### THE OCCURRENCE OF INDIGENOUS EDIBLE MUSHROOMS IN KAYA CHIVARA FOREST IN KILIFI, KENYA

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### Abstract

Kaya forests are pockets of natural rainforests in the lower coastal regions of Kenya. These forests have for generations supported livelihoods of communities living around them. Mushrooms have seasonally been harvested from these forests but the harvests have dwindled with time because of population pressure and frequent human traffic that disturbs the breeding grounds. Harvesting wild edible mushrooms requires skills to distinguish edible from poisonous species. The local communities have however acquired skills to identify edible mushrooms. Sadly, these skills remain a preserve of the older generation because the youth are not interested. The community therefore risks losing these skills if not documented. This study was therefore intended to characterize indigenous edible mushrooms from Kaya Chivara and document indigenous skills in wild mushroom gathering. To achieve this, local skilled mushroom collectors were engaged to guide the team to identify indigenous edible species from the mushroom-rich Kaya Chivara. A purposive sampling was adopted where edible mushrooms were collected from the canopies of Brachystegia speciformis. Gilled mushrooms with insect or rodent damages, those with non-sporulated caps and had local names were pointed out for harvesting as edible mushrooms. Images were taken in-situ before collecting the mushroom samples for further analyses. Morphological characteristics were used to partially identify the mushroom species based on image and literature matching. Toxicological assays were then conducted using the purge and trap Gas Chromatography Mass Spectrometry (GC-MS) system from AGILENT® Technologies. Twenty indigenous edible mushroom samples were collected. These were further categorized into eight (8) species as; Mwasi (Pleurotus spp.), Hakoranyani (Rusulla spp.), Choga sikiro reruhe (Pleurotus spp.) and Mbwate (Agaricus spp.), Choga rerema (Agaricus spp.), Muhama (Rusulla spp.), Choga Kadzonzo (Agaricus spp.) and Choga nyama (Agaricus spp.). Mwasi and Hakoranyani occurred frequently at 30%, Mbwate and Choga rerema occurred at 10% while Muhama, Choga Kadzonzo and Choga nyama occurred at 5% each. Toxicological analyses indicate that these mushrooms species are edible and had a variety of medicinal constituents. Communities living around Kaya Chivara possess skills that are useful in identifying edible wild mushrooms. Communities have names for mushroom species they have frequently interacted with. This is key in the identification and gathering process. GC-MC analyses reveal immense medicinal potentials of the study mushroom species. It is therefore prudent to conserve theses germplasm for possible domestication and ultimately commercialization.

Keyword: Indigenous mushrooms, Toxicology, Kaya Chivara

# INTRODUCTION

The Kenyan coastal line is dotted with over 50 pockets of forests referred to as the Kaya forests. These forests are sacred grounds for prayer and rituals for the Mijikenda

community. Recently, eleven (11) Kaya forests were recognized as centers for conservation of biodiversity by UNESCO (2008). However, population pressure, mining and settlements have increasingly threatened

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the existence of some of the Kaya forests (Rajat *et al.*, 2018). In Kilifi, Kaya Fimboni remains the largest with more than 400ha of forested land (Gebara & Gaard, 2018). Kaya Chivara which has remained uniquely undisturbed is the fourth largest after Kaya Fungo and Kaya Chonyi. Cultural norms among the Mijikenda community have maintained a rich biodiversity in these forests despite immense pressure for settlement and open-cast mining that is currently happening around them (Rajat *et al.*, 2018).

Kaya Chivara sits in Jaribuni region of Kilifi County. It is an evergreen natural forest rich in biodiversity. Despite the local and international efforts in conservation, Kaya Chivara remains under threat due to encroachment and settlements (Centre, 2008). livelihoods, This forest supports to communities living in its proximity as a source of food, fuelwood and medicine. It is also a home to both several edible and toxic mushrooms because natural forests provide ambient conditions for mushroom growth (García-Montero et al., 2012). Recent encroachments and increased pressure on the forest coupled with global warming and shortened rain seasons has seen mushroom harvests dwindle in quantity and in lengths of harvest seasons. The increased human traffic and fuelwood collection has also interfered with the mushroom breeding grounds. The decayed wood that usually provide shelter and nutrients for growth are removed before the mushroom colonies establish. This ultimately terminates the sporulation cycle leading to loss of subsequent generation of that mushroom species (Tsing, 2012). For this reason, a number of mushroom species previously harvested in Kaya Chivara are no longer available and probably these germplasms have been lost forever.

Mushrooms make nutritious and delicious meals. They contain Carbohydrates, fiber,

protein, Vitamin D, Selenium, Phosphorous and Folate that make them bioactive and healthy food products (Hunter et al., 2019). They activate multiple antioxidants like polyphenols, polysaccharides, ergothioneine, glutathione, selenium and vitamin C which reduce risks of cancer to consumers. The antioxidants also help in controlling aging and reduce risk of developing cardiovascular diseases (Kemp & Pratt, 2019). The main phenolic compounds in mushrooms include flavonoids and phenolic acids which act as antioxidants and pro-oxidants. Polysaccharides such as beta-glucan stimulate the immune system for better defense against harmful pathogens (Zaynab et al., 2019). Mushroom therefore form a unique protein supplement to the diets of communities living around Kaya Chivara.

There is a thin line between edible and toxic mushrooms in wild harvests. This poses great danger to those with little experience in identification mushroom and gathering. Harvesting wild edible mushrooms therefore requires skills that come with experience and frequent interactions to develop markers for poisonous and edible mushroom species. Communities in Kaya Chivara have with time acquired inherent skills to identify edible mushrooms. Sadly, these skills remain a preserve of the older generation because the youth are not interested. These communities therefore risk losing these useful skills if they are not documented. This study was therefore designed to characterize indigenous edible mushrooms from Kaya Chivara with further intent to domesticate and commercialize unique species as efforts in conservation. Documentation of indigenous knowledge and skills was ultimately intended for prosperity of this community.

### MATERIALS AND METHODOLOGY

### **Study Site**

Kaya Chivara is located at S3 47 55.00 and E39 30 52.00 within the Kilifi County of Kenya. Its natural biodiversity has remained intact because it holds a sacred groove managed by the Jibana community. The forest is divided into four sub-sections; South Eastern, South West, North East and South West. The South Eastern part of the forest was surveyed for mushrooms in this study because the guides reported the existence of more breeding mushroom grounds under Brachystegia speciformis that dominated this part of the forest.

### **Sample Collection**

A group of skilled elders and staff from the National Museums of Kenya guided the survey and collection of edible mushrooms. Α purposive sampling method was adopted where mushrooms were collected under the canopies of Brachystegia speciformis that housed breeding more grounds. Morphological traits such as color, shape and size of caps and stipes were used as markers to identify mushroom species for sampling. Further, gilled mushrooms with insect or rodent damages, smooth caps with no spores, those without latex when pricked and species with local names were considered edible and marked for collection. Images of sited edible mushrooms were taken in-situ, together with GPS coordinates before harvesting. The local names and related literature on edibility, taste and preparation methods for each collected sample were recorded from the elders for further verification. The collected samples were transported in cooler boxes and stored at 4°C for domestication trials. Samples for molecular and biochemical analyses were stored at-20°C until further use.

# Partial identification of Mushroom Samples

The color, size and shape of the mushroom caps and stipes, color, texture and pattern of

gills were used as markers and matched with existing image and literature to proximate the genus of the mushroom sample. The samples were compared with existing literature on mushroom identification guides (Marklud & Holmberg, 2013, Jordan, 2016). Collected mushroom images were also run through google and other available image search platforms for proximate matching with previously described mushroom species.

# **Toxicological Analyses**

Toxicological analyses adopted the methodology used by Timmons (1994) with slight adjustments. About 5g of each mushroom sample was oven dried in a crucible at 110°C for 30 minutes. Five (5) grams of NaOH were added to the dried mushroom sample in the crucible followed by few drops of sterile distilled water (SDW) to dissolve. Diethyl ether (3ml) was then added to the paste and the sample homogenized for 5 minutes. The suspension was then transferred into a test-tube and concentrated using a stream of Nitrogen gas at -40°C for 30 minutes. About 3ml of Dichloromethane was then added and shaken for 5 seconds then filter centrifuged. The collected extract was then loaded and run through the Gas Chromatography Mass Spectrometry (GC-MS) system (AGILENT® Technologies, Santa Clara, USA). The resultant unique GC-MS peaks for each mushroom species were then run through the natural product library (database) to identify the compounds present in each peak. Based on the GC-MS output and literature match, inferences on toxicities of the collected mushroom samples were made.

# Data Collection and Analysis

The collected edible mushrooms were grouped based on their local names. A simple frequency of occurrence was then determined. Inferences on toxicity of the collected mushroom samples were made based on the identified compounds in the GC-MS profiles using the natural products library.

### RESULTS

# Morphological characteristics of indigenous edible mushrooms from Kaya Chivara

A total of twenty (20) mushroom samples were collected from Kaya Chivara. From

these, eight (8) distinct mushroom species were locally identified as *Mwasi* with 6 samples, *Hakoranyani* (6), *Choga sikiro reruhe* (2), *Muhama* (2), and one (1) sample each for *Mbwate Choga rerema*, and Choga *Kadzonzo*.



**Fig. 1** Morphological characteristics of collected mushroom species from Kaya Chivara 1). *Mwasi*, 2). *Hakoranyani*, 3). *Muhama*, 4). *Mbwate*, 5). *Choga sikiro reruhe*, 6) *Choga rerema* 7). *Choga nyama*, 8). *Choga Kadzonzo* 

Mwasi (1) has brown-redish caps, whitecreamy gills with deep mushroom aroma. The stipes are stout and white in color. Hakoranyani (2) has deep reddish-brown caps with creamy gills. The caps of Muhama (3) are small, with smooth rounded edges. The creamy brown caps are tough with white compacted gills supported by stout stipes. Mbwate (4) is a smooth brown mushroom at sprouting stage but the caps develop a browngrey hue at maturity while Choga sikiro reruhe (5) has white caps with variegated edges. The caps have distinct brown marking at the apex. The fleshy caps have creamy gills but are supported by relatively thin stipes. Choga rerema (6) has relatively big white caps with a brown tint at the apex. The soft, fleshy gills have a creamy hue. The caps can grow to more than 20cm in diameter. Choga nyama (7) matures into large soft mushrooms of 15-20cm in diameter. The flat brown-creamy caps have creamy fleshy gills and are supported by thick stipes. Choga *Kadzonzo* (8) are small cappy mushrooms that grow in clusters of several mushrooms. The small white caps with gills are supported by white thin stipes. (Fig. 1)

# Occurrence and partial identification of collected mushroom species

*Mwasi* and *Hakkoranyani* were the most (30%) frequently occurring mushroom species in Kaya Chivara followed by Mbwate and *Choga rerema* at 10% occurrence. *Mbwate, Choga rerema,* and Choga *Kadzonzo* occurred at 5% frequency. There was a statistically significant difference (p = 0.5) in the

distribution of these mushroom species in Kaya Chivara.

Based on the morphology of the collected mushroom species and available literature matching, the 20 mushroom samples were partially identified into eight (8) species as *Rusulla spp.* for *Hakoranyani*, *Pleurotus spp.* (*Mwasi*), *Rusulla spp.* (*Muhama*), *Agaricus spp.* (Mbwate), *Pleurotus spp.* (Choga sikiro reruhe), *Agaricus spp.* (Choga rerema), *Agaricus spp.* (Choga nyama.) and *Agaricus spp. for Choga Kadzonzo. Hakoranyani* and *Mwasi* were the most dominant indigenous mushroom in Kaya Chivara forest. The GCMS profiles of the 8 indigenous mushrooms displayed quite some variations in the number of peaks for each species (Fig. 3). The identified micro-compounds in these peaks include; Diethyl ester, Tetradecanoic acid, Hexadecanoic acid, 9-octadecanoic acid, methyl strerate, Trans-1,3-Octadecenoic acid, Oleic acid, Ergosterol, Methyl tetradecanoate, Heptadecanoic acid. Palmitic acid. 11-Octadecenoic acid. 24. 25-Dihydroxycholecalciferol, Isopatchoulane and Calarene epoxide.



Fig. 2 Occurrence frequency of indigenous edible mushrooms in Kaya Chivara GCMS profiles of collected indigenous edible mushrooms



**Fig. 3: GC-MS profiles of indigenous edible mushroom of Kaya Chivara** 1).Hakkoranyani, 2). Mwasi 3). Muhama, 4). Mbwate, 5). Choga sikiro reruhe, 6). Choga rerema 7). Choga Kadzonzo 8). Choga nyama

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Individual variations in GC-MS profiles and subsequent micro-molecule content were noted across the species. Hakkoranyani had five (5) major peaks which were identified as Diethyl ester (1), Hexadecanoic acid (2), Trans-1,3octadecenoic acid (3), Oleic acid (4) and Ergosterol in peak 5. The four (4) peaks in Mwasi were identified as Diethyl ester (1), Tetradecanoic acid (2), Hexadecanoic acid (3), 9-Octadecenoic acid (4) and Methyl stearate in peak 5. Muhama the compact white gilled mushroom had Diethyl ester in peak 1, Hexadecanoic acid (2), 9,12-Octadecadienoic acid (3), 11-Octadecenoic acid (4) and Ergosterol at peak 5. Meanwhile Mbwate had 6 peaks that were identified as Diethyl ester Hexadecanoic (1), acid (2),9,12-Octadecadienoic acid (3), 11-Octadecenoic acid (4), Heptadecanoic acid (5), and 24, 25-Dihydroxycholecalciferol at peak 6.

*Choga sikiro reruhe* contains Diethyl ester in peak 1, Isopatchoulane (2), Calarene epoxide (3), Hexadecanoic acid (4) and 11-Octadecenoic acid at peak 5. Seven (7) peaks noted in *Choga rerema* were identified as Diethyl ester (1), Methyl tetradecanoate (2), Hexadecanoic acid (3), Palmitic acid (4), 10-Octadecenoic acid (5), Oleic acid (6), and Ergosterol at peak 7. Choga Kadzonzo, contains Diethyl ester in peak (1), Methyl tetradecanoate (2), Hexadecanoic acid (3), 9-Octadecenoic acid (4) and Heptadecanoic acid at peak 5. The GC-MS profile of Choga nyama revealed seven (7) peaks identified as Diethyl ester (1), 9-Hexadecenoic acid (2), Hexadecanoic acid (3), 9, 12-Octadecenoic acid (4), 11-Octadecadienoic acid (5), Heptadecanoic acid (6) and Ergosterol at peak 7. All surveyed mushrooms had Diethyl ester and Tetradecanoic acid in common. However, unique were observed patterns in Hakkoranyani which was the only mushroom with Trans-1,3-Octadecenoic acid. Similarly, Mwasi uniquely has Methyl strerate, while Choga rerema uniquely posted Palmitic acid, Isopatchoulane and Calarene epoxide. While Choga Kadzonzo, uniquely had Heptadecanoic acid (Table 1). The eight GC-MS profiles did not identify any toxic micro-molecules of concern. This validates the edibility of these eight (8) mushrooms species.

	Micro-molecules	Hakko ranyani	Mwasi	Muhama	Mbwate	Choga sikiro reruhe	Choga re re ma	Choga nyama	Choga Kadzonzo
1	Diethyl ester	+	+	+	+	+	+	+	+
2	Tetradecanoic acid		+	-	-	-	-	-	-
3	Hexadecanoic acid	+	+	+	+	+	+	+	+
4	Heptadecanoic acid	-	-	-	-	-	-	-	+
5	Mythyl tetradecanoate	-	-	-	-	-	+	-	+
6	9-12 Octadecanoic acid	-	+	+	-	-	-	+	-
7	10-Octadecenoic acid	-	-	-	-	-	+	-	-
8	11- Octadecenoic acid	-	-	+	+	+	-	+	-
9	Trans-1,3-Octadecenoic acid	+	-	-	-	-	-	-	-
10	24,25-Dihydroxycholecalciferol	-	-	-	+	-	-	-	-
11	Methyl strerate	-	+	-	-	-	-	-	-
12	Oleic acid	+	-	-	-	-	+	-	-
13	Ergosterol	+	-	+	-	-	-	+	-
14	Palmitic acid	-	-	-	-	-	+	-	-
15	Isopatchoulane	-	-	-	-	+	-	-	-
16	Calarene epoxide	-	-	-	-	+	-	-	-

Table1. Profile of micro-molecules present in the sampled mushrooms

#### **DISCUSSION**

Indigenous edible mushrooms belonging to Lactarius spp., Rusulla spp., Agaricus spp.,

Pleurotus spp., Volvariella spp., and Coprinus spp. have been reported in South Africa (Rasalanavho et al., 2019), in Cameroon (Kinge *et al.*, 2011) and in Tanzania (Mshandete and Cuff, 2007). Most indigenous mushrooms are used for food, medicine and for mythological purposes. This study reports eight (8) indigenous edible mushroom species of the genus *Rusulla spp.*, *Pleurotus spp.and Agaricus spp.*, where four (4) out of the eight (8) species belonged to the Genus Agaricus. A rich biodiversity of mushrooms species was noted in a single forest out of more than fifty (50) Kaya forests yet to be exploited.

Communities of Kaya Chivara have interacted with indigenous mushrooms for decades. Through this interaction, inherent skills to identify edible mushroom have been developed. Markers to identify edible mushrooms in the wild have been passed down through generations. Mushrooms with insect or rodent damages, smooth caps without spores, gilled mushroom, those without latex when damaged and generally those with pleasant mushroom aroma are considered edible. Candidates that pass these initial criteria are then further tested by rubbing the mushroom against the skin. Any species that causes irritation is suspected poisonous and is avoided. Tongue testing is the final step in the verification process. Mushrooms with pungent smell and unpleasant taste are considered poisonous and are therefore omitted from the diets.

Coded names have been given to edible mushrooms based on their color, morphology and taste. Hakkoranyani literarily refers to the brightly red-colored rumps of baboons when they are on heat. This mushroom species has a reddish-brown hue that appears bright under the tree canopy. Choga nyama means meatwhile Choga mushroom sikiro reruhe describes a white mushroom cap with a lobed (ear) shape. Choga sikiro reruhe and Choga nvama were described as the most delicious mushroom bv the community while Hakkoranvani and Mwasi are the most abundant mushroom species in Kaya Chivara. Choga nyama is a valued mushroom species because of its beefy flavor when cooked. These species have moderate aroma and are either roasted or cooked in stew. Mbwate is a cook-only mushroom because it is too soft and fragile for open-fire roasting. Most edible mushrooms are either roasted or cooked as part of a mixed stew in the diet. Raw consumption of mushrooms is discouraged and this is quite understandable as a caution to avoid poisoning.

Mushrooms are healthy fungal vegetables that protein, vitamins, contain minerals. and antioxidants that have various health benefits. They are rich in important healthy nutrients like Selenium, Choline, Vitamins B and its complexes, Vitamin D, Beta-Glucans, Potassium and fiber (Jayachandran et al., 2017). On consumption, mushrooms actively reduce stress and risks of several types of cancer, heart complications, diabetes, improve muscle movements, learning and memory. Edible mushrooms provide folate (folic acid) necessary in pregnancy to boost fetal health and generally improve fertility. Beta-glucans, a dietary fiber abundant in mushrooms, lowers blood cholesterol levels (Kemp & Pratt, 2019). Yuan et al., (2017) reported amino acids, proteins and minerals in Pleurotus spp and Lentinula edodes. Further, high levels of fatty acids with health benefits such as oleic, linoleic and palmitic acid are found in mushrooms (Kalač, 2010). Incidentally, mushrooms are the only vegetables that provide Vitamin (Cardwell et al., 2018).

Variations in micro-molecules content across species may be connected to the distinct flavors and culinary characteristics of each mushroom species. This study revealed sixteen (16) micro-molecules across the eight (8) species of mushrooms sampled (Table 1). Some of the identified micro-molecules have medicinal benefits that can find applications in

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natural and alternative medicine. Antiinflammatory properties of Hexadecanoic acid and Diethyl ester have been reported (Aparna *et al.*, 2012). These micro-molecules can find applications in treatment of rheumatoid conditions. Similarly, Sulijaya *et al.*, (2018) demonstrated that 11-Octadecenoic acid (Keto-C) suppresses pro-inflammatory cytokinines. Keto-C is therefore a promising bioactive anti-inflammatory agent against periodontal diseases.

Methyl tetradecanoate is reportedly an antibiotic, anti-oxidant, and cytotoxic agent that has been used in anti-plasmodial applications (Maroyi, 2018). Its derivative Methyl stearate also has antioxidative and antifungal properties (Pinto et al., 2017). Isopatchoulane that was uniquely noted in Choga rerema is also known for its antifungal, growth regulating, analgesic, antidiabetic and hypotensive properties (Kumar et al., 2017). Ghavam et al., (2021)) also reported antifungal activity of Octadecenoic acid and its derivatives with further possible applications in food preservation. Ergosterol is an amphotericin compound with high antifungal activity that terminates spore formation process in fungi. It is therefore regarded as an essential compound in the pharmaceutical industry (Gray et al., 2012). Bactericidal properties of 10-Octadecenoic acid (Kasanah et al., 2019) and Trans-1,3-Octadecenoic acid (Hameed et al., 2018) point at possible use of micro-molecule these against bacterial infections. Trans-1,3-Octadecenoic acid also has anti-hepatic, and antioxidant effects.

Some of these micro-molecules also find therapeutical applications in oncology, diabetic and ulcer control. Heptadecanoic acid significantly promotes apoptosis of carcinoma cell and inhibit their activation, proliferation and migration. (Xu *et al.*, 2019). Calarene epoxide also has anti-cancer, anti-diabetic, anti-ulcer activity and also aid in wound healing process (Bainsal *et al.*, 2016). Oleic Acid increases efficiency of pulmonary actions during an injury or shock to the cardiovascular system by decreasing the alveolar-arterial oxygen difference (Bleyl *et al.*, 2002). 24,25-Dihydroxycholecalciferol commonly elevates the activation of vitamin D and calcium in the body which promotes bone metabolism (Kappelle *et al.*, 1989). The natural occurrence of these medicinally bioactive molecules in the sampled indigenous edible mushrooms reveals immense medicinal potentials in these species.

### CONCLUSION

The line between edible and toxic mushroom is very thin. However, communities worldwide have developed physical markers that enable them to identify useful mushrooms they have interacted with. Loosely, mushrooms in the wild that have local names are usually safe for consumption because the respective communities have interacted with them for ages and are comfortable to consume them. Similarly, toxic mushrooms have been coded with harsh names to warn communities of the dangers that lie within these beautiful fungi. Other features reported are; insect and rodent damages, absence of spores on caps, mushrooms devoid of latex when pricked, smooth soft caps that are not leathery, and those with nice mushroom aroma may pass the first test for consideration. Further tests on the skin and tongue are necessary. Any sample with irritation when applied to the skin is definitely not edible. Further, samples that cause irritation when tip-tongue tested, are definitely not suitable for the table. Mushroom poisoning remains a public health challenge in developing countries and providing basic guidelines to determine edible from poisonous is therefore essential. mushrooms Communities in Kaya Chivara have developed these inherent skills to harness wild mushroom to supplement their diets.

In this study, the collected indigenous mushrooms were partially classified into eight (8) species proposed as Rusulla ametica for Hakoranyani, Pleurotus eryngii (Mwasi). fragrantisma (Muhama), Agaricus Rusulla (Mbwate), Pleurotus populinus arvensis (Choga sikiro reruhe), Agaricus bisporus (Choga rerema), Agaricus (Choga spp. nyama.) and Agaricus xanthoderma for Choga Kadzonzo. Abundance of mushroom breeding sites was noted under the canopies of Brachystegia speciformis. This points at possible mycorrhizal association between these mushroom species and Brachistegia spp. The eight GC-MS profiles did not identify any toxic compounds of concern which validates the edibility of the sampled mushrooms species. Although the GC-MS profiles only provided a qualitative presence of the vital micro-molecules in these mushroom species, the results suggest immense potential for medicinal applications of these species in conventional and alternative medicine. The study however could not retrieve a number of edible mushroom species this community had previously harnessed for their diets. It is possible that these germplasms have been lost from this ecosystem.

# RECOMMENDATIONS

Morphological identification provided proximate identification of the sampled mushroom species. Further molecular work is required to ascertain the actual species these edible mushrooms belong too. The GC-MS profiles provided a qualitative presence of micro-molecules in these mushrooms. A quantitative analysis shall provide more insights on the content of these biomolecules for purposes of bioprospecting. The survey indicated that a number of mushroom species previously harnessed by the community have become extinct and could not be retrieved. It is therefore prudent to conserve these eight (8) retrieved mushroom species for future prosperity of these communities through domestication and commercialization.

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# **CONFLICT OF INTEREST**

The authors declare no conflict of interest in this work

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