EISSN: 2707-0425

East African Journal of Science, Technology and Innovation, Vol. 6 (2): March 2025

Antibacterial and antifungal Activities of Crude Ethanolic Extracts of Wild Edible Mushrooms Found in Morogoro Municipality, Tanzania

[1*]*KIBONA V J.*, [2]*LUPALA A S.*, [2]*MWEGA E D.*, [1]*NONGA H E.*, [1]*MDEGELA R H.*, [2]*SANGA G J.*, [2]*NJAU E P*

[1]Department of Veterinary Medicine and Public Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P. O. Box 3021, Chuo Kikuu, Morogoro, Tanzania
[2]Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, P.O. Box 3019, Chuo Kikuu, Morogoro, Tanzania

***Corresponding Author**: kibonavumilia@gmail.com

Abstract

The increasing occurrence of antimicrobial resistance necessitates search for alternative bioactive compounds against microbes, in particular natural products from plants and mushrooms. A cross-sectional investigation was carried out from November 2023 to March 2024 to investigate the antimicrobial properties of crude ethanolic extracts of wild edible mushrooms in Morogoro Municipality. The crude ethanolic extracts tested were from eight wild edible mushrooms namely *Afrocantharellus platyphyllus*, *Amanita* sp, *Cantharellus* sp, *Cantharellus luteopunctatus*, *Craterellus* sp, *Lactarius kabansus*, *Lentinus* sp, and *Termitomyces* sp. The mushroom ethanolic extracts were tested against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Salmonella typhi* (ATCC 33459) and *Candida albicans* (ATCC 90028) using agar well diffusion and tetrazolium microtiter plate bioassay methods. Five of the eight crude ethanolic extracts of mushroom species tested revealed promising antibacterial and antifungal activity. The mean zone of inhibition for extracts of *Craterellus* sp, *Cantharellus* sp, *C. luteopunctatus*, *A. platyphyllus*, and *L. kabansus* ranged from 9.70 ± 0.33mm (Mean ± StdDev) to 17.00 ± 0.57 mm. The recorded minimum inhibitory concentrations (MIC) value for these extracts varied from 5.2 ± 1.11 to 266.67 ± 66.70 mg/ml. *Staphylococcus aureus*, *S. typhi*, *P. aeruginosa*, and *C. albicans* were more susceptible to ethanolic extracts of *Craterellus* and *Cantharellus* sp. None of the extracts demonstrated an inhibitory effect on the growth of *B. subtilis*. This study indicates that some wild edible mushrooms from the Morogoro Municipality exhibit potential antimicrobial effects against both bacterial and fungal species. Therefore, additional research is advised to isolate and identify the bioactive compounds and to evaluate their efficacy and toxicity in animal models to confirm their antibacterial and antifungal properties.

Keywords: *Antimicrobials; antimicrobial resistance; ethanolic extract; Morogoro; wild edible mushrooms*

Received: 24/02/25
Accepted: 11/03/25
Published: 28/03/25

Cite as, *Kibona et al., (2025)*. Antibacterial and antifungal Activities of Crude Ethanolic Extract of Wild Edible Mushrooms Found in Morogoro Municipality, Tanzania. *East African Journal of Science, Technology and Innovation 6(2).*

Introduction

Mushrooms are the distinctive large fruiting bodies of macrofungi belonging to the phylum *Basidiomycota* class *Agaricomycetes*. They can be categorized as edible *(Lactarius edulis, Cantharellus isabelinus,* and *Amanita masasiensis*), inedible (*Boletus* sp), medicinal, or poisonous (Härkönen *et al.,* 1994; Miles and Chang, 2004). Wild mushrooms can thrive in a variety of habitats ranging from aquatic to terrestrial environments and are most abundant in damp and forested areas. Species of mushrooms differ in terms of flavor, texture, size, and shape. Edible mushrooms are a nutritional source in most regions of the world, and their worth has grown over time, particularly due to their potential medical uses (Hamza *et al.,* 2024). Their use in promoting and maintaining good health, and traditional disease treatment, dates back to ancient times, especially in Asia (Venturella *et al.,* 2021).

In Tanzania, indigenous mushrooms have been harvested for a long time and utilized as a crucial source of food and income (Chelela *et al.,* 2014; Marealle *et al.,* 2021). Over 80 species of mushrooms have been identified in the country, and are predominantly collected in the wild during the rainy season (Tibuhwa, 2011; Chelela *et al.,* 2014). *Amanita masasiensis, L. edulis, and C. isabelinus* are examples of edible mushrooms found in Tanzania (Qwarse *et al.,* 2021; Nyamoga, 2023). Secondary metabolites such as alkaloids, tannins, flavonoids, terpenoids, and polysaccharides are produced by some mushroom species as; (i) a defence mechanism against pathogenic microbes and predators, (ii) inducing alteration of population structures to gain ecological advantage, and (iii) signalling allelopathy and chemotaxis to establish trophic relationships with other mushrooms or plants (Tibuhwa, 2014; Chelela *et al.,* 2016; Osivand *et al.,* 2018; Vamanu *et al.,* 2018; Pérez-Moreno, 2021). In humans, these bioactive substances are utilized for a variety of pharmacological purposes, including antibacterial, hepatoprotective, cytotoxic, immunomodulatory, antidiabetic, anti-inflammatory, and antioxidant activities (Thakur and Singh, 2013; Wasser, 2014).

Antimicrobial resistance can occur naturally, arising through genetic changes in microorganisms, or result from the misuse of antimicrobials. In 2021, WHO reported an alarming increase in the misuse of antimicrobial drugs in the livestock sector, particularly in food animals, contributing significantly to the development and transmission of resistant strains to humans. This resistance leads to the failure of traditional antimicrobial treatments, making common infections harder to treat, prolonging infections, increasing medical costs, raising mortality rates, and posing a serious threat to public health. This highlights the necessity of exploring bioactive compounds from mushrooms as potential alternatives to conventional antimicrobial drugs (Gebreyohannes *et al.,* 2019). With the emergence of novel strains of antimicrobial-resistant bacteria and fungi, it is essential to look for new substances with antimicrobial effects in natural products, including mushrooms (Roca *et al.,* 2015; Ferri *et al.,* 2017; Noman *et al.,* 2022). Despite the growing global interest in natural antimicrobial compounds, research on mushrooms as alternative sources of bioactive metabolites remains limited, particularly in Tanzania and Africa at large. Many studies have focused on plants and synthetic compounds, while the antimicrobial potential of fungi, especially locally available and traditionally used mushrooms, remains largely unexplored. Historical and anecdotal reports indicate that people in Africa have used mushrooms for food, medicinal purposes, recreational activities, myths and cultural beliefs, and source of income for poor households (Tibuhwa, 2013). Most research in Tanzania focuses on mushroom diversity, taxonomy, biology, and the study of bioactive compounds derived from mushrooms (Härkönen *et al.,* 2003; Magingo *et al.,* 2004; Kivaisi, 2007; Kivaisi *et al.,* 2009; Tibuhwa, 2012). Nonetheless, mushrooms can serve as alternative resources for combating disease pathogens, including bacteria and fungi. However, limited studies have investigated the antimicrobial activities of mushrooms in Tanzania (Baraza *et al.,* 2009; Chelela *et al.,* 2014; Tibuhwa, 2017). Hence, there was a need to investigate the antibacterial and antifungal activity of mushrooms as an alternative to commercial drugs used in humans and livestock. The

purpose of this study was to determine the antimicrobial potential of wild edible mushrooms found in Morogoro Municipality, Tanzania. The baseline results provide a foundation for further research on natural products with the potential to combat antimicrobial resistance (AMR). Additionally, the findings contribute to Tanzania's National Development Vision 2025 and the National Action Plan on Antimicrobial Resistance (AMR) 2023-2028.

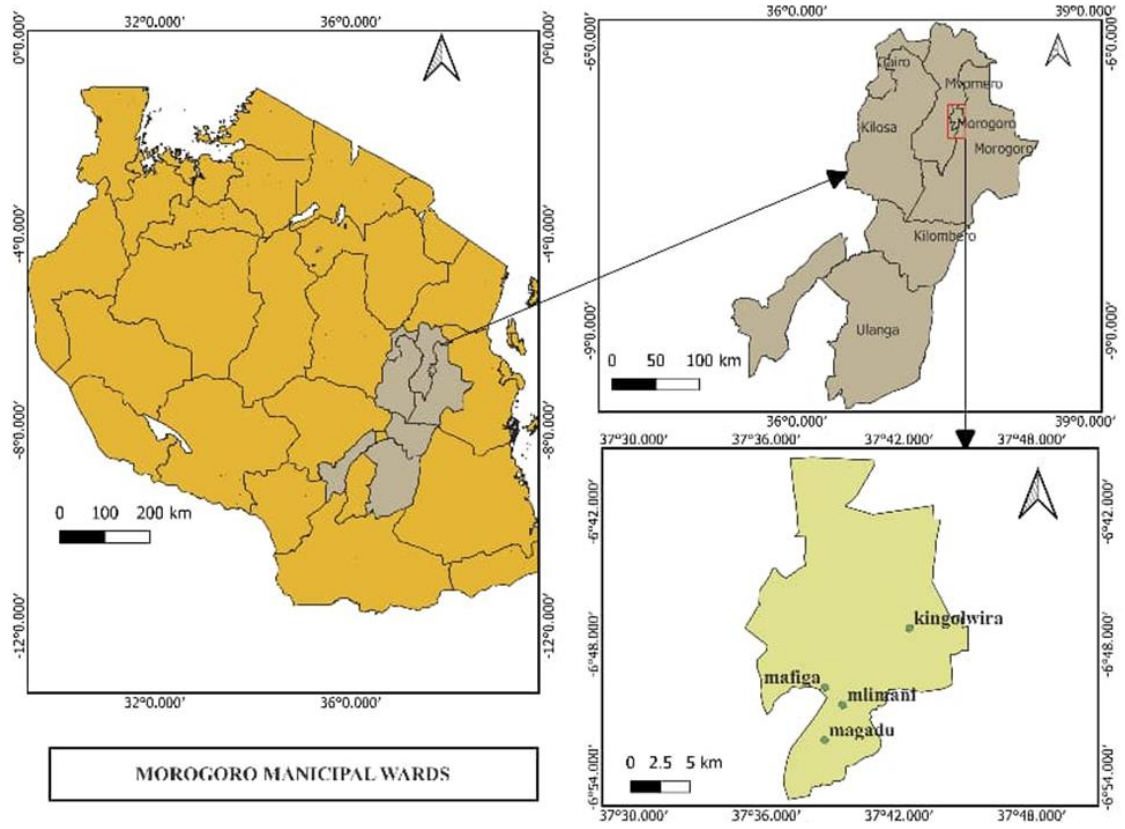
Material and Methods

Study area

The research was carried out in Morogoro Municipality, in the Morogoro region, of Tanzania. The Municipality has 29 wards and a human population of 471,409 according to the 2022 census (Population and Housing Census Tanzania, 2022). Morogoro Municipality is located 200 kilometers west of Dar es Salaam and it lies in the eastern part of Tanzania, at longitude 37°39′40″ E and latitude 6°49′15″ S (Figure1). The average annual temperature of 20-30°C and annual rainfall is between 821 - 1505 mm (URT, 2024). Morogoro experiences a tropical climate with distinct wet and dry seasons, including a long rainy season from March to May and a short one from October to December (Balama *et al*., 2016). Four wards namely Kingolwira, Magadu, Mafiga, and Mlimani were purposively selected based on the availability of mushrooms in the locality.

Figure 1

A Map of Morogoro Municipality, Tanzania, showing Wards in Which Samples Were Collected



Study design and sampling strategies

A cross-sectional study design was used, with study wards selected purposively and through the snowball sampling method. This approach relied on knowledgeable and experienced individuals involved in wild edible mushrooms.

Inclusion criteria were based on the abundance and presence of edible wild mushrooms in the surrounding areas. Since no prior literature on the diversity and abundance of wild edible mushrooms in Morogoro was available to determine a predefined sample size, we employed an exploratory approach. The sample size was determined by the number of wild edible mushroom species successfully identified and collected during the study period. Additionally, as mushroom fruiting is highly dependent on seasonal and ecological conditions, our sampling was limited to species available during the collection period. This approach ensured the inclusion of all accessible species for a comprehensive assessment of their antimicrobial potential. A total of eight wild edible mushroom species were collected and analyzed in this study.

Mushroom collection and identification

Previously identified mushrooms (Kibona *et al.*, in press) based on morphological features i.e. their body form, cap, spore-bearing surface, stipe, stem, odor, color, gills, presence or absence of the ring, volva and bulbous base structure, ornamentation, and texture. The morphological features of the mushrooms were compared to reference keys in field guidebooks, online resources (e.g., the CABI Bioscience database at https://www.speciesfungorum.org/), and fungi nomenclature standards (Lodge and O'Dell, 2004; Walshaw, 2004; Tibuhwa, 2012; Tibuhwa, 2013). Molecular techniques targeting the rDNA-ITS and nrLSU region were used to complement morphological identification (Schoch *et al.,* 2012). Eight wild edible mushroom species namely; *Afrocantharellus platyphyllus*, *Amanita* sp, *Cantharellus luteopunctatus*, *Cantharellus* sp, *Craterellus* sp, *Lactarius kabansus*, *Lentinus* sp, and *Termitomyces* sp were tested for their antibacterial and antifungal effects as described by (Andrews, 2001; Sai Latha *et al.,* 2018; Gebreyohannes *et al.,* 2019). The laboratory analysis was done at the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Tanzania.

Mushroom extract preparation

The mushrooms were first washed using distilled water and then oven-dried at 50°C for 8 hours followed by air-drying at room temperature for 2-3 days. The dried mushroom samples were ground into a fine powder using a Sathiya blender. Extraction of bioactive compounds from mushrooms was done using ethanol analytical grade (Oxoid® Ltd., Basingstoke, Hampshire, England, UK). Briefly, the powdered mushrooms were weighed and mixed with 99.9% ethanol at a 1:4 ratio for extraction over 72 hours. The obtained extracts were passed through Whatman filter paper and then concentrated using a rotary evaporator to eliminate the ethanol. A water bath was used to remove excess water for 24 -72 hours. Finally, the crude ethanolic extract was stored in a refrigerator at -4°C for subsequent antibacterial and antifungal analysis.

Preparation of test organism

Two Gram-positive bacteria namely *B. subtilis* (ATCC 6633) and *S. aureus* (ATCC 25923), and three Gram-negative *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853) and *S. typhi* (ATCC 33459) and one fungal species *C. albicans* (ATCC 90028) was used as test organisms. The organisms were obtained from the archive of the Microbiology laboratory at the Sokoine University of Agriculture, Department of Microbiology, Parasitology and Biotechnology. The test bacteria were cultured on nutrient agar (Oxoid® Ltd., Basingstoke, Hampshire, England, UK) at 37°C for 24 hours while *C. albicans* was cultured on Sabouraud dextrose agar (SDA) at 37°C for 48 hours. The inoculum concentration for each organism was standardized to match a 0.5 McFarland standard for consistency (Andrews, 2001).

In vitro antimicrobial activity testing

Agar well diffusion test

The antimicrobial properties of the crude ethanolic extract from eight mushroom species were screened using the agar well diffusion method (Sai Latha *et al.,* 2018). An aliquot of crude ethanolic extract of each of the eight wild edible mushroom species was weighed and added with 3% dimethyl sulfoxide (DMSO) to get 80% concentration as a stock solution that was used in antibacterial and antifungal testing. Nutrient agar and Sabouraud dextrose (Oxoid® Ltd., Basingstoke, Hampshire, England, UK) agar were prepared for the antimicrobial screening of the mushroom extracts. The concentration of the test organism was prepared from overnight

cultures of the microbial isolates and emulsified with sterile sodium chloride to obtain turbidity similar to 1.5 x 10^8 CFU (McFarland standard). The inoculum was then spread uniformly on the agar plates using a sterile swab. Five wells were made in each plate using a cork borer. Then, 100 µL of 80%, 40%, 20%, and 20% of the ethanolic extract of wild edible mushroom was added to each well using a pipette. Gentamicin (10 µg) and nystatin (15 µg/mL) were used as positive control drugs for bacteria and yeast respectively and dimethyl sulfoxide (DMSO) was used as negative control. The plates, preloaded with the respective extracts and test bacteria were incubated at 37°C overnight, and for 48 hours for fungi. The experiment was conducted in independent triplicates, and the inhibition zone diameter was measured in millimeters using a ruler and analyzed according to the Clinical Laboratory Standard Institute (CLSI, 2024). The diameter of the well at 80% was recorded and used for analysis.

Minimum Inhibitory Concentration (MIC), and Minimum bactericidal and fungicidal concentration (MBC/MFC)

The MIC was determined according to Appiah *et al.* (2017) and Gebreyohannes *et al.* (2019) for all mushroom extracts that exhibited antimicrobial activities after performing the agar well diffusion technique. The MIC was determined by the tetrazolium microtiter plate bioassay method. The stock solution of Mueller Hinton broth and mushroom extracts were prepared and used in the test. Fifty microliters of Mueller Hinton broth (Oxoid® Ltd., Basingstoke, Hampshire, England, UK) were aseptically dispensed into each well of a microtiter plate. The mushroom extract stock solution was introduced into wells in column one, mixed, and serially diluted in twofold dilutions up to wells of column nine and discarded 50 µL of the mixture of Mueller Hinton broth and mushroom extracts. Wells in columns 12, 11, and 10 served as negative control, quality control, and positive control, respectively. In each case, with the exception of well number 11, 50 µL of the test organism suspended in normal saline was inoculated into wells 1 through 12. Gentamicin and nystatin were diluted in sterile distilled water to achieve a final concentration of 0. l µg/mL and 3/µg/mL, which were used as a positive control for bacterial and fungal species respectively. The microtiter plate was covered and cultured at 37°C for bacteria and at 30°C for yeast species for 24 hours. After 24 hours of incubation, 50 µL of 0.2% p-iodo nitro tetrazolium chloride (INT) indicator dye was added into each well of the microtiter plate to determine the lowest concentration that inhibited the growth of microorganisms. The INT dye was used to confirm the presence of microorganisms. The microtiter plate was re-incubated for three hours at 37°C and 30°C for bacterial and yeast species, respectively (Maregesi *et al.*, 2008). The color change of the INT dye was monitored every 30 minutes over 3 hours. The results were recorded by a color change of the dye from clear/pale yellow indicating no growth to pinkish red/purple indicating growth of microbes. The minimum inhibitory concentration was identified as the lowest concentration that exhibited no visible growth after the addition of INT dye. The minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) were determined by a loopful of the content from wells showing no color change and transferring it to Sabouraud dextrose agar (SDA) for yeast and nutrient agar (NA) plates for bacterial growth. All plates were incubated for 24 hours at 37°C for bacteria and 30°C for yeast. The MBC and MFC were identified as the lowest crude extract concentrations that prevented any visible bacterial or yeast growth.

Data analysis

Statistical analysis was conducted using a Statistical Package for Social Science software version two (IBM Corporation, Armonk, NY, USA) at 95% confidence intervals (CI). Descriptive statistics were calculated for the inhibition zones, including mean and standard deviation. The Kruskal-Walli's test was used to determine significant differences in the antimicrobial efficacy of crude ethanolic extracts against test organisms because our data were not normally distributed. Pairwise comparisons between the mushroom extracts for each microorganism were made using the Mann-Whitney U and Bonferroni correction tests.

Ethical clearance

We received ethical approval from the Institutional Review Board of the Sokoine

University of Agriculture with DPRTC/R/186/33 as reference number. Furthermore, permission to conduct the research in the area was obtained from the Morogoro Municipality administrative authority, which initially allowed the conduct of this research activity in all the respective study sites (wards).

Results

Antimicrobial activity of ethanolic extract of wild edible mushroom

The results of antifungal and antibacterial activities of crude ethanolic extracts of wild edible mushrooms are summarized in Table 1. Five out of the eight wild edible mushrooms tested namely *Cantharellus luteopunctatus*, *Cantharellus* sp, *Craterellus* sp, *Lactarius kabansus*, and *Afrocantharellus platyphyllus* demonstrated promising antimicrobial activities, with different levels of inhibition against four bacteria species *Staphylococcus aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), *S. typhi* (ATCC 33459), and *C. albicans*. The highest zone of inhibition observed was 17.00 ± 0.57 mm of *Craterellus* sp against *S. aureus,* while the lowest was 9.70±0.33 mm of *A. platyphyllus* against *E. coli*. The *C. luteopunctatus* extracts showed a wide inhibition zone greater than the positive control (Nystatin) against *C. albicans*. *Bacillus subtilis* was not sensitive to all the wild mushroom crude ethanolic extracts. Crude ethanolic extract of *Amanita* sp, *Termitomyces* sp, and *Lentinus* sp were ineffective against all the tested microorganisms.

Table 1

Antimicrobial Activity of 8 Wild Edible Mushrooms' Crude Ethanolic Extract Through Determination of Zone of Inhibition (mm) in Morogoro Municipality, Tanzania

Mushrooms species	***S. aureus***	***E. coli***	***S. Typhi***	***P. aeruginosa***	***B. subtilis***	***C. albicans***
C. luteopunctatus	13.33 ± 0.33	Niz	12.33 ± 0.33	13.33 ± 0.33	Niz	15.33 ± 0.33
Amanita sp	Niz	Niz	Niz	Niz	Niz	Niz
A. platyphyllus	12.67 ± 0.3	9.70 ± 0.33	12.33 ± 0.33	12.33 ± 0.33	Niz	12.33 ± 0.33
Cantharellus sp	13.33 ± 0.33	13.33 ± 0.33	13.33 ± 0.33	12.33 ± 0.33	Niz	12.67 ± 0.33
Craterellus sp	17.00 ± 0.57	13.00 ± 0.57	14.33 ± 0.33	12.33 ± 0.33	Niz	12.67 ± 0.33
L. kabansus	15.33 ± 0.33	11.67 ± 0.33	13.33 ± 0.33	13.00 ± 0.57	Niz	13.33 ± 0.33
Lentinus sp	Niz	Niz	Niz	Niz	Niz	Niz
Termitomyces sp	Niz	Niz	Niz	Niz	Niz	Niz
Gentamicin/Nystatin	22.00 ± 0.00	18.33 ± 0.00	19.33 ± 0.33	18.33 ± 0.33	21.67 ± 0.33	14.67 ± 0.33

Note: The zone of inhibition, expressed as the mean ± standard deviation in millimeters (mm), represents the diameter of microbial growth inhibition. "Niz" denotes the absence of an inhibition zone.

Figure 2

Minimum Inhibitory Concentration of Crude Ethanolic Extract of Cantharellus sp Using Tetrazolium Microtiter Plate Bioassay in Morogoro Municipality, Tanzania

Note: Numbers 1 to 9 at the top of the microtiter plate indicate different concentrations of *Cantharellus* sp extract from i.e. well 1=4g/mL, well 2=2g/mL, well 3=1g/mL, well 4=0.5g/mL, well 5= 0.25g/mL, well 6=12.5mg/mL, well 7=6.3mg/mL well 8=3.1mg/mL, well 9= 1.55mg/mL, well 10=positive control, well11=quality control, well 12=negative control (3% DMSO). Numbers 1-5 in the column represent the test organism i.e. 1=*S. aureus*, 2=*E. coli*, 3=*S. typhi*, 4=*P. aeruginosa*, 5=*C. albicans*

Minimum Inhibitory Concentration (MIC) Using ITN (mg/ml)

The minimum inhibitory concentration (MIC) was assessed for all crude ethanolic extracts of wild edible mushrooms that demonstrated antimicrobial activity during the agar-well diffusion screening. *Cantharellus* sp extract exhibited the highest antimicrobial activity among the extracts, with the lowest MIC values of 5.2 ± 1.10 mg/ml against *S. typhi*. In contrast, the highest MIC recorded was 266.67 ± 66.70 mg/ml of *A. platyphylus* against *P. aeruginosa* (Table 2, Figure 2). All mushroom crude ethanolic extracts had the same antimicrobial efficacy against *S. aureus* (p= 0.061). For the other test organisms, crude ethanolic extracts displayed a difference in antimicrobial efficacy and the p-value was less than 0.05 (Table 2).

Table 2

The Minimum Inhibitory Concentration of Five Crude Ethanolic Extracts of Wild Edible Mushrooms in mg/mL in Morogoro Municipality, Tanzania

Bacterial / Yeast	Mushroom species						
	C. luteopunctatus	*A. platyphyllus*	*Cantharellus* sp	*Craterellus* sp	*L. kabansus*	Gentamicin/ Nystatin	p-value
S. aureus	16.7 ± 4.2	16.7 ± 4.2	12.9 ± 4.2	8.3 ± 2.0	10.4 ± 2.1	0.0001 ± 0.00	0.061
E. coli	np	41.7 ± 8.3^{a}	20.8 ± 4.2^{a}	33.3 ± 8.3^{a}	33.3 ± 8.3^{a}	0.0001 ± 0.00^{b}	0.014
S. Typhi	10.4 ± 2.1^{a,b}	16.7 ± 4.2^{a}	5.2 ± 1.1^{b}	20.8 ± 4.2^{a}	20.8 ± 4.2^{a}	0.0001 ± 0.00^{c}	0.015
P. aeruginosa	33.3 ± 8.3^{a}	266.67 ± 66.7^{b}	33.3 ± 8.3^{a}	83.3± 16.6^{a}	41.7 ± 8.3^{a}	0.0001 ± 0.00^{c}	0.008
C. albicans	10.4 ± 2.1^{a}	41.7 ± 8.3^{b}	14.6 ± 5.5^{a,b}	33.3 ± 8.3^{b}	20.8±4.2^{a,b}	0.003 ± 0.00^{c}	0.016

Note: Each value is expressed as mean n=3 and standard deviation, (np) indicates not performed, values with different superscripts (a, b, c) are statistically significantly different from each other ($p \leq 0.05$).

Minimum bactericidal and fungicidal concentration MBC/FC (g/mL)

All five mushroom extracts showed minimum bactericidal and fungicidal in at least one species of tested microorganism. Although all five mushroom extracts demonstrated inhibitory effects on the growth of tested organisms few were capable of killing at their highest concentration. *Craterellus* sp showed the lowest bactericidal activity of 3.33 ± 0.7g/mL and fungicidal 2.67 ± 0.7 g/mL against *S. typhi* and *C. albicans* respectively. Crude extracts of *A. platyphylus*, *Cantharellus* sp, and *L. kabansus* showed no bactericidal against *E.coli* except for *Craterellus* sp at 4g/ml. (Table 3).

Table 3

Minimum bactericidal and fungicidal concentration of five mushroom extracts (MBC/MFC) in g/mL in Morogoro Municipality, Tanzania

Bacteria/ Yeast	Mushroom species					
	C. luteopunctatus	*A. platyphyllus*	*Cantharellus* sp	*Craterellus* sp	*L. kabansus*	Gentamicin/ Nystatin(mg/mL)
S. aureus	4.00±0.0	4.00±0.0	4.00±0.0	3.33± 0.7	4.00±0.0	0.0001±0.00
E. coli	-	-	-	4.00±0.0	-	0.0001±0.00
S. typhi	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0	4.00±0.0	0.0001±0.00
P. aeruginosa	-	-	4.00±0.0	4.00±0.0	4.00±0.0	0.0001±0.00
C. albicans	4.00±0.0	4.00±0.0	3.33±0.7	2.66±0.7	4.00±0.0	0.003±0.00

Note: Each value represents mean and standard deviations, - (no MBC/MFC).

Discussion

Antimicrobial resistance (AMR) is a global and complex health challenge affecting public health, livestock, and agriculture, primarily caused by the indiscriminate use of antibiotics. Recently, many studies have been conducted to search for alternative drugs to combat the ever-increasing threat of AMR. (Sánchez, 2017; Murugaiyan *et al.*, 2022). Despite these efforts, no new antibiotics have been discovered. Based on their biological nature, mushrooms remain a promising alternative source of bioactive compounds with potential antimicrobial activities. This study evaluated the antimicrobial properties of eight wild edible mushrooms collected from Morogoro Municipality, Tanzania. Generally, the findings showed that *Cantharellus* sp and *Craterellus* sp exhibited the highest antimicrobial activity against both Gram-negative and positive bacteria and *Candida albicans*. This was followed by *Cantharellus luteopunctatus*, and *Lactarius kabansus*, while *Afrocantharellus platyphyllus* demonstrated the least antimicrobial activity. These preliminary results demonstrate that some wild edible mushrooms from the Morogoro Municipality possess potential antimicrobial properties against fungal and bacterial species which warrants further research to identify and characterize the bioactive compounds and assess their effect and toxicity in animal models to determine their antibacterial and antifungal properties.

The highest antimicrobial activity of *Cantharellus* sp. against both fungal and bacteria pathogens can be attributed to its bioactive compounds; terpenoids, phenolic compounds, and

polysaccharides which are known to have strong antimicrobial effect (De Carvalho Cruz and Sheashea, 2023; Kumar *et al.,* 2025). These compounds weaken the microbial cell membranes, inactivate necessary metabolic enzymes, and prevent biofilm formation (Ogidi *et al.,* 2019; Moussa *et al.,* 2022). Additionally, *Cantharellus* sp's unique ecological needs for nutrient-rich substrate and mutualistic mycorrhizal relationships with plants enhance its capacity to produce these metabolites as a soil defense mechanism against microbial competition (Boer *et al.,* 2005; Ge *et al.,* 2023). These findings concur with other studies previously reported by (Dulger *et al.,* 2004; Barros *et al.,* 2008; Ozen *et al.,* 2011; Tsungai *et al.,* 2016a). This suggests that *Cantharellus* species possess genes that trigger the production of bioactive compounds with antimicrobial activities, making them potential raw materials for the development of antimicrobial agents in the pharmaceutical industry.

Furthermore, it was found that ethanolic extracts of *Craterellus* species exhibited higher antimicrobial activity against *S. aureus* and *S. typhi*. This enhanced antibacterial effect may be attributed to their rich composition of bioactive compounds, particularly phenolic acids such as quercetin, ferulic acid, gallic acid, and p-coumaric acid (Borges *et al.,* 2013; Kosanić *et al.,* 2019; Moussa *et al.,* 2022). According to Tridip *et al.* (2023), water and methanolic extract of *Craterellus cornucopioides* exerted antibacterial activity against *B. subtilis*, *Enterococcus faecalis*, *B. licheniformis*, *E. coli*, and *S. aureus* with inhibition zones similar to the current study implying that the mushroom has a conserved antimicrobial effect within the genus. Moreover, Özcan and Ertan. (2018), reported *C. cornucopioides* extracts inhibited the growth of *S. aureus* and *Klebsiella pneumonia* at the highest concentration of 200 mg/mL different from this study where *S. aureus* was inhibited at a concentration of 16.6 ± 4.1 mg/mL.

The variation in antimicrobial efficacy among crude ethanolic extracts of various wild edible mushrooms in this study can be explained by the differences in biochemical constituents of the species of mushrooms in terms of the presence and concentrations of the bioactive compounds such as phenolics, flavonoids, and organic acids (Chelela *et al.,* 2016; Zhou *et al.,* 2024). Research has revealed that mature fruiting bodies of mushrooms show higher levels of antibacterial activity than immature ones as they contain higher levels of bioactive compounds (Barros *et al.,* 2007; Kaewsaen *et al.,* 2024). Environmental factors, such as climate, soil composition, and symbiotic relationships, also affect antimicrobial compound synthesis, leading to interspecies variability (Pawlowska, 2024). For example, the antimicrobial properties of *Lactarius* sp and *Termitomyces* sp have been linked to environmental conditions (Kostić *et al.,* 2023; Paloi *et al.,* 2023). The current study used mature fruiting bodies of mushrooms which may further account for high efficiency in antibacterial and antifungal activities as previously reported in other studies (Chelela *et al.,* 2014; Chelela *et al.,* 2016; Hamza *et al.,* 2024)

It was further established that the least antimicrobial activity was observed with the ethanolic extract of *A. platyphyllus*. This can be due to its specific chemical composition of bioactive compounds which are known to exhibit selective efficacy against certain pathogens, such as *Mycobacteria* sp, and less effective against other bacteria and fungi (Qwarse *et al.,* 2024). Additionally, extraction procedures and solvent type may influence on the availability of bioactive compounds, therefore affecting the observed antimicrobial activity (Lee *et al.,* 2024). Other species of *Afrocantharellus* such as *A. splendens* and *A. symoensii* are reported to possess strong antioxidants which are linked with antimicrobial activity (Tibuhwa, 2014; Hussein *et al.,* 2015; Juma, 2016; Bach *et al.,* 2019).

In the current study, *Bacillus subtilis* was found to be non-sensitive to all crude ethanolic extracts of the tested wild edible mushrooms. This finding is contrary to the study by Deshmukh *et al.* (2014) and Kolundžić *et al.* (2017) which reported antibacterial activity against *B. subtilis* using aqueous extracts, cyclohexane, methanol, and dichloromethane of *Cantharellus cibarius*. This variation can be due to differences in experimental protocols, such as solvent, concentration, and testing conditions, the efficacy of bioactive compounds could further explain the lack of activity observed in this study. Moreover,

variations in the *B. subtilis* strain, including differences in resistance genes and their expression levels, and bacteria's inherent defense mechanisms, may have influenced the observed results (Dulger *et al.,* 2004; Abriouel *et al.,* 2011; Deshmukh *et al.,* 2014). Additionally, *B. subtilis* resistance to crude extracts of mushrooms may also be attributed to its inherent antimicrobial defense mechanisms. These include biofilm formation, efflux pumps that expel toxic compounds, and a robust cell wall that limits extract penetration (Reygaert, 2018). Genetic adaptation, including mutations that enhance survival against antimicrobial compounds, may also play a role (Fayanju *et al.,* 2024). Additionally, *B. subtilis* produces antimicrobial peptides such as subtilin, which inhibit the activity of bioactive compounds in the extracts (Iqbal *et al.,* 2024)

Amanita sp, *Lentinus* sp, and *Termitomyces* sp showed no antimicrobial effects against the tested organisms. However, previous studies demonstrated that these mushroom species have promising antimicrobial activities against *Bacillus cereus*, *C. albicans, E. coli*, *P. aeruginosa*, *S. typhimurium*, and *S. aureus* (Chelela *et al.,* 2014; Tsungai *et al.,* 2016; Sevindik, 2018; Nhi *et al.,* 2022). The lack of antimicrobial activity in *Amanita* sp., *Lentinus* sp., and *Termitomyces* sp. could be attributed to the absence or low concentrations of bioactive compounds with antibacterial properties, differences in extraction efficiency, or the selective specificity of their metabolites against certain microorganisms as well as experimental factors such as media, pH, and reagents used(Santoyo *et al.,* 2009; Smolskaitė *et al.,* 2015; Nhi *et al.,* 2022). Moreover, ethanolic extraction, while effective in isolating various compounds, may not extract all bioactive compounds in the mushrooms thereby reducing its bioavailability in the crude extract and affecting the observed results. Some bioactive compounds require enzymatic activation or synergistic interactions for full antimicrobial potential (Boa, 2004). Additionally, the tested organisms may possess resistance mechanisms such as efflux pumps, enzymatic degradation, or biofilm formation, which reduce the efficacy of the extracts (Reygaert, 2018; Anusiya *et al.,* 2021). The ability of crude ethanolic mushroom extracts to inhibit the growth of tested organisms, while rarely exhibiting bactericidal or fungicidal activity, may be attributed to the nature and concentration of their bioactive compounds. In this study, most ethanolic extracts demonstrated bacteriostatic or fungistatic effects, a finding consistent with previous reports (Appiah *et al.,* 2017; Sułkowska-Ziaja *et al.,* 2023).

Interestingly, *C. luteopunctatus* showed a greater inhibition zone against *C. albicans* at a concentration of 200mg/mL than Nystatin which was used as a positive control. This observation may be explained by the presence of effective and potent bioactive compounds like phenol, tannin, terpenoid, and alkaloid present in the mushroom extract that exert strong antimicrobial properties. Studies have shown that extracts from *Cantharellus* species contain a number of compounds with significant antimicrobial activity (Dulger. *et al.,* 2004). Therefore, *C. luteopunctatus* can be used as a potential candidate in treatment of candida infections in animals and humans. However, further work on *Cantharellus* species is recommended using purified and characterized bioactive compounds to elucidate their anti-antifungal properties.
One limitation of this study is the use of ethanol as the sole extraction solvent, which may not have effectively extracted all bioactive compounds. Although ethanol has been widely recognized as an efficient solvent for mushroom extraction, studies have shown that other solvents such as methanol, water, acetone, or ethyl acetate may enhance the recovery of certain bioactive metabolites (Tsungai *et al.,* 2016). Another limitation is the absence of ethanol as solvent control in the MIC and MBC experiments, which prevents definitive confirmation that the observed antimicrobial effects were solely due to the mushroom extracts rather than residual ethanol. However, dimethyl sulfoxide (DMSO) was used as a negative control, as it served as the solvent for both the crude extracts and positive controls. Despite this, incorporating ethanol as a separate solvent control in future studies would help eliminate potential solvent interference and provide a more precise assessment of antimicrobial activity.

Conclusion

Edible mushrooms from the Morogoro Municipality exhibit significant antibacterial and

anti-fungal potential. The findings obtained in this study are consistent with previous research; however, this study did not directly assess their traditional medicinal use. While the results suggest a possible link to their ethnopharmacological relevance, further validation is needed. In the context of rising antimicrobial resistance, these findings highlight the potential of mushrooms as a source of natural antimicrobial agents, contributing to ongoing efforts to explore alternative therapeutic options

Recommendation

Based on these findings, further research should focus on isolating and characterizing the bioactive compounds in mushrooms responsible for the observed antimicrobial effects, assessing their mechanism of action, and evaluating their efficacy and safety in animal models for potential therapeutic applications. In addition, future studies should also incorporate ethnopharmacological surveys to systematically document the traditional uses of these mushrooms in antimicrobial applications. This will provide valuable insights into their historical medicinal relevance and complement laboratory findings on their bioactivity.

Acknowledgments

The authors sincerely acknowledge the Sokoine University of Agriculture for providing the necessary resources and facilities and heartfelt appreciation to the Ministry of Education, Morogoro Region, Municipality Council for permitting this study. The authors also sincerely acknowledge the financial support provided by the SUA Research and Innovation Support III (SUARIS 3) for funding this research project. Their generous contribution has been instrumental in facilitating the successful execution of this study, and we deeply appreciate their commitment to advancing research and innovation.

References

Abriouel, H., Franz, C. M. A. P., Omar, N. B., & Gálvez, A. (2011). Diversity and applications of *Bacillus* bacteriocins. *FEMS Microbiology Reviews*, *35*(1), 201–232. https://doi.org/10.1111/j.1574-6976.2010.00244.x

Andrews, J. M. (2001a). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, *48*(suppl_1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5

Andrews, J. M. (2001b). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, *48*(suppl_1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5

Anusiya, G., Gowthama Prabu, U., Yamini, N. V., Sivarajasekar, N., Rambabu, K., Bharath, G., & Banat, F. (2021). A review of the therapeutic and biological effects of edible and wild mushrooms. *Bioengineered*, *12*(2), 11239–11268. https://doi.org/10.1080/21655979.2021.2001183

Appiah, T., Boakye, Y. D., & Agyare, C. (2017). Antimicrobial Activities and Time-Kill Kinetics of Extracts of Selected Ghanaian Mushrooms. *Evidence-Based Complementary and Alternative Medicine*, *2017*(1), 4534350. https://doi.org/10.1155/2017/4534350

Bach, F., Zielinski, A. A. F., Helm, C. V., Maciel, G. M., Pedro, A. C., Stafussa, A. P., Ávila, S., & Haminiuk, C. W. I. (2019). Bio compounds of edible mushrooms: In vitro antioxidant and antimicrobial activities. *LWT*, *107*, 214–220. https://doi.org/10.1016/j.lwt.2019.03.017

Balama, C., Augustino, S., Mwaiteleke, D., Lusambo, L. P., & Makonda, F. B. S. (2016). Economic Valuation of Nontimber Forest Products under the Changing Climate in Kilombero District, Tanzania. *International Journal of Forestry Research*, *2016*, 1–13. https://doi.org/10.1155/2016/7893143

Baraza, L., Joseph, C., Moshi, M., & Nkunya, M. (2009). Chemical constituents and biological activity of three Tanzanian wild mushroom species. *Tanzania Journal of*

Science, *33*(1). https://doi.org/10.4314/tjs.v33i1.44280

Barros, L., Calhelha, R. C., Vaz, J. A., Ferreira, I. C., Baptista, P., & Estevinho, L. M. (2007). Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms methanolic extracts. *European Food Research and Technology*, *225*, 151–156.

Barros, L., Venturini, B. A., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2008). Chemical Composition and Biological Properties of Portuguese Wild Mushrooms: A Comprehensive Study. *Journal of Agricultural and Food Chemistry*, *56*(10), 3856–3862. https://doi.org/10.1021/jf8003114

Boa, E. (2004). Wild Edible Fungi: A Global Overview of Their Use and Importance to People. In *Food and Agriculture Organization of the United Nations*.

Boer, W. D., Folman, L. B., Summerbell, R. C., & Boddy, L. (2005). Living in a fungal world: Impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews*, *29*(4), 795–811. https://doi.org/10.1016/j.femsre.2004.11.005

Borges, A., Ferreira, C., Saavedra, M. J., & Simões, M. (2013). Antibacterial Activity and Mode of Action of Ferulic and Gallic Acids Against Pathogenic Bacteria. *Microbial Drug Resistance*, *19*(4), 256–265. https://doi.org/10.1089/mdr.2012.0244

C Reygaert, W. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, *4*(3), 482–501. https://doi.org/10.3934/microbiol.2018.3.482

Chelela, B., Chacha, M., & Matemu, A. (2014). *Antibacterial and antifungal activities of selected wild mushrooms from Southern Highlands of Tanzania*.

Chelela, B. L., Chacha, M., & Matemu, A. (2016). Chemical Composition of Ethanolic Extracts of Some Wild Mushrooms from Tanzania and Their Medicinal Potentials. *International Journal of Medicinal Mushrooms*, *18*(5), 457–464. https://doi.org/10.1615/IntJMedMushrooms.v18.i5.90

De Carvalho Cruz, R., & Sheashea, M. (2023). *Cantharellus cibarius* Fr./ *Cantharellus subalbidius* / *Cantharellus lateritius* Singer (Chanterelle). In A. Sharma, G. Bhardwaj, & G. A. Nayik (Eds.), *Phytochemistry and Nutritional Composition of Significant Wild Medicinal and Edible Mushrooms* (pp. 116–141). Royal Society of Chemistry. https://doi.org/10.1039/BK9781837672097-00116

Deshmukh, L. O. K. N. A. T. H., Kumar, S. U. N. E. E. L., Aharwal, R. P., Rajak, R. C., & Sandhu, S. S. (2014). Study on in-vitro antibacterial activity of mushrooms collected from Jabalpur region. *International Journal of Pharmacy and Pharmaceutical Sciences*, *6*(9), 143-146.

Dulger. B , A. G., & . F. G. (2004). Antimicrobial Activity of the Macrofungus Cantharellus cibarius. *Pakistan Journal of Biological Sciences*, *7*(9), 1535–1539. https://doi.org/10.3923/pjbs.2004.1535.1539

Fayanju, A. M., Akinyele, B. J., Oladejo, B. O., & Osunla, A. C. (2024). Genetic composition, virulence factors, and antimicrobial resistance profiles of Bacillus cereus and Bacillus subtilis isolates from food vendors in Ondo State, Nigeria: Implications for food safety. *Bulletin of the National Research Centre*, *48*(1), 90. https://doi.org/10.1186/s42269-024-01245-8

Ferri, M., Ranucci, E., Romagnoli, P., & Giaccone, V. (2017). Antimicrobial resistance: A global emerging threat to public health systems. *Critical Reviews in Food Science and Nutrition*, *57*(13), 2857–2876. https://doi.org/10.1080/10408398.2015.1077192

Ge, W., Ren, Y., Dong, C., Shao, Q., Bai, Y., He, Z., Yao, T., Zhang, Y., Zhu, G., Deshmukh, S. K., & Han, Y. (2023). New perspective:

Symbiotic pattern and assembly mechanism of Cantharellus cibarius-associated bacteria. *Frontiers in Microbiology*, *14*, 1074468. https://doi.org/10.3389/fmicb.2023.1074468

Gebreyohannes, G., Nyerere, A., Bii, C., & Berhe Sbhatu, D. (2019). Determination of Antimicrobial Activity of Extracts of Indigenous Wild Mushrooms against Pathogenic Organisms. *Evidence-Based Complementary and Alternative Medicine*, *2019*, 1–7. https://doi.org/10.1155/2019/6212673

Gebreyohannes, G., Nyerere, A., Bii, C., & Sbhatu, D. B. (2019). Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. *Heliyon*, *5*(8), e02192.

Hamza, A., Mylarapu, A., Krishna, K. V., & Kumar, D. S. (2024). An insight into the nutritional and medicinal value of edible mushrooms: A natural treasury for human health. *Journal of Biotechnology*, *381*, 86–99. https://doi.org/10.1016/j.jbiotec.2023.12.014

Härkönen, M., Niemelä, T., & Mwasumbi, L. (2003). *Tanzanian mushrooms: Edible, harmful and other fungi*.

Härkönen, M., Saarimäki, T., & Mwasumbi, L. (1994). Tanzanian mushrooms and their uses 4. Some reddish edible and poisonous *Amanita* species. *Karstenia*, *34*(2), 47–60. https://doi.org/10.29203/ka.1994.306

Hussein, J. M., Tibuhwa, D. D., Mshandete, A. M., & Kivaisi, A. K. (2015). Antioxidant properties of seven wild edible mushrooms from Tanzania. *African Journal of Food Science*, *9*(9), 471–479. https://doi.org/10.5897/AJFS2015.1328

Iqbal, S., Malik, M., Mustafa, B., & Ahmed Janjua, H. (2024). Clinical and Therapeutic Potential of Bacillus subtilis Antimicrobial Peptides. In *Bacillus subtilis – Functionalities and Emerging Applications [Working Title]*. Intech Open. https://doi.org/10.5772/intechopen.1007960

Juma, I. (2016). Assessment of antioxidant potentials of the wild and domesticated saprophytic edible mushrooms from Tanzania. *Current Research in Environmental & Applied Mycology*, *6*(1), 1–10. https://doi.org/10.5943/cream/6/1/1

Kaewsaen, R., Wichienchot, S., Thayanukul, P., Charoensiddhi, S., & Chanput, W. P. (2024). Chemical Profile and In Vitro Gut Microbiota Modulation of Wild Edible Mushroom Phallus atrovolvatus Fruiting Body at Different Maturity Stages. *Nutrients*, *16*(15), 2553. https://doi.org/10.3390/nu16152553

Kolundžić, M., Stanojković, T., Radović, J., Tačić, A., Dodevska, M., Milenković, M., Sisto, F., Masia, C., Farronato, G., Nikolić, V., & Kundaković, T. (2017). Cytotoxic and Antimicrobial Activities of *Cantharellus cibarius* Fr. (*Cantarellaceae*). *Journal of Medicinal Food*, *20*(8), 790–796. https://doi.org/10.1089/jmf.2016.0176

Kosanić, M., Ranković, B., Stanojković, T., Radović-Jakovljević, M., Ćirić, A., Grujičić, D., & Milošević-Djordjević, O. (2019). *Craterellus cornucopioides* Edible Mushroom as Source of Biologically Active Compounds. *Natural Product Communications*, *14*(5). https://doi.org/10.1177/1934578x19843610

Kostić, M., Ivanov, M., Fernandes, Â., Calhelha, R. C., Glamočlija, J., Barros, L., Soković, M., & Ćirić, A. (2023). A Comparative Study of *Lactarius* Mushrooms: Chemical Characterization, Antibacterial, Antibiofilm, Antioxidant and Cytotoxic Activity. *Journal of Fungi*, *9*(1), 70. https://doi.org/10.3390/jof9010070

Kivaisi, A. K. (2007). Mushroom cultivation in Tanzania–a new industry. *Opera Mycologia*, *1*, 12-22.

Kivaisi AK: Uujue uyoga na namna ya kulima aina mbalimbali kwa kutumia tekinolojia

rahisi. 2009, Dar es Salaam University Press, 59-ISBN 978-9976- 60-488-2.

Kumar, A., Devi, R., Dhalaria, R., Tapwal, A., Verma, R., Rashid, S., Elossaily, G. M., Khan, K. A., Chen, K., & Verma, T. (2025). Nutritional, Nutraceutical, and Medicinal Potential of *Cantharellus cibarius* Fr.: A Comprehensive Review. *Food Science & Nutrition*, *13*(1), e4641. https://doi.org/10.1002/fsn3.4641

Lee, J.-E., Jayakody, J., Kim, J.-I., Jeong, J.-W., Choi, K.-M., Kim, T.-S., Seo, C., Azimi, I., Hyun, J., & Ryu, B. (2024). The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. *Foods*, *13*(19), 3151. https://doi.org/10.3390/foods13193151

Lodge, A., & O'Dell, M. (2004). *Collecting and Describing Macrofungi*.

Magingo, F. S., Oriyo, N. M., Kivaisi, A. K., & Danell, E. (2004). Cultivation of *Oudemansiella tanzanica* nom. Prov. On agricultural solid wastes in Tanzania. *Mycologia*, *96*(2), 197–204. https://doi.org/10.1080/15572536.2005.11832967

Marealle, A. I., Moshi, M., Innocent, E., Qwarse, M., & Andrae-Marobela, K. (2021). Ethnomedical survey of plants used for the management of HIV and AIDS-related conditions in Mbulu District, Tanzania. *Journal of Medicinal Plants Research*, *15*(1), 1–21.

Maregesi, S. M., Pieters, L., Ngassapa, O. D., Apers, S., Vingerhoets, R., Cos, P., Berghe, D. A. V., & Vlietinck, A. J. (2008). Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *Journal of Ethnopharmacology*, *119*(1), 58–66. https://doi.org/10.1016/j.jep.2008.05.033

Miles, P. G., & Chang, S.-T. (2004). *Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact*. CRC press.

Moussa, A. Y., Fayez, S., Xiao, H., & Xu, B. (2022). New insights into antimicrobial and antibiofilm effects of edible mushrooms. *Food Research International*, *162*, 111982. https://doi.org/10.1016/j.foodres.2022.111982

Murugaiyan, J., Kumar, P. A., Rao, G. S., Iskandar, K., Hawser, S., Hays, J. P., Mohsen, Y., Adukkadukkam, S., Awuah, W. A., Jose, R. A. M., Sylvia, N., Nansubuga, E. P., Tilocca, B., Roncada, P., Roson-Calero, N., Moreno-Morales, J., Amin, R., Kumar, B. K., Kumar, A., … Van Dongen, M. B. M. (2022). Progress in Alternative Strategies to Combat Antimicrobial Resistance: Focus on Antibiotics. *Antibiotics*, *11*(2), 200. https://doi.org/10.3390/antibiotics11020200

Nhi, N. T. N., Khang, D. T., & Dung, T. N. (2022). Termitomyces mushroom extracts and its biological activities. *Food Science and Technology*, *42*, e125921. https://doi.org/10.1590/fst.125921

Noman, E. A., Radin Mohamed, R. M. S., Al-Gheethi, A. A., Al-shaibani, M. M., Al-Wrafy, F. A., Al-Maqtari, Q. A., & Vo, D.-V. N. (2022). Antibiotics and antibiotic-resistant bacteria in greywater: Challenges of the current treatment situation and predictions of future scenario. *Environmental Research*, *212*, 113380. https://doi.org/10.1016/j.envres.2022.113380

Nyamoga, G. Z. (2023). Edible Miombo Wild Mushrooms and Its Potential for Improving Livelihoods of People in Songea and Tabora Districts, Tanzania. *AFRICAN JOURNAL OF APPLIED RESEARCH*, *9*(1), 20–37. https://doi.org/10.26437/ajar.v9i1.519

Ogidi, O. I., Omu, O., & Ezeagba, P. A. (2019). Ethno pharmacologically active components of Brassica Juncea (Brown Mustard) seeds. *International Journal of Pharmaceutical Research and Development*, *1*(1), 9–13.

Osivand, A., Araya, H., Appiah, K. S., Mardani, H., Ishizaki, T., & Fujii, Y. (2018). Allelopathy of Wild Mushrooms—An Important Factor for Assessing Forest

Ecosystems in Japan. *Forests*, *9*(12), 773. https://doi.org/10.3390/f9120773

Özcan, Ö., & Ertan, F. (2018). Beta-glucan Content, Antioxidant and Antimicrobial Activities of Some Edible Mushroom Species. *Food Science and Technology*, *6*(2), 47–55. https://doi.org/10.13189/fst.2018.060201

Ozen, T., Darcan, C., Aktop, O., & Turkekul, I. (2011). Screening of Antioxidant, Antimicrobial Activities and Chemical Contents of Edible Mushrooms Wildly Grown in the Black Sea Region of Turkey. *Combinatorial Chemistry & High Throughput Screening*, *14*(2), 72–84. https://doi.org/10.2174/138620711794474079

Paloi, S., Kumla, J., Paloi, B. P., Srinuanpan, S., Hoijang, S., Karunarathna, S. C., Acharya, K., Suwannarach, N., & Lumyong, S. (2023). Termite Mushrooms (Termitomyces), a Potential Source of Nutrients and Bioactive Compounds Exhibiting Human Health Benefits: A Review. *Journal of Fungi*, *9*(1), 112. https://doi.org/10.3390/jof9010112

Pawlowska, T. E. (2024). Symbioses between fungi and bacteria: From mechanisms to impacts on biodiversity. *Current Opinion in Microbiology*, *80*, 102496. https://doi.org/10.1016/j.mib.2024.102496

Pérez-Moreno, J. (2021). Global perspectives on the ecological, cultural and socioeconomic relevance of wild edible fungi. *Studies in Fungi*, *6*(1), 408–424. https://doi.org/10.5943/sif/6/1/31

Qwarse*, M., Julius Moshi, M., Joel Mihale, M., Ignace Marealle, A., Selemani Omari Nondo, R., Sempombe, J., & Mugoyela, V. (2024). Antimycobacterial Activity and Brine Shrimp Toxicity of Wild Mushrooms Used by Communities in Southern Tanzania. *Research Journal of Pharmacognosy*, *11*(2). https://doi.org/10.22127/rjp.2024.419439.2241

Qwarse, M., Moshi, M., Mihale, M. J., Marealle, A. I., Sempombe, J., & Mugoyela, V. (2021). Knowledge on utilization of wild mushrooms by the local communities in the Selous-Niassa Corridor in Ruvuma Region, Tanzania. *Journal of Yeast and Fungal Research*, 12(1):8-19. http://doi.org/10.5897/JFR20200203.

Roca, I., Akova, M., Baquero, F., Carlet, J., Cavaleri, M., Coenen, S., Cohen, J., Findlay, D., Gyssens, I., Heure, O. E., Kahlmeter, G., Kruse, H., Laxminarayan, R., Liébana, E., López-Cerero, L., MacGowan, A., Martins, M., Rodríguez-Baño, J., Rolain, J.-M., … Vila, J. (2015). The global threat of antimicrobial resistance: Science for intervention. *New Microbes and New Infections*, *6*, 22–29. https://doi.org/10.1016/j.nmni.2015.02.007

Sai Latha, S., Naveen, S., Pradeep, C. K., Sivaraj, C., Dinesh, M. G., & Anilakumar, K. R. (2018). Toxicity Assessment of Wild Mushrooms from the Western Ghats, India: An in Vitro and Sub-Acute in Vivo Study. *Frontiers in Pharmacology*, *9*, 90. https://doi.org/10.3389/fphar.2018.00090

Sánchez, C. (2017). Bioactives from Mushroom and Their Application. In M. Puri (Ed.), *Food Bioactives* (pp. 23–57). Springer International Publishing. https://doi.org/10.1007/978-3-319-51639-4_2

Santoyo, S., Ramírez-Anguiano, A. C., Reglero, G., & Soler-Rivas, C. (2009). Improvement of the antimicrobial activity of edible mushroom extracts by inhibition of oxidative enzymes. *International Journal of Food Science and Technology*, *44*(5), 1057-1064.

Schoch, C. L., Seifert, K. A., Huhndorf, S., Robert, V., Spouge, J. L., Levesque, C. A., Chen, W., Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List, Bolchacova, E., Voigt, K., Crous, P. W., Miller, A. N., Wingfield, M. J., Aime, M. C., An, K.-D., Bai, F.-Y., Barreto, R. W., Begerow, D., … Schindel, D. (2012).

Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for *Fungi*. *Proceedings of the National Academy of Sciences*, *109*(16), 6241–6246. https://doi.org/10.1073/pnas.1117018109

Sevindik, M. (2018). Investigation of Antioxidant/Oxidant Status and Antimicrobial Activities of *Lentinus tigrinus*. *Advances in Pharmacological Sciences*, *2018*, 1–4. https://doi.org/10.1155/2018/1718025

Smolskaitė, L., Venskutonis, P. R., & Talou, T. (2015). Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species. *LWT - Food Science and Technology*, *60*(1), 462–471. https://doi.org/10.1016/j.lwt.2014.08.007

Sułkowska-Ziaja, K., Trepa, M., Olechowska-Jarząb, A., Nowak, P., Ziaja, M., Kała, K., & Muszyńska, B. (2023). Natural Compounds of Fungal Origin with Antimicrobial Activity—Potential Cosmetics Applications. *Pharmaceuticals*, *16*(9), 1200. https://doi.org/10.3390/ph16091200

Thakur, M. P., & Singh, H. K. (2013). Mushrooms, their bioactive compounds and medicinal uses: A review. *Medicinal Plants - International Journal of Phytomedicines and Related Industries*, *5*(1), 1. https://doi.org/10.5958/j.0975-6892.5.1.004

Tibuhwa, D. D. (2011). Substrate specificity and phenology of macrofungi community at the University of Dar es Salaam Main Campus, Tanzania. *J. Appl. Biosci*, *46*, 3173–3184.

Tibuhwa, D. D. (2012). Folk taxonomy and use of mushrooms in communities around Ngorongoro and Serengeti National Park, Tanzania. *Journal of Ethnobiology and Ethnomedicine*, *8*(1), 36. https://doi.org/10.1186/1746-4269-8-36

Tibuhwa, D. D. (2013). Wild mushroom-an underutilized healthy food resource and income generator: Experience from Tanzania rural areas. *Journal of Ethnobiology and Ethnomedicine*, *9*, 1–14.

Tibuhwa, D. D. (2014). A Comparative Study of Antioxidant Activities between Fresh and Dry Mushrooms in the Genera *Cantharellus* and *Afrocantharellus* from Tanzania. *Food and Nutrition Sciences*, *05*(02), 212–221. https://doi.org/10.4236/fns.2014.52026

Tibuhwa, D. D. (2017). Cytotoxicity, antimicrobial, and antioxidant activities of boletus bicolor, basidiomycetes mushroom indigenous to Tanzania. *Tanzania journal of science 43(1)*.

Tridip Boruah, A., Prastuti Parashar, B., Debasish Das, C., Himashri Devi, D., Abhinob Kr Nath, E., Aparna Barman, F., & Hangsha Barman, G. (2023). *Craterellus cornucopioides* (L.)/ *Craterellus odoratus* (Schwein.) Fr. (Black Chanterelle). In A. Sharma, G. Bhardwaj, & G. A. Nayik (Eds.), *Phytochemistry and Nutritional Composition of Significant Wild Medicinal and Edible Mushrooms* (pp. 142–164). Royal Society of Chemistry. https://doi.org/10.1039/BK9781837672097-00142

Tsungai, R., Chenjerayi, K., Catherine, C., Mudadi, A. B., & Takafira, M. (2016a). Antibacterial properties of wild edible and non-edible mushrooms found in Zimbabwe. *African Journal of Microbiology Research*, *10*(26), 977–984. https://doi.org/10.5897/AJMR2016.8052

Tsungai, R., Chenjerayi, K., Catherine, C., Mudadi, A. B., & Takafira, M. (2016b). Antibacterial properties of wild edible and non-edible mushrooms found in Zimbabwe. *African Journal of Microbiology Research*, *10*(26), 977–984. https://doi.org/10.5897/ajmr2016.8052

Vamanu, E., Pelinescu, D., & Avram, I. (2018). Antioxidative effects of phenolic compounds of mushroom mycelia in simulated regions of the human colon, in vitro study. *Polish Journal of Food and Nutrition Sciences*, *68*(1).

Venturella, G., Ferraro, V., Cirlincione, F., & Gargano, M. L. (2021). Medicinal mushrooms: Bioactive compounds, use, and clinical trials. *International Journal of Molecular Sciences*, *22*(2), 634.

Walshaw, S. C. (2004). Tanzanian Mushrooms. Edible, Harmful and Other Fungi. *Economic Botany*, *58*(3), 487–487. https://doi.org/10.1663/0013-0001(2004)058[0487:DFABRE]2.0.CO;2

Wasser, S. (2014). Medicinal mushroom science: Current perspectives, advances, evidences, and challenges. *Biomedical Journal*, *37*(6).

Zhou, Y., Chu, M., Ahmadi, F., Agar, O. T., Barrow, C. J., Dunshea, F. R., & Suleria, H. A. R. (2024). A Comprehensive Review on Phytochemical Profiling in Mushrooms: Occurrence, Biological Activities, Applications and Future Prospective. *Food Reviews International*, *40*(3), 924–951. https://doi.org/10.1080/87559129.2023.2202738