Evaluation of the antioxidant activities of some wild edible indigenous Ghanaian mushrooms

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ABSTRACT
Wild edible mushrooms have been reported to contain high phenolic contents which confer free radical scavenging ability. This study investigated antioxidant potentials of wild edible mushrooms from the Ashanti region (Kumasi) in Ghana. Cultivated strain of Pleurotus ostreatus (control) and five wild edible mushrooms (*Termitomyces sp., Volvariella sp., Termitomyces shimperi, Auricularis auricular, and Volvariella volvaceae*) were evaluated. Total phenolic content, Glutathione content and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical experiments were run on aqueous and ethanolic extracts of each mushroom. Ethanolic and aqueous extracts of *T. shimperi* showed the highest antioxidant activity in vitro, with their EC50 values of 0.699mg/ml and 0.47mg/ml respectively. Their EC50 values were greater than the standard, butylated hydroxy toluene (BHT) value of 0.526mg/ml. Ethanolic extracts of *Volvariella sp* had the significantly ($p<0.05$) higher phenolic content (861.3 mg/GAE, $p <0.01$) than the cultivated control, P. Ostreatus (aqueous extracts at 284.1695 mg/GAE and ethanolic extracts at 167.7287 mg/GAE). *Termitomyces sp., A. auricular, and V. volvaceae* had lower antioxidant activities than the BHT control. Two wild varieties (*Termitomyces shimperi and Volvariella sp.*) of mushroom assayed showed significantly ($p<0.05$) higher antioxidant ability. Consumption of these varieties of wild edible mushrooms can therefore be recommended.

Keywords: Antioxidants, Mushrooms, Free radicals, Phenolics, Glutathione.

1. INTRODUCTION

Edible mushrooms are consumed both locally and internationally. They have nutritional and medicinal values. It has been reported that some mushrooms species possess benefits that include antimicrobial, antioxidant, cholesterol lowering, immune-stimulatory and anticancer effects (Oyetayo, 2009 ;Barros *et al.*, 2007). Hence, natural antioxidants can be obtained from mushroom species can be a source of, taken as food supplements or potentially usable in the pharmaceutical industry. Mushrooms may also possess a many secondary metabolites which include compounds such as polyketides, phenols, terpenes, and steroids. However, there is very wide diversity of mushroom species ranging from fleshy and edible, medicinal, poisonous and other species of wild edible mushrooms (Ainsworth *et al.*, 1995).

Studies have shown significant antioxidant activity of wild edible mushrooms (Keleş *et al.*, 2011), and some have shown that their total phenolic content has been associated with their antioxidant activity (Barros *et al.*, 2007). Therefore, they can possibly be a natural antioxidants' source because they are available and acceptable to the public. Often, wild edible mushrooms are picked and sold in the market places without proper identification and classification. With the increase in environmental pollutions that affect the oxidative balance of the human body, there becomes an imperative to study antioxidants of natural sources such as can be found in mushrooms, and to analyze these wild mushrooms to ascertain their nutritional, antioxidant and other medicinal properties. Additionally, more information would be available to the public on the potential health benefits of these wild edible mushrooms, and their inclusion in the diets of Ghanaians can be encouraged.

2. METHODS

2.1 Sample Determination

Wild indigenous edible mushrooms were sampled from the local market in Kumasi metropolis and were identified by a botanist. The control sample was gotten from the local market. After taxonomical identification, the samples were identied as: *Plerotus ostreatus* (control), *Termitomyces sp., Volvariella sp., Termitomyces shimperi, Auricularis auricular, Volvariella volvaceae*.

2.2 Sample preparation

The fruiting bodies were washed with distilled water to remove any excess soil matter on the fruiting body. The samples were then cut into bits prior to storage in a deep freezer, then the samples were lyophilized using a freeze-dryer at the Crop Research Institute at Fumesua, Kumasi, Ghana. After the samples were freeze-dried, they were blended into a fine and smooth powder with the use of a kitchen dry blender and then stored in plastic zip lock bags, prior to the sample extraction.

2.3 Sample extraction

According to the demonstration of Ferrari *et al.* (2012) who stated that extraction yield increases significantly in a mixture of organic solvent with water, fifty percent of ethanol was employed

for the sample extraction instead of absolute ethanol. Additionally, distilled water was also used in preparation of aqueous sample extracts. In total 12 extractions were made (see Table 1 below), six for ethanolic and another six for the aqueous extracts in 500 ml conical flasks for each of the extracts. After the entire extraction procedure, the samples were then taken to the crop research institute at Fumesua for freeze drying again. The freeze dried extracted samples were then put into zip-locked for subsequent analysis.

Table 1. Study groups for plant extracts based on the specie and solvent

Wild mushroom varieties	**ID.**	**Aqueous extracts**	**Ethanolic extracts**
Pleurotus ostreatus (control)	S1	S1A	S1E
Termitomyces sp.	S2	S2A	S2E
Volvariella sp.	S3	S3A	S3E
Termitomyces shimperi	S4	S4A	S4E
Auricularis auricular	S5	S5A	S5E
Volvariella volvaceae	S6	S6A	S6E

2.4. Total phenolic content determination

Analysis of the total phenolic content was undertaken at Noguchi memorial institute for medical research (NMIMR), Legon. 100ml of Na_2CO_3 solution was prepared 24 hours before the assay. The methodology employed in this assay was done according to modified Folin-Ciocalteau's colorimetry (Singleton *et al.*, 1999). Results of the Total Phenolic content assay were then expressed as mg of Gallic acid equivalents (GAE) per 100 grams of extracts because gallic acid represented the standard curve.

2.5 Free radical (DPPH (Dipicrylphenylhydrazine)) scavenging activity determination

Five serial dilutions were prepared for the standard, butylated hydroxytoluene (BHT) from a stock solution of 20mg/ml, the same preparation was followed for the 12 extracts. Absolute methanol was employed in the preparation. One hundred µl of the extract or BHT (positive control) was added into the well plates, then a 100 µl of the DPPH free solution (0.5mM) was pippeted into each of the well plates. The plates were then covered with foil and incubated in the dark for 20 minutes. Absorbance for all test samples (BHT and all 12 extracts) was measured in triplicate at 517nm.

2.6 Determination of glutathione (GSH) content

The determination of the glutathione content of the samples was also undertaken at NMIMR, Legon, Ghana. 200µl of glutathione buffer was prepared to be mixed with each of the samples

and another eppendorf tube mixture was prepared for the glutathione. 10 µl of OPT (optaldehyde) was added to the mixture, then the mixtures were incubated at room temperature for 15 minutes. Finally the excitation and emission wavelengths of 350nm and 420nm respectively were used to estimate the fluorescing capacities of each of the subjects.

2.7 Analysis of data

The data obtained were reported as plots and analyzed using the boniferrori test for two way analysis of variance. The concentrations of the glutathione and total phenolic content of the samples were gotten from the equation of the line of their respective standard curves. Statistical difference was indicated when P value is < 0.05.

3. RESULTS

3.1. Total phenolic content (TPC) of extracts

After reading the absorbances of each of the mushroom sample extracts, their concentrations were evaluated from the gallic acid standard curve and then expressed as gallic acid equivalents (GAE/100g). Aqueous extracts of *Volvariella sp.* (S3A), ethanolic extracts of *Volvariella sp.* (S3E) and ethanolic extracts of *Termitomyces shimperi* (S4E) all possessed a significantly higher phenolic content than the cultivated control, *Pleurotus ostreatus* with $p<0.05$, $p<0.01$, $p<0.001$, respectively (Figure 1).

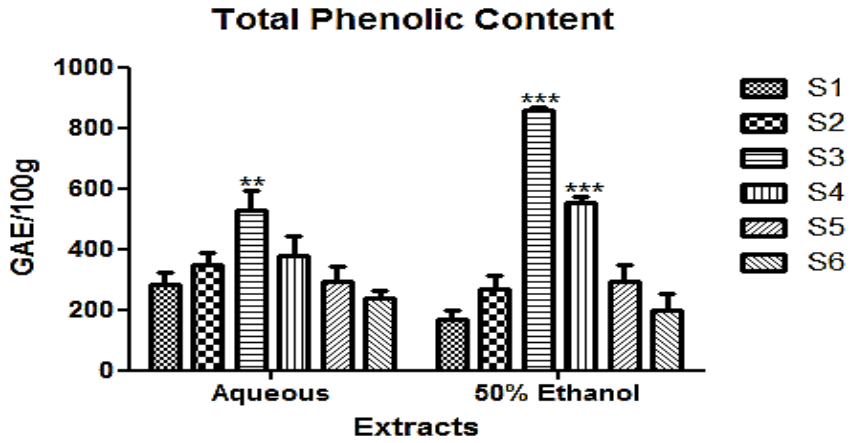

Figure 1. TPC of aqueous and ethanolic extracts of wild edible (S2-S6) and cultivated mushroom extracts (S1-control).

3.2. Free radical (DPPH) scavenging activity

3.2.1. DPPH scavenging activity of extracts of Pleurotus ostreatus (control)

The DPPH scavenging activity of mushroom extracts were shown as EC50 values. A lower EC50 value shows higher DPPH scavenging activity of the extracts. In figure 2 below, S1A (Aqueous) & S1E (50% Ethanolic) shows the concentration of extracts that extinguished fifty percent of DPPH, this is the metric of antioxidant activity. The aqueous extracts of the sample showed a higher antioxidant potential than that of the positive control (BHT) (EC50 value: 0.526 mg/ml).

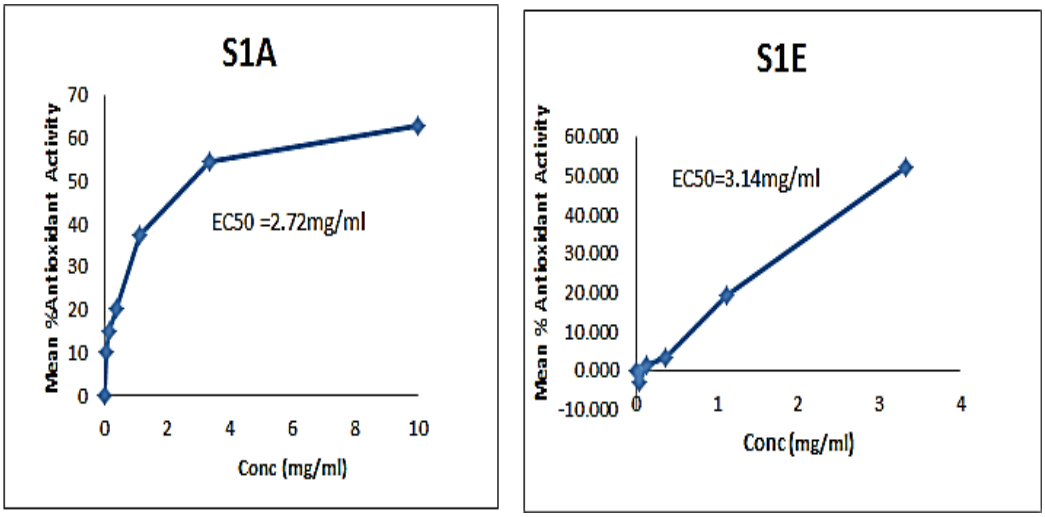

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Fig. 2. DPPH scavenging activity of ethanolic and aqueous extracts of *Pleurotus ostreatus.*

3.2.2. DPPH scavenging activity of extracts of Termitomyces sp.

Figure 3 shows the curve for ethanolic extract (S2E) and aqueous extracts (S2A) with EC50 value of 1.52 mg/ml.

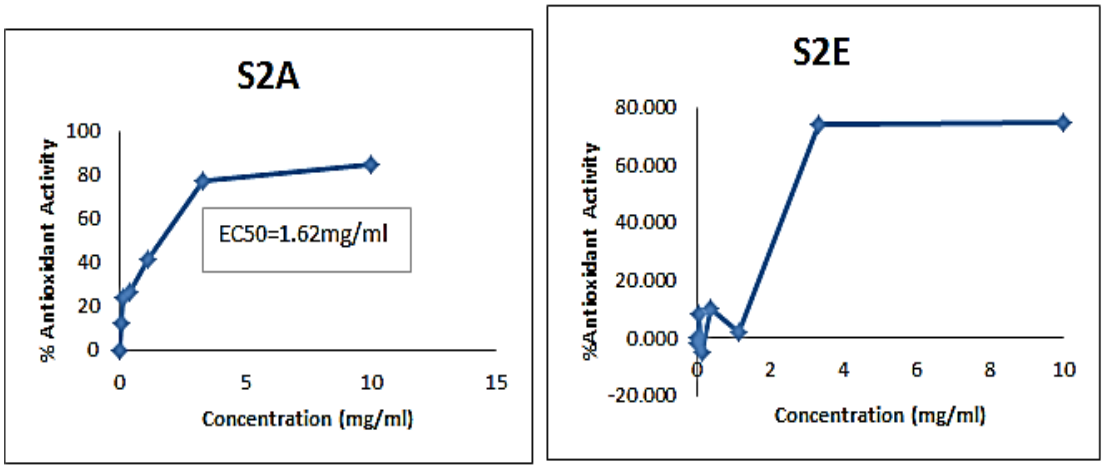

Fig. 3. DPPH scavenging activity of ethanolic and aqueous extracts of *Termitomyces sp*.

3.2.3. DPPH scavenging activity of extracts of Volvariella spp.

Figure 4 shows the antioxidant activity of ethanolic extracts (S3E) and aqueous extracts of *Volvariella sp.,* S3A.

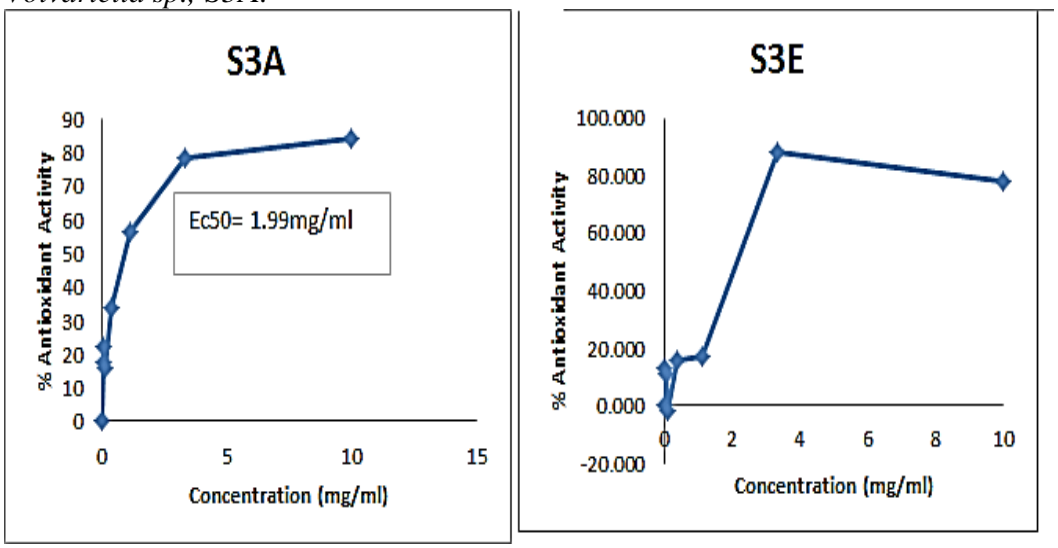

Fig.4. DPPH scavenging activity of ethanolic and aqueous extracts of *Volvariella spp.*

3.2.4. DPPH scavenging activity of extracts of Termitomyces shimperi.

Figure 5 shows the DPPH scavenging abilities of the aqueous (S4A) and ethanolic (S4E) extracts of *Termitomyces shimperi* with EC50 values of 0.47mg/ml and 0.699mg/ml respectively. The ethanolic extracts possessed a slightly higher EC50 value and the aqueous extracts possessed a lower EC50 value than the standard BHT (0.526 mg/ml).

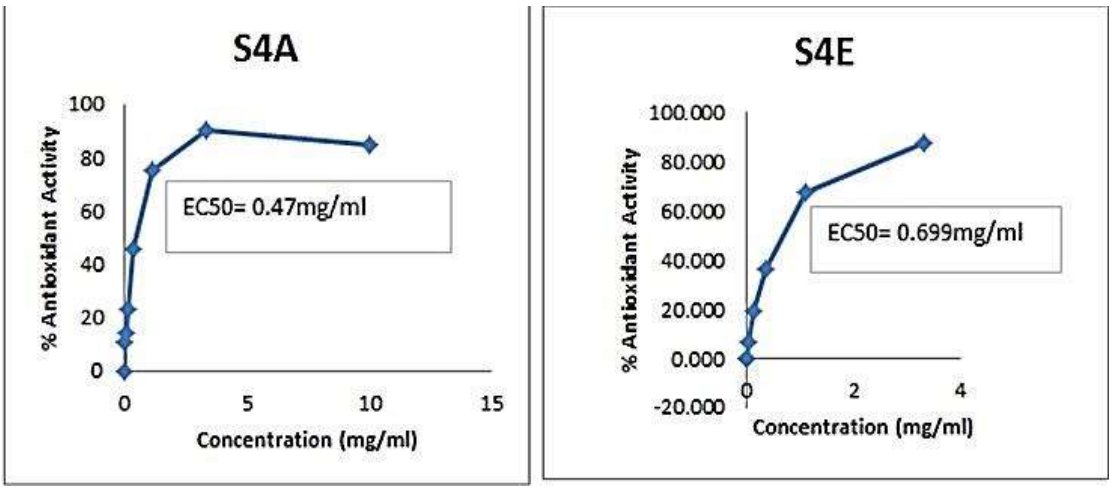

Fig. 5. DPPH scavenging activity of ethanolic and aqueous extracts of *Termitomyces shimperi.*

3.2.5. DPPH scavenging activity of extracts of Auricularis auricular.

Figure 6 below shows the antioxidant activities of ethanolic and aqueous extracts of *A. auricular.* The aqueous (S5A) and ethanolic (S5E) extracts had EC50 values of 4.806mg/ml and 3.240 mg/ml respectively.

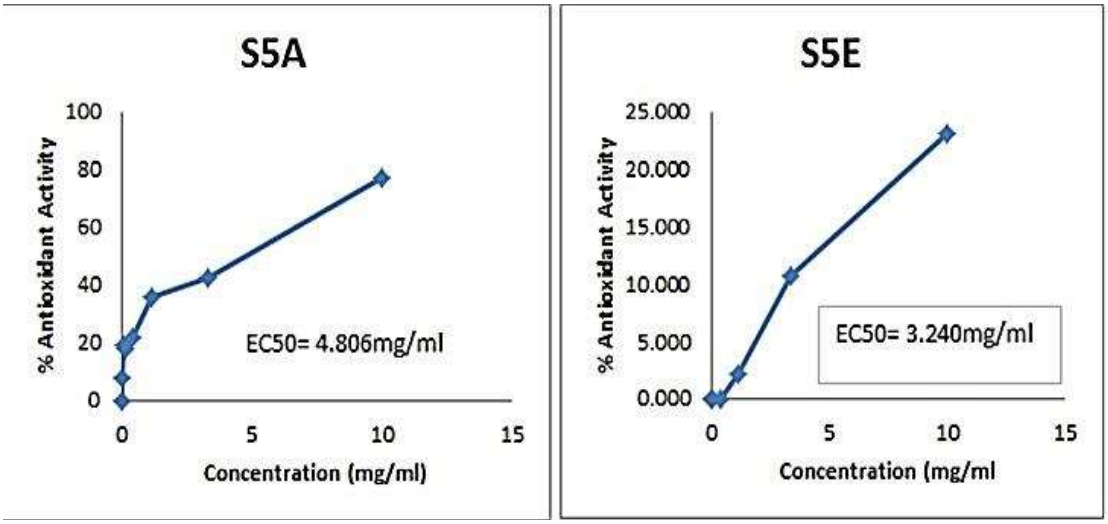

Fig. 6. DPPH scavenging activity of ethanolic and aqueous extracts *of Auricularis auricular.*

3.2.6. DPPH scavenging activity of extracts of Volvariella volvaceae

No valid value was computed for EC50 for antioxidant activity of *Volvariella volvaceae.*

3.3. Glutathione (GSH) content of samples

3.3.1 GSH content of sample extracts

Figure 7 below shows the glutathione content of each of the extracts and cultivated control (*Pleurotus ostreatus*). Aqueous extracts of samples 2, 3, 4 and 6 possessed a significantly lower glutathione content than the cultivated control at p values, $p<0.05$, $p<0.01$, $p<0.001$, $p<0.001$ respectively.

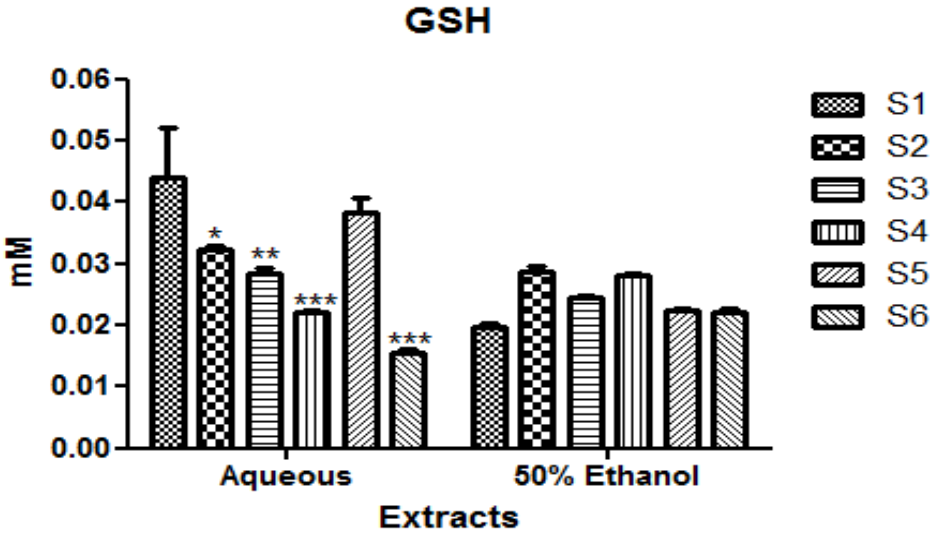

Fig. 7. Glutathione content of mushroom extracts of wild edible (S2-S6) and cultivated mushroom extracts (S1-control) with statistical difference: *p<0.05, **p<0.01, ***p<0.001 against the control).

4. DISCUSSION

This study assessed antioxidant activity of some wild edible mushrooms in Kumasi. Aqueous and ethanolic extracts of *Volvariella sp.* (S3A and S3E) and the ethanolic extract of *Termitomyces shimperi* (S4E) possessed significantly higher total phenolic content than the cultivated control, *Pleurotus ostreatus*. The rest of the extracts either possessed insignificantly higher total phenolic content or a lower phenolic content than the control. Ethanolic extracts of *Volvariella sp.* had the most remarkable content of 861.3 mg/GAE (100g) which was slightly higher than the cultivated control which was at 284.17 mg/GAE (100g). It is also important to note that extracts of *Volvariella sp.* possessed higher total phenolic content but lower glutathione content than *Pleurotus ostreatus*. Amidst the plethora of compounds with antioxidant activity, the polyphenols have become relevant as a result of the milieu of biological activity which they possess and include free radical scavenging ability (Rodrigo and Bosco, 2006).

Our study was similar to other reported studies of high phenolic content in mushrooms (Barros *et al*., 2007; Keleş *et al.* (2011). The very high phenolic content may have been contributed by some natural microbial content or growth during the extraction, literature has established that some mushroom species possess significant antimicrobial activity *in vitro*. Glutathione content of Aqueous extracts of *Termitomyces sp., Volvariella sp., Termitomyces shimperi and Volvariella volvaceae* were lower than the control. This is apparently as a result of the heat labile nature of the enzymes coupled and possible denaturation of a portion of the total enzyme constituents. The EC50 value of each subject is the concentration of the extract at which fifty percent of the free radical has been extinguished. Aqueous extracts of *Termitomyces sp* and *Volvariella sp*. had lower values than cultivated mushroom (2.72 mg/ml) but relatively higher than the standard hence they still possess antioxidant activity.

Constantly, the human body is exposed to free radicals, according to Gilbert (2000), free radicals possess an electron which is unpaired in the outermost orbit of their electron shell; free radical species include include hydroxyl, superoxide, hydroperoxyl and peroxyl radicals. These free radicals act as oxidative agents and as inhibitors of enzymes that possess an iron-sulphur centre. They also lead to the oxidation of critical molecules of the body namely, proteins, DNA and lipids which can lead to injury of the cell and its death (McCord, 2000;Freidovich, 1999). Antioxidants play have a responsibility to maintain the health of the human body because they can extinguish free radicals in the human body by donating electrons to the free radical. The body has its own defense system to extinguish free radicals and they include the superoxide dismutase, glutathione peroxidase and catalase enzymes (Halliwell and Cross, 1994). Olajire and Azeez (2011) further stated that antioxidants are beneficial because they inhibit the initiation or interrupt the oxidation of biomolecules.

Interestingly, we found out from out study that *Termitomyces shimperi* showed the highest antioxidant activity against DPPH free radicals *in vitro*. The ethanolic and aqueous extracts of *Termitomyces shimperi* showed EC50 values of 0.699mg/ml and 0.47mg/ml respectively for the free radical scavenging assay, both the aqueous and ethanolic extracts of sample 4 (*Termitomyces shimperi*) possessed lower EC50 values than the control; the aqueous extracts of the sample possessed a lower EC50 than the standard (BHT- a synthetic antioxidant) which

stands at an EC50 value of 0.526mg/ml. From literature, mushrooms have more nutritional values than most vegetables. In the sense that mushroom contains some vitamins such as thiamine (vitamin B1), riboflavin (vitamin B2), cobalamin (vitamin B12), ascorbic acid (vitamin C), contains lot of minerals and have low calorific value (Rai and Arumuganthan, 2008). Mushrooms also have a rich supply of folic acid which is more than any other vegetable or meat with the exception of liver (Oei, 1991). On the average, dry mushrooms contain between 19-40% high quality proteins that possess there entire nine essential amino acids. The fat content of mushroom is low (1-8% dry weight) i.e. it contains mostly of unsaturated fatty acids, hence they have no health implications as opposed to saturated fatty acids of animal origin (Oei, 1991). With these nutritional content and reported findings in our study and other studies of high antioxidant activities, it is apparent that wild mushrooms are very beneficial to man.

5. CONCLUSION

Remarkably, ethanolic and aqueous extracts of wild edible *Termitomyces shimperi* and Volvariella *sp.* possess high antioxidant activity *in vitro* than commercially cultivated mushroom and can be recommended for inclusion into Ghanaian diets because of their potential health benefits. Based on the fact that free radicals are highly dangerous to normal human physiology, high phenolic content of this plant can have great health benefits to man.

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