* (clinical isolate) were obtained from Muhimbili University of Health and Allied Sciences (MUHAS). Fungi strains *Candida albicans (*ATCC 90028) and *Cryptococcus neoformans* (clinical isolate) were obtained from Muhimbili University of Health and Allied Sciences (MUHAS). Nutrient agar, nutrient broth, Sabouraud dextrose agar and Saboraud dextrose broth (liquid media) were purchased from HiMedia Laboratories, India. Iodo nitrotetrazolium chloride (INT) was from (Sigma-Aldrich, USA). All other reagents and chemicals were of analytical grade.

### Wild Mushroom Extracts Preparation

Fresh whole mushrooms (25 g) were ground using a blender (Singsung - Singapore) and extracted sequentially by soaking twice in 250 mL of ethanol and chloroform for 48 h. The extracts were filtered using a filter paper and further concentrated in vacuum evaporator (Heildolph, Germany). The concentrates were then stored at 4° C for further bioassays.

### Antimicrobial Assay

Antimicrobial activity was determined according to the method of (Eloff, 1998). Sub-culturing of bacteria and fungi strains was done using Nutrient agar and Sabouraud dextrose agar respectively. Mycobacteria sub-culturing and testing was done using middle brook broth unlike to other bacteria and fungi strains. A 50 µL of the broth media were uploaded in each well of the 96 micro plate followed by 50 µL of the extract into the first wells of each row making a total volume of 100 mL.

Serial dilution was done by thorough mixing the broth and extracts in the first wells and then 50 µL were drawn from each of the first row wells and put into the next row. The procedure was repeated down the columns of the plate to the last wells at the bottom end where at last 50 µL was discarded (Maregesi *et al*., 2013). Subsequently, 50 µL of the test microbe's suspension 0.5 Mac Farhland standard turbidity (Maregesi *et al*., 2013) was prepared by adding microbe's inoculum into sterile distilled water and adjusted to get the right turbidity then added in each well to make the final volume of 100 µL. A positive standard Gentamycin was used and Dimethyl sulphoxide (DMSO) was taken as negative control. The plates were then incubated at 32 °C for 24 h while those of mycobacteria were incubated at 37 °C for 24 h. The Minimum Inhibition Concentrations (MIC's) for each extract were determined by using 40 µL of 0.02% p-iodo nitrotetrazolium (INT) chloride dye dissolved in water was and then added in each well and incubated at 32 °C for 1 h (Eloff, 1998). Microbial growth was indicated by a colour change to pink and the lowest concentration which showed no microbial growth was regarded as MIC (Mwangomo *et al*., 2012; Maregesi et al., 2013).

**Data Analysis**

Results represent triplicate determinations. For the antimicrobial test, the minimum inhibitory concentration (MIC) which killed all the organisms was recorded.

**RESULTS**

The antimicrobial activities of crude extracts of one edible (*Lactarius densifolius)* and five inedible *(Lactarius sp, Lactarius gymnocarpoides, Russula kivuensis, Amanita muscaria* and *Amanita phalloide*s) species of wild mushrooms against bacteria and fungi strains are shown in **Table 1 & 2**. The *A. muscaria* petroleum ether extract (MS1PE) showed moderate inhibitory activity against *S. flexneri*, and *K. oxytoca* with MIC of 1.56 mg/mL, however, it was weak against *S. pyogens* (MIC = 3.13 mg/mL) **(Table 1)**. Chloroform (MS1C) and ethanol (MS1E) extracts of *A. muscaria* showed weak activity against *V. cholera* with MIC of 3.13 mg/mL. *Amanita phalloides* ethanol extract (MS2E) possessed weak activity against *E. coli* (MIC = 3.13 mg/mL). Other tested wild mushroom extracts (MS2E, MS2C, BM2E, MS4C and BM8C) possessed no activity against *S. flexneri, S. typhi, S. pyogens, V. cholera, K. pneumonia, P. aeriginosa, S. kisarawe* and *K. oxytoca* **(Table 1)**. Also, all species of mycobacteria (*M. madagascariensis* and *M. indicuspranii*) were resistance to all wild

mushrooms extracts tested (**Table 1 & 2**). Some of the tested mushroom extracts possessed inhibitory activity against fungi strains. *Amanita muscaria* ethanol extract (MS1E) exhibited moderate inhibitory activity against *C. albicans* with MIC of 0.78 mg/mL however, it was not active against *C. neoformans* (MIC = 12.50 mg/mL). *Amanita. muscaria* chloroform extract (MS1C) was moderate active against both *C. albicans* and *C. neoformans* (MIC = 1.56 mg/mL) besides, its petroleum ether extract (MS1PE) possessed weak activity against both *C. albicans* and *C. neoformans* (MIC = 6.25 mg/mL). All other wild mushroom extracts showed no inhibitory activity against both fungi species **(Table 2)**.

**Table 1. Inhibitory activity (MIC) of wild mushroom crude extracts against selected human pathogenic bacteria**

| Bacteria | Wild mushroom crude extracts (mg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **MS1PE** | **MS1C** | **MS1E** | **MS2E** | **MS2C** | **BM2E** | **MS4C** | **BM8C** | **Gentamycin** |
| *S. flexneri* | 1.56 | 12.50 | 6.25 | 25.00 | 12.50 | 25.00 | 12.50 | 12.50 | 0.0015 |
| *S. typhi* | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 12.50 | 0.0061 |
| *V. cholera* | 12.50 | 3.125 | 3.125 | 6.25 | 12.50 | 6.25 | 6.25 | 6.25 | 0.0015 |
| *E. coli* | 12.50 | 25.00 | 12.50 | 3.125 | 6.25 | 6.25 | 6.25 | 6.25 | 0.0015 |
| *S. kisarawe* | 12.50 | 12.50 | 6.25 | 12.50 | 25.00 | 25.00 | 25.00 | 25.00 | 0.0061 |
| *S. pyogens* | 3.125 | 6.25 | 6.25 | 6.25 | 6.25 | 25.00 | 25.00 | 25.00 | 0.0015 |
| *K. pneumoniae* | 12.50 | 6.25 | 6.25 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 0.0030 |
| *P. aeriginosa* | 6.25 | 6.25 | 6.25 | 25.00 | 25.00 | 25.00 | 25.00 | 25.00 | 0.0015 |
| *K. oxytoca* | 1.56 | 25.00 | 25.00 | 12.50 | 25.00 | 12.50 | 25.00 | 25.00 | 0.0030 |
| *M. madagascariensis* | 12.50 | 25.00 | 25.00 | 12.50 | 12.50 | 12.50 | 25.00 | 25.00 | NT |
| *M. indicuspranii* | 12.50 | 25.00 | 25.00 | 25.00 | 25.00 | 12.50 | 25.00 | 25.00 | NT |

**Table 2: Inhibition activity (MIC) of wild mushroom crude extracts against selected human pathogenic fungi**

| Fungi | Wild Mushrooms crude extracts (mg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **MS1C** | **MS1E** | **MS1PE** | **BM2E** | **MS4PE** | **MS4C** | **BM1C** | **BM8C** | **Fluconazole** |
| *C. albicans* | 1.56 | 0.78 | 6.25 | 12.50 | 25.00 | 25.00 | 25.00 | 25.00 | 0.006 |
| *C. neoformans* | 1.56 | 12.50 | 6.25 | 12.50 | 12.50 | 25.00 | 25.00 | 25.00 | 0.003 |

**Key:** MS1PE = *Amanita muscaria* petroleum ether extract, MS1C = *Amanita muscaria* chloroform extract, MS1E = *Amanita muscaria* ethanol extract, MS4PE = *Russula kivuensis* petroleum ether extract, MS2E = *Amanitha phalloides* ethanol extract, MS2C = *Amanita phalloides* chloroform extract, BM2E = *Lactarius gymnocarpoides* ethanol extract, BM8C = *Lactarius densifolius* chloroform extract, MS4C = *Russula kivuensis* chloroform extract, BM1C = *Lactarius sp.* chloroform extract, BM2C = *Lactarius gymnocarpoides* chloroform extract, MS4E = *Russula kivuensis* ethanol extract, BM1E = *Lactarius sp.* ethanol extract and NT = Not tested.

## DISCUSSION

Microbial infections possess a very serious public health problem all over the world especially in poor countries (Agada *et al*. 2012). The demonstration of antibacterial activity against both gram positive and gram-negative bacteria may be indicative of the presence of broad-spectrum antibiotic compounds (Tassou *et al*, 1995; Agada *et al*, 2012). According to Algiannis *et al*. (2001), proposed classification based on MIC values is as follows; extracts with MIC up to 0.5 mg/mL are considered as strong inhibitors, 0.6 - 1.5 mg/mL as moderate inhibitors and those with MIC values above 1.6 mg/mL as weak inhibitors. From this study, it was shown that some extract of wild mushrooms, exhibited moderate inhibitory activity against one or more bacterial and fungal species tested, whilst some showed weak or no activity to the bacteria and fungi species. In particular, among all extracts, petroleum ether and ethanol extracts of some wild mushrooms possessed moderate antimicrobial activity against tested microorganisms. The weak or inactive activity of *S. pyogens, K. pneumonia, P. aeriginosa, S. kisarawe* and *S. typhi* against wild mushroom extracts is similar to the findings reported by Priya and Srinivasan (2013), where *Ganoderma* showed medium inhibitory action against

*Bacillus* sp., and *Staphylococcus aureus*, low inhibitory action against *E. coli* and *K. pneumoniae* however, no inhibition zone was observed against *P. aeruginosa*. Additionally, *Lentinus edodes*, *Pleurotus obstreactus* and *Agaricus bisporus* strains showed no inhibition zone after 48 h of incubation against *Bacillus spp*., *S. aureus*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. This suggests that some of the tested microbes were resistant to wild mushroom crude extracts. The microbial resistance of *E. coli*, *S. typhimurium*, *P. aeruginosa* and *A. niger* was also reported by Maregesi *et al*. (2008) in which the strains were resistant to most of the plant extracts from *Barleria eranthemoides* (leaves) and *Lonchocarpus eriocalyx* (stem bark).

On the other hand, mycobacteria (*M. madagascariensis* and *M. indicuspranii)* were more resistant to all mushroom crude extracts. The observed inhibitory activities of crude extracts were lower as compared to Gentamycin and Fluconazole. Suay *et al.*, 2000, reported that crude form of the extracts or presence of many compounds may provoke each other and reduce activity. Thus, further studies on isolation, purification and screening of active compounds are necessary. Best to the knowledge, no studies have been done to evaluate antibacterial and antifungal activities of the wild mushroom species tested. Therefore, findings from this study showed that wild mushroom crude extracts may also exhibit antibacterial activity as compared to antifungal activity. Salahuddin (2008) reported that the *Schizophyllum commune* mushroom had more antibacterial activity than antifungal activity. Also, antibacterial activity of *Polypores* and gilled (*Agaricales*) mushrooms had more pronounced activity than antifungal Suay *et al*. (2000). Jonathan and Fasidi (2003), also suggested that the antifungal activities of the mushrooms *Lycoperdon pusilum* and *Lycoperdon giganteum* extracts against pathogenic tested fungi were very low. Also, Lauer *et al*. (1991) observed that *Lentinus adherens* mushroom extracts were less effective against pathogenic fungi compared to pathogenic bacteria. Generally, it is observed in many studies that the fungal and yeast strains are more resistant to antimicrobial compounds than bacterial strains (Nishizawa *et al.* 1990; Papadopoulou *et al*. 2005).

## CONCLUSION

The wild mushrooms crude extracts showed moderate to weak activities against some species of bacteria and fungi. Also, some of the crude extracts exhibited no activity against some bacteria and fungi species. All extracts showed no inhibitory activity against two species of mycobacterium. Therefore, some species of wild mushrooms collected from Southern Highlands of Tanzania has a high potential to be used as natural antimicrobial agents. Hence, further studies on isolation and

screening of the active compounds might deliver a better source for emerging new therapeutic agents and therefore find its application in nutraceuticals and pharmaceuticals industries.

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