CYTOTOXICITY, ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF *BOLETUS BICOLOR*, A BASIDIOMYCETES MUSHROOM INDIGENOUS TO TANZANIA.

Donatha D Tibuhwa

Department of Molecular Biology and Biotechnology, University of Dar es Salaam,
P.O. Box 35179, Dar es Salaam, Tanzania
Tel: +255 22 2410501-08, dtibuhwa@yahoo.co.uk

ABSTRACT

Boletus bicolor commonly known as coloured bolete is a basidiomyctes mushroom species widely used as a delicacy and its extract has been used traditionally to treat several human ailments in Tanzania. This study evaluates its extracts in order to provide a scientific foundation on the possible application in folk medicine system based on the biochemical evaluation. Standard microbes Bacillus subtilis and Candida albicans were used to test, the antifungal and antibacterial activity. Cytotoxicity test was also done on the extracts using brine shrimp test. Besides, the extracts were also used to investigate for antioxidant activity using DPPH (1, 1-diphenyl-2-picrylhydrazyl radical). Quantitative analyses for ß-carotene, lycopene, total phenolic compounds and vitamin C contents were also determined using spectrophotometric assay at 515 nm. The study result revealed B. bicolor methanol extracts to exhibit positive antimicrobial activity against Bacillus subtilis forming an inhibition zone of up to 2.3 mm/2days while it had no effect on Candida albicans. It further portrayed relatively mild cytotoxic activity with the LC_{50} value of 113.75µg/ml. The phenolic compounds were recorded high up to 187.45 GAE mg/g, ß-carotene 15.7 mg/g, Lycopene 23.81 mg/g and Vitamin C content 5.09 mg/100g. Likewise the radical scavenging ability was also high and concentration dependent with maximum ability at 1 ml/100g and EC_{50} value of 0.046 mg/ml. The findings from this study supports the traditional medicinal use of this mushroom and envisage a purposeful thoroughly study for isolating the bioactive compounds, up-scaling and possible developments into nutraceutics.

Keywords: mushroom, *Boletus*, antimicrobial antifungal, inhibition, cytotoxicity, basidiomycetes

INTRODUCTION

Searching for new drugs of pharmacological value has been tremendously increasing for the last three decades due to increased resistance of microorganisms to drugs in use. Plants and fungi especially fungi which form fruiting bodies, have been extensively researched on their health benefits Imtiaj and Lee (2007), Fagbohun et al. (2012), Tibuhwa (2014). Bioactive compounds from plants and mushrooms with medicinal value require chemical properties analysis for safety and efficiency. In developing countries, about 80% of individuals use them as traditional medicine (Gurib-Fakim 2005, WHO 2002-2005).

It is well documented that mushrooms play great roles in folk medicines in different parts of the world. Mushrooms are known to prevent diseases such as hypertension, hypercholesterolemia, cancer and as well as other great medicinal roles including immune-modulatory, liver protective, anti-fibrotic, anti-inflammatory, anti-diabetic, antiviral and antimicrobial activities (Breene 1990, Chang1991). Mushrooms have been also reported to be source of essential nutritive factors that include essential amino

acids and other medicinal compounds (Bonatti et al. 2004, Agrahar-Murugkar and Subbulakshmi (2005), Cheung, and Cheung 2005, Tibuhwa 2014, Hussein et al. 2015, Ibrahim et al. 2016).

Boletus are cosmopolitan mushroom genus belonging to basidiomycetes not well known in mythical, pseudo-science and science in producing novel mycochemicals. These mushrooms are mainly white rots that are involved in the fundamental process of lignocellulose degradation in nature. In Africa, the genus *Boletus* is among the major genera found in the ectomycorrhizal vegetations of the African Miombo woodlands (De Crop et al. 2012). The woodland is characterized by the local dominance of ectomycorrhizal trees of different genera. These includes *Caesalpinaceae*, especially *Julbernardia*, *Brachystegia* and *Isoberlinia*, as well as trees of the genus *Uapaca* of the family Phyllantaceae as detailed in Tibuhwa et al (2008) and De Crop et al (2012).

A two weeks fieldwork was done in the Southern part of Tanzania in Mtwara region with patches of Miombo woodland. This was followed by another two weeks fieldwork in the Kigoma region in North-West Tanzania. Kigoma region is situated at the border of Burundi and Lake Tanganyika, which is rich in miombo forest (Figure 1). Despite its richness in miombo woodland yet it is poorly explored for the presence and use of edible fungi due to political instability surrounding the region. The trips discovered a highly liked Boletus mushroom generally compared with liver meat due to its soft fleshy and delicacy. The mushroom not only is used for food in the studied area but also traditionally is considered for medicinal applications, which draw an attention to the researcher hence, this study. To the best knowledge of this study, there is no study that has been done to explore and establish the antioxidant activities and other medicinal properties of this well-eaten mushrooms in communities mainly living near the studied miombo woodlands in Tanzania. Therefore, this work is aimed at: i) Establishing its antioxidant potential, ii) Its free radical scavenging abilities and iii) Evaluate the antimicrobial activity of its extracts against microbes of medicinal importance represented by a fungal strain *Candida albicans* and a Gram positive bacteria *Bacillus subtilis* in order to establish if there is any scientific proof for its wide application in folk medicine system in the studied area.

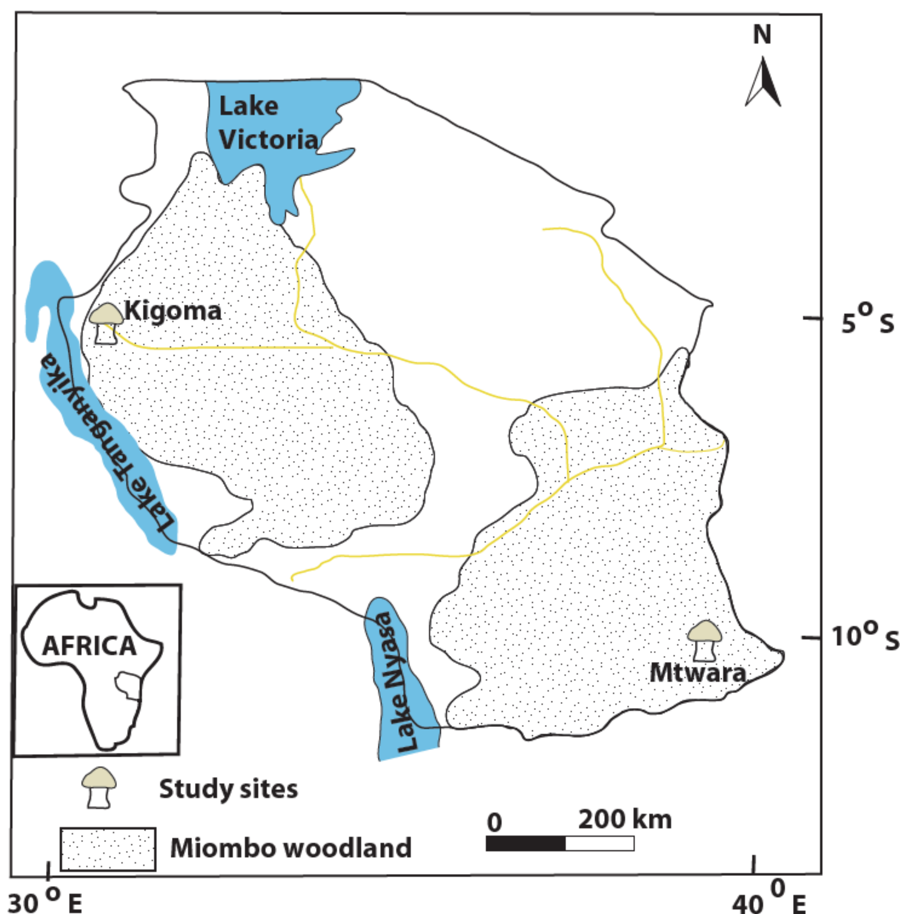

Figure 1: Map of Tanzania showing the sampling sites; The Southern part in Mtwara region and North-West Tanzania in Kigoma region**.** (Source: Google map, modified by the researcher by inserting the study sites)

MATERIALS AND METHODS

Collection of mushroom and Identification

Studied mushroom fruitbodies (Figure 2) were collected in Newala-Mtwara region and Kigoma region in Northwest Tanzania. The author identified the mushroom and the voucher specimens DDT 182.2015, DDT 193.0011, are kept in the mycological herbarium of the University of Dar es Salaam (DSM). The mushroom is among the dominant *Boletus* species fruiting out in clusters from grounds in Mtwara and Kigoma Miombo woodland. The fungi nomenclature was based on Kirk and Ansell (1992) as well as the web site of CABI bioscience databases (http://www.speciesfungorum.org/Names/Names.asp). The mushroom fruitbodies were photographed *in situ*, prior to picking from its substrate (Figure 2). Picking was done with the aid of the scalpel. Picked mushrooms were then packed into collecting plastic bags which were correctly labeled

with collection number, collecting date, name of the collector as well as few field identification tips. The tips included sporocarp shape, colour, smell, colour changing on bruising, and tentative names. It was then brought to the department of Molecular Biology and Biotechnology laboratory at the UDSM for further identification. Identification was done using available colored field guide books/ monographs such as described by Arora (1986), Härkönen et al. (1995, 2003), Kirk et al. (2001), Lodge et al. (2004).

Extracts preparation

Mushroom extracts were prepared following the methods detailed in Tibuhwa (2012) whereby air-dried fruitbodies of the studied mushroom powder was prepared by cutting the mushroom into smaller pieces using a coping saw and then grinding it with a mortar and pestle. It was then subjected to extraction using methanol for 48 hours incubation after which the mixture was filtered using cheesecloth. The filtrate was then allowed to evaporate by aid of vaporizer to get the crude extract, which was then weighed using weighing balance and found to be 0.5 gram.

Serial dilution of crude extracts

About 0.3 g of crude extract was dissolved in 10 ml of DMSO to get a stock solution to g/ml. Serial dilutions were then made from a stock solution to obtain a concentration of 15 mg/ml, 7.5 mg/ml, 3.75 mg/ml, 1.875 mg/ml and 0.9375 mg/ml by taking 1.0 ml the sock solution and diluted in DMSO.

Brine shrimp test (BST)

Brine shrimp eggs were bought from Dohse Acquaristic, Bonn (Aus Dem Hause Dohse Acquaristik), Germany. Sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast. The BST was carried out to screen for the cytotoxic activity of the *Boletus bicolor* crude extract. The procedures are as detailed in Tibuhwa (2016) and Sosovele et al. (2012), which included dissolving the crude extracts in dimethylsulfoxide (DMSO) to make a concentration of 4 mg/ml (stock solution) for each sample. One teaspoon of brine shrimp eggs was gently poured in a 300 ml of conical flasks full of filtered seawater. It was then allowed to shake for 48 hours while illuminated using an electric bulb, which helped attracting the hatched shrimps. Using 100 µl pipette, 10 hatched shrimp larvae were selected and transferred into different sample wells, which contained 1.0 ml of each dilution of extract with one control with only DMS. The mixture were mixed gently to allow good mixing and incubated at room temperature for about 24 hours. Using the microscope the mortality of larvae in each well was determined by counting the number of survivors and dead nauplii. Based on the concentration that killed 50% of the brine shrimp nauplii. LC_{50} in µg/ml was determined from the logarithmic plot as described in Throne et al (1995). The percentage mortality were obtained by using the formula:

$$\% \text{ Mortality} = \frac{\text{number of dead nauplii} \times 100\%}{\text{Total number of nauplii}}$$

Antimicrobial activity test

Preparation of Water extract

An amount of 4.0g of the dried mushroom samples were weighed and soaked in 30 ml of distilled water at 60 ºC for 4 hours. It was then filtered with Whatman No 42 filter paper and the filtrate dried at 50 ºC for 72 hours. A standard concentration of 20 mg /ml of the filtrate was then prepared using sterile distilled water as detailed in Tibuhwa (2012).

Preparation of tested Microorganisms and antimicrobial assay:

A Gram-positive bacterium, *Bacillus subtilis* (DSM 347) and a yeast *Candida albicans*

(ATCC 90028) both obtained from the Department of Molecular Biology and Biotechnology (MBB), University of Dar es Salaam were used. The bacteria were grown in a nutrient broth while the fungal on malt extract agar and were incubated at 37°C for 24 h. A volume of 10.0 ml of the pure culture were then centrifuged using sterile physiological saline water the cells were then rinsed twice and the suspension adjusted to optical density of 0.1 at 600 nm, equivalent to a cell population of 106 cells/ml based on McFarland standard. The suspension was kept in the test tube and stored at 4°C until used. The assay for antibacterial activities of each extract was determined by agar well diffusion method of Stoke and Ridgway (1980). The fungal cultures were prepared using peptone agar media. Both bacterial and fungal cultures were seeded into Mueller Hinton agar and Sabourad agar plates respectively. Wells measuring 7.0 mm diameter was made into each petri dish plates using sterile cork borer. About 30 µl of the extract was introduced into bore agar wells using sterile micropipette. The plates were kept inside the refrigerator at 4ºC for 3 hours to allow proper diffusion of the extracts into the medium. The zones of inhibition produced were measured in millimeters (mm) with all tests carried out in duplicates and their means recorded as explained in Hirasawa et al. (1999). Positive and negative controls were also set using the same conditions.

Antioxidant activity assay:

All chemicals used in this work were analytical grade and purchased from Sigma Aldrich Co. (St Louis, MO, USA). The antioxidant ability was analyzed using DPPH radical and antioxidant properties were analysed by determining the polyphenols (Total phenolic compounds, vitamin C, and Carotenoids (ß -carotene, lycopene) as detailed in Singleton 1999.

Mushroom extracts preparation and yield

Methanolic extractions were carried out using 25 g of the whole mushroom fruit body and weighed using analytical balance (METTLER TOLEDO) at room temperature. The mushrooms were powdered in a motor using pestles and soaked in 250 ml of absolute methanol and extraction proceeded as described in Tibuhwa (2012) adopted from Jaita et al. (2010). The mixture was kept stirring for 48 hrs at room temperature then filtered using Whatman No 42 filter paper. The filtrates were evaporated to dryness at 40 ºC in a rotary evaporator with 90 rpm (Labrota 4001, Heidolph® Essex Scientific Laboratory Supplies LTD) under reduced pressure. The obtained concentrated extracts were stored in dark at 4 ºC until further analysis. The percentage yield extracts were calculated based on dry weight as:

$$\text{Yield (\%)} = \frac{(W_1 \times 100)}{W_2}$$

Where W_1= weight of extract after methanol evaporation

W_2= Weight of the extracted mushroom.

Total phenolic contents determination

The total phenolic content was determined using the Folin-Ciocalteu colorimetric method (Tibuhwa 2012). Each 0.1 gm of extract was diluted with 5.0 ml of absolute methanol. An amount of 200 µl of the mushroom extract was transferred into a test tube then mixed thoroughly with 1.0 ml of Folin-Ciocalteau reagent. After 3 min, 0.8 ml of 7.5% (w/v) sodium carbonate was added to the mixture. The mixture was agitated for further 30 minutes in the dark and centrifuged at 3300 g for 5 minutes. The absorbance of mushroom extract and prepared blank were measured at 515 nm using spectrophotometer (UV-VIS model 6305 Jenway UK). The total phenolic content in the mushroom extract was expressed as milligram of gallic acid equivalent per 100 g weight of mushroom

using the linear equation obtained from standard gallic acid calibration curve.

ß -carotene and Lycopene antioxidant activity assays

The assay was carried out according to the method of Nagata and Yamashita (1992). The mushroom extract (100 mg) was shaken with 10 ml of acetone-hexane mixture (92:3) for 1 minute and filtered through Whatman No 42 filter paper. The absorbance of the filtrate was measured at 453, 505 and 663 nm. The ß-carotene and Lycopene content were calculated as:

Lycopene mg/100 mg = $0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$

ß-carotene mg/100 mg = $0.216\ A_{663} - 0.304 A_{505} + 0.452 A_{453}$

Determination of Vitamin C

The vitamin C content was determined titrametrically using 2.6 dichlorophenol indophenol methods following the method outlined by Tibuhwa et al. (2012). Twenty five gm of grounded sample was mixed with 25 ml of 5% (w/v) metaphosphoric acid solution and shaken for 30 min. The mixture was then filtered through Whatman No. 42 filter paper using suction pump. 10 ml was pipetted from the extract in 250 ml conical flask and titrated against 0.025% of 2.6 dichlorophenol indophenol reagents. The amount of vitamin C in each extract was calculated using the following equation:

mg of ascorbic acid per 100 g =

$$\frac{A x I x V1 x 100}{V_2 x W}$$

Where A = quantity of ascorbic acid (mg) reacting with 1 ml 0f 2.6 dichlorophenol indophenol

I = volume of indophenol (in ml) required for the completion for the titration with extract

V_1 = Total volume of extract

V_2 = Volume of aliquot

W = Weight of the mushroom sample extracted.

DPPH free radical scavenging activity assays

The qualitative assays were performed according to the method of Masuda et al. (1999), Jaita et al. (2010). A series of extracts with methanol ranging from 1:10-1:10^7 were prepared. A volume of 1.0 ml of the mushroom extract was mixed with 1.0 ml of 0.4 $mmol^{-1}$ absolute methanolic solution containing 1:1- diphenyl-2-picryhydrazyl (DPPH) reagent. Each mixture was left in the dark for 30 minutes and the absorbance was measured at 515 nm. This assay was performed in triplicates while

the percentage of DPPH radical scavenging activity of each extract was calculated as follows:

DPP radical scavenging activity =

$$\left(\frac{A_0-(A_1-A_s)}{A_0}\right)*100$$

Where A_0=Absorbance of the control solution containing only DPPH

A_1=absorbance in the presence of mushroom extract in DPPH solution and

A_s = the absorbance of the sample extract solution without DPPH

The EC_{50} value (total antioxidant necessary to decrease the initial DPPH radical concentration by 50%) was determined from plotted graph of scavenging activity against the concentration of extracts. A lower EC_{50} value means better radical scavenging activity

***Boletus bicolor* and Tanzanian folk medicine**

Guided and focused face to face interview was used to collect the information from fifty individuals from the studied area. The interviewed personnel were selected based on the pre-information from the local field

guides to only involve people who are likely to have good knowledge on mushrooms. So interviewed personnel involved most of traders who were found in local market selling mushrooms, known mushroom pickers and some local traditional healers. Information was solicited in an open-ended fashion with a guiding question form prepared by the author as detailed in Tibuhwa (2012, 2013, 2016). Through this questionnaire, the interviewees were asked if they knew the mushroom, its local name, where does it grow mostly? Any use of the studied mushroom they know, and what pretreatment (if any) are carried out before its use.

Data analysis

Data analysis was carried out using Analysis Systems (SAS) computer programme for Probit analysis according to Throne et al (1995). The lethal concentration values that kill 50% of the shrimps (LC_{50}) were determined for each fungal extract by graphical method where by a graph of percentage mortality of brine shrimp larvae against logarithmic Concentrations was used. The LC_{50} values were directly determined from probit analysis or calculated by substituting 50% for "y" into the curve equation in the graph.

RESULTS AND DISCUSSION

Identification of the studied mushroom

The results of this study found a Tanzania indigenous mushroom *Boletus bicolor* Perk (Figure 2) was distinctively found growing on soils remnant of the miombo-woodland in the Southern Tanzania, Mtwara region (10° 21' 12" S° 09' 58" E) and the Kigoma region (04°52'57" S, 29°48'41" E) in the North-West Tanzania situated at the border of Lake Tanganyika and Burundi (Figure 1). The mushroom was identified based on the following morphological characters: As the name depicts *it is a beautiful and totally contrasting red and yellow colors with large Basidiocarp, strongly incurved yellow margins, very showy with reddish mixed with yellow and pink lilacs when young then becoming more reddish that darkens as it matures. Margin smooth, distinctive yellow contrasting the rest of the basidiocarp surface. Hymenium well developed with tubes, with olive to light yellow basidiospores. Stipe central yellow, quite thick, club shaped and occasionally splitting. Flesh yellow slightly darkening to bluish brown on long exposure by bruising or cut*. **Comments:** This edible species (*Boletus bicolor*) differ from the closely resembling species *Boletus sensibilis* and *Boletus subvetipes*, which are inedible and poisonous by the former not immediately staining blue on bruising.

Anti-microbial activity

The methanol extracts of *Boletus bicolor* collected from both sites (Kigoma and Mtwara) were effective against a Gram-positive bacteria *Bacillus subtilis* showing clear zones of inhibition while showed no effect against a standard fungal strain *Candida albicans* (Table 2). The extract had an inhibition zone of 2.30 ± 0.55 mm/2 days and 2.28 ± 0.65 mm/2 days for the Kigoma and Mtwara sites compare to the positive control (streptomycin) with an inhibition zone of 4.7 mm/2 days. The results showed that streptomycin and ketoconazole had stronger activity than tested extracts as shown in Table 1 while a negative control; DMSO had no inhibitory effect (0.00 mm/2 days) on the tested organisms. The obtained results are in line with other numerous studies intending for screened of antimicrobial activity from mushroom in search of the new antimicrobial agents. For example, Ponugupati (2015) did the similar study in USA using methanolic extracts from three mushrooms against *Bacillus subtilis.*

Figure 2: *Boletus bicolor* locally known as uyoga maini: (a & b) a young fleshy fruitbodies (c & d) Old fruitbodies growing on soil in the remnant of Miombo woodland in Newala district- Mtwara region, Tanzania. (All photos taken by the author).

The study findings established the variation on the portrayed inhibition zones. *Lentinus edodes* was found having the largest mean clear zone diameter of 2.0 mm followed by *Pluerotus ostreatus* (1.8 mm) while the least was portrayed by *Agaricus bisporus* with mean clear zone of (1.6 mm) all relatively less compared to the portrayed inhibition zone of 2.3 mm. Other similar studies were also done by Ramesh and Pattar (2010), Gezer et al. (2006), Mercan et al. (2006) who also came up with the varied activity against the pathogens from different mushroom extracts. From these studies and comparing to this study results, it is clear that different mushroom species exhibit different antimicrobial activity as it has been also noted in Marjana et al. (2012); who also noted that the intensity of the antimicrobial effect from mushroom is dependent on the extract concentration and the respective tested organism. As established, the results in this study revealed antibacterial against a standard Gram-positive bacteria *Bacillus subtilis* while showed no antifungal activity against a standard fungal strain *Candida albicans* (Table 1). This might be due to the fact that bacteria are more sensitive to antibiotic compared to fungi (Hugo and Russel 1983) or due to their generic differences in their cell wall. The Gram-positive bacteria consist of peptidoglucans (mureins) and teichoic acids while fungi cell wall consists of polysaccharides such as chitin and glucan (Jean 2001, Farkaš 2003).

Table 1. Inhibition zone exhibited by Methanol extracts of *Boletus bicolor* against standard

Inhibition zone in mm/2days after application of antibiotic/antimicotic					
Tested Organism	***B. bicolor* extracts Kigoma sample**	***B. bicolor* extracts Mtwara sample**	**Streptomycin**	**Ketaconazole**	**DMSO**
Bacillus subtillis	2.30 ± 0.55	2.28 ± 0.65	4.70 ± 0.47	–	–
Candida albicans	–	–	–	6.89 ± 1.76	–

Doses of Mushroom extracts and used standard drugs each (30 µl/discs); Results are shown as mean± SD

***Boletus bicolor* cytotoxicity assay**

The brine shrimp lethality bioassay using *Boletus bicolor* extracts portrayed significant cytotoxic activities of LC_{50} at 113.75 µg/ml, which is by far much greater than 100 µg/ml, thus making it non-toxic and safe. The LC_{50} of the control had LC_{50} value of 256 µg/ml. Literature states that any extract is regarded as highly toxic if it portrays LC_{50} less than 1.0 µg/ml while for those with less than 100 µg/ml are referred to as mildly toxic and non-toxic as detailed in Sosovele et al. (2014).

It was interesting to note that mortality rate of the brine shrimp in this study increased relatively with the increase in the concentration of the extracts thus noted as concentration dependent. The observations of this dependence have been also noted in other studies but using extracts from different organism. For example Sosovele et al. (2014) reported the same tendency in actinomycetes extracts isolated along the coast of Indian Ocean, Tibuhwa (2016) in a medicinal plant *Oxalis corniculata* from Tanzania, while Krishnaraju (2005) noted the same during assessment of medicinal plants from India.

Antioxidant activities and free radical scavenging ability

Results from this study showed *Boletus bicolor* portraying good antioxidant activities depicted by high phenolic compounds up to 187.45 GAE mg/g, ß-carotene 15.7 mg/g, Lycopene 23.81 mg/g and Vitamin C content 5.09 mg/100g. Moreover, the radical scavenging ability was also high and it was concentration dependent. The maximum ability was at 1.0 ml/100g with EC_{50} value of 0.046 mg/ml. Different *Boletus* species have been widely investigated for their potential therapeutic benefits and antioxidant potentialities. For example Marijana et al. (2012) investigated mushrooms as possible antioxidant and antimicrobial agents of the two boletus mushrooms *Boletus aestivalis* and *Boletus edulis* and another different mushroom *Leccinum carpinihave* from Serbia while from Croatia other two boletus *Boletus edulis*, and *Boletus auranticus* were investigated for antioxidant properties Senka et al. (2010) and established that they portrayed high antioxidant potential and significant antimicrobial activities.

Electron donating ability of the given compound indicates its reducing power thus provides a mechanism for testing its ant oxidative ability. In this study, the obtained results in the order of their EC50 values shows that the reducing ability was dose dependent. The total antioxidant necessary to de-crease the initial DPPH radical concentration by 50% did not differ markedly with samples collected from two different sites, which are far apart, by more

than 1000 km (Figure 1). For example the highest ability was observed in both samples from Kigoma and Mtwara (EC_{50} < 0.175 mg/ml and EC_{50} < 0.178 mg/ml) respectively. These results reveal that the portrayed scavenging ability is not much influenced by the habitat it grows. It is however interesting to note that the studied mushrooms have high scavenging ability compared to abilities observed in well-known medicinal plants. For example in the study by Mon et al. 2011, they established that medicinal plants *Ardisia japonica, Ardisia conyzoides* and *Cocculus hirsutus* possess scavenging ability of EC_{50} = 12.72, 15.9 and 10.68 µg/ml, respectively. It is well known that human body produces free radicals during normal cellular metabolism that sometime exceed the natural endogenous defense mechanisms to eliminate them. The studied mushrooms have been found to possess potential anti-oxidants in high amount. Supplying our body with natural antioxidants for supporting our natural endogenous system.

***Boletus bicolor* and Tanzanian folkloric uses**

The results from this study reveals that *Boletus bicolor* locally known as '*uyoga maini'* in swahili meaning 'liver meat like' to possess high antioxidant potentials, which might be associated with its folkloric uses. The mushroom in Ha language known as 'bhoba bhitiku' literally with same meaning as in Swahili language 'liver meat like' is traditionally used to cure different diseases including, gastric ulcers, hypercholesterolemia, malnutrition and improving of healthy in long ill people and lactating mothers. It is commonly boiled or fried mixed with onions and took as stew in believes that it promotes health and improve immune system apart from being taken as delicacy food. The cytotoxicity screening in this study found it with mild cytotoxic activity against brine shrimp larvae LC_{50} value of 113.75 µg/ml while having strong antioxidant activities. For example the phenolic content which is well known for it anticancer properties was found to be high up to up to 187.45 GAE mg/g while ß-carotene which plays a big role in human healthy by providing vitamin A were also found to be relatively high up to 15.7 mg/g, (Table 2). Vitamin A is very important for vision while other carotenoids influence the human immune function and gap-junctional communication (Graßmann 2005). All these might attribute to its traditional medicinal uses by the studied communities.

Table 2. Antioxidant potentials in *Boletus bicolor*

Potential antioxidants	**Kigoma Samples**			**Mtwara Samples**		
	Sample C1	**Sample C2**	**Average**	**Sample C3**	**Sample C4**	**Average**
Total Phenols (Gallic Equivalence GAE/gdw)	179.34	187.45	183.395	184.95	184.68	184.815
ß-carotene mg/100g	15.7	15.5	15.6	16.2	15.2	15.7
Lycopene mg/100g	23.87	23.81	23.84	23.31	22.31	22.81
Vitamin C	5.09	5.09	5.09	4.86	5.08	4.97

The high levels of antioxidant activities are well known to contribute towards anti cancer properties (Russell and Paterson 2006), suppress excess free radical species which cause smash-up of cells by chain reactions, such as lipid peroxidation or formation of DNA adducts that could cause cancer-promoting mutations (Banerjee et al.2012, Filipa et al. 2011).

CONCLUSION

The present study results thus shows that tested mushroom species demonstrated a strong antioxidant and antimicrobial activities as well as less toxic to the cell. This suggests that *Boletus bicolor* mushroom may be used as good sources of natural antioxidants and for pharmaceutical purposes in treating of various diseases. The result thus seconds the folkloric uses of the studied mushrooms in promoting different health benefits.

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