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Nutritional and Bioactive Constituents of *Hypsizygus ulmarius* (Bull.:Fr.) Redhead Fruit Bodies Cultivated on Some Agro-wastes

John Victor Sunday*, Okwulehie Ikechukwu Cyriacus, Bassey Hasadiah Okon

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria

Abstract

This study investigated the myco-chemical constituents of *Hypsizygus ulmarius* fruit bodies cultivated on some agro-wastes. The agro-wastes include: coconut fibre (CF), maize straw (MS), sugarcane bagasse (SB), coconut fibre + maize straw (CF+MS), coconut fibre + sugarcane bagasse (CF+SB), maize straw + sugarcane bagasse (MS+SB) and coconut fibre + maize straw + sugarcane bagasse (CF+MS+SB). Completely randomized design with seven treatments and four replicates was used. Data obtained were statistically analyzed using analysis of variance and the mean were separated using Duncan's Multiple Range Test at 0.05 level of significance. The analyses indicated the presence of nutritional and bioactive compounds at different concentrations to include vitamins such as vitamin A (5.77 in SB - 8.33mg/100g in CF), vitamin B1 (0.78 in MS - 0.94mg/100g in SB), vitamin B2 (0.29 in CF - 0.50mg/100g in CF+MS), vitamin B3 (1.72 in CF 2.81mg/100g in MS+SB), and vitamin C (7.57 in CF - 8.64mg/100g in MS). Minerals such as Calcium (198.36 in MS - 290.5lmg/l00g in CF), Magnesium (72.00 in MS - 144.44mg/100g in SB), Sodium (6.72 in MS - 9.36mg/100g in CF), Potassium (314.41 in CF+SB - 395.63mg/100g in CF) and Phosphorus (152.17 in SB - 195.23mg/100g in CF). Heavy metal concentrations which included zinc (1.13 in MS - 2.40 mg/kg in CF), iron (6.28 in SB - 16.98 mg/kg in MS), lead (0.17 in MS+SB - 0.25 mg/kg in CF), cadmium (0.09 in MS - 0.15 mg/kg in CF+SB) and copper (0.12 in MS - 0.21 mg/kg in CF). Proximate analyses which include protein (19.25 in SB - 24.50% in CF), carbohydrate (39.59 in MS - 43.25% in CF+MS), fibre (14.98 in CF - 19.73% in SB), ash (7.96 in CF - 10.50% in MS), moisture content (4.33 in CF+MS+SB - 6.28% in CF) and fat (3.80 in CF - 4.20% in MS and MS+SB). The bioactive compounds which include tannin (0.56 in CF+MS - 0.74mg/g in MS+SB), alkaloid (4.80 in SB - 7.90mg/g in CF), phenol (2.82 in CF+MS - 4.55mg/g in SB), flavonoid (3.80 in SB - 4.30mg/g in CF), steroid (0.16 in SB - 0.75mg/g in CF), hydrogen cyanide (0.01 in SB - 0.07mg/g in CF+MS+SB), anthocyanin (0.22 in MS - 0.35mg/g in CF) and saponin (2.60 in SB – 10.00mg/g in MS). The result showed that the mushroom is of good quality. It contained nutrients which can help to improve the micronutrients and general well-being of the people.

Keywords

Mushroom, Nutritional, Bioactive, Fruit Bodies, *Hypsizygus ulmarius*

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1. Introduction

The quest for food sources by man has been consistent in other to improve his biological functions, health and general well-being. Fungi represented by yeast, mushroom, and bracket fungi have been widely utilized as food since the origin of mankind [59]. The medicinal as well as nutritional values of edible mushrooms have a long history and they

* Corresponding author
E-mail address: victorjohn21666@gmail.com (J. V. Sunday)

contained valuable bioactive compounds [4, 34]. In many countries, especially Europe and Asia, mushrooms are well known and considered as favourite delicacy. They are usually consumed either in the fresh or processed forms and are considered valuable mainly due to their flavour and palatability. Edible mushrooms have the tendency to contribute greatly to food value by supplying both macro and micro-nutrients in our diet [10, 61]. A good number of mushroom species are consumable and has a good profile of vital nutrients which include vitamins, minerals, carbohydrates, low fat and oil content, various amino acids and fibres [10, 35, 47]. The nutritional importance of mushrooms can be compared to those of fruits, eggs, cereal, milk, and meat [42].

Beyond the nutritional importance of mushrooms, they also possess and provide medicinal relevance and are thus considered as functional food [7]. They are found to be source of bioactive compounds such as phenols, steroids, flavonoids, terpenoids, sterols, ascorbic acid, ergothioneine and carotenoids [6, 17]. Mushrooms health promoting properties records such as anti-tumour, anticancer, cholesterol lowering, anti-genotoxic, antioxidant, antimicrobial, and immune stimulatory effects have been documented for some mushrooms species [26, 33, 35, 37, 39]. This study aims at examining the myco-chemical properties of *Hypsizygus ulmarius* fruit bodies cultivated on some agro-wastes with a view to increase the awareness of people about the significance of this food item.

2. Materials and Methods

2.1. Source of Materials

The spawn of the mushroom species *Hypsizygus ulmarius* used for this study was obtained from Dr. Magnus Nwoko of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike. The coconut fibre was collected from Ikot Mbang market, Ibiono, Akwa Ibom State, maize straw was collected from a farmland at Amaoba village in Abia State and the sugarcane bagasse was collected from Nassarawa road, Itam, Akwa Ibom State.

2.2. Preparation of the Substrates and Inoculation

Cultivation of *Hypsizygus ulmarius* was carried out by following the method of Karthika and Murugesan (2015) but with little modification [28]. The substrates used were: coconut fibre (CF), maize straw (MS), sugarcane bagasse (SB), coconut fibre and maize straw (CF + MS), Coconut fibre and sugarcane bagasse (CF + SB), maize straw and sugarcane bagasse (MS + SB), Coconut fibre and maize straw and Sugarcane bagasse (CF + MS + SB). These mixtures were made in the ratio of 50:50 and 50:50:50 respectively. The substrates were first chopped to convenient sizes and were then moistened in fresh water overnight in a container. The moistened substrates which were kept overnight were then pasteurized using steam for 2h in a closed chamber. The pasteurized substrates were removed from the closed chamber and placed in a clean container inside the room and allowed to cool at room temperature. During inoculation, all the instruments used were first sterilized with alcohol while the perforated buckets were rinsed with dilute solution of hypo-chloric acid (5%). Thirty grams (30g) of grain based spawn of *Hypsizygus ulmarius* was poured at the bottom of the sterilized perforated bucket followed by filling the same bucket with 50g of pasteurized substrate. Also, 30g of grain based spawn was spread again on the surface of the substrate. Again, the substrate was then filled to cover the spawn and pressed lightly. This method was repeated four times until the perforated bucket was filled and four replicate were made for each substrate. The perforated buckets were covered with lid. Perforation of bucket was for aeration of inoculated substrate. After inoculation, the buckets were kept in the ventilated room and covered with black polyethylene bag.

2.3. Determination of Vitamins

The vitamin A (retinol), B1 (thiamin), B2 (riboflavin) and B3 (niacin) content of *Hypsizygus ulmarius* fruit-bodies were analyzed using spectrophotometric method as described by Association of Official Analytical Chemists (AOAC) [3] and vitamin C (ascorbic acid) was analyzed using EDTA/TCA (tricyclic antidepressant) extraction methods [3].

2.4. Determination of Minerals

Mineral compositions of dried mushroom samples were determined by wet-ashing method. The solutions of ash obtained from the samples was dissolved in a drop of trioxonitrate (v) acid made up to 50ml with deionized water and analyzed for Calcium (Ca) and Magnesium (Mg) using vanadate ethyldiamine-tetra acetic acid (EDTA) complexometric titration method according to AOAC [3]. Sodium (Na) and Potassium (K) were estimated using flame photometer as described by Onwuka (2005) [54] while Phosphorus (P) was determined using UV-visible spectrometer after making Ammonium vanado-molybdate at 436nm according to the established procedures of AOAC [2].

2.5. Determination of Heavy Metals Concentration

The concentrations of iron, copper and zinc in the sample were determined by Energy Dispersive X-ray Fluorescence (EDXRF) technique according to the method of Stihi *et al.*

(2011) [61]. The concentration of cadmium and lead in the sample was determined by Atomic Absorption spectrometry (AAS) [10], using the AVANTA GBC spectrometer (GBC Scientific equipment Pty Ltd, Australia) with flame and hollow cathode lamps (HCL).

2.6. Proximate Analysis

Proximate analyses which consist of protein, moisture, ash, crude fibre, fat and carbohydrate contents of *Hypsizygus ulmarius* fruit-bodies were determined using the method described by Association of Official Analytical Chemists [3]. The content of the available carbohydrate the mushroom was calculated by the following equation [58]: Carbohydrate (g/10n g sample) = 100 - [(moisture + fat + protein + ash + crude fiber) g/100g]

2.7. Determination of Bioactive Compound

2.7.1. Determination of Alkaloids

A measured weight of the processed sample (5g) was dispersed in l00mls of 10% acetic acid in ethanol solution. The mixture was well shaken and allowed to stand for 4 hours at room temperature being shaken every 30min. The mixture was filtered through what man filter paper. The alkaloids were determined using alkaline precipitation gravimetric method described by AOAC [2].

2.7.2. Determination of Saponins

This was determined by the method described by Okeke and Elekwa (2002). 5g of the sample was boiled with 100ml of 20% ethanol in a water bath for l: 30mins and filtered while still hot. The filtrate was collected and heated for 30mins in 40ml of ether, and then poured into a separating funnel, thereafter the lower part of the filtrate in the separating funnel was collected and 60ml of n-butanol was added and the upper layer/part was then collected while the lower part was discarded. The filtrate was then evaporated to dryness using steam bath at 70°C in an oven, and then cooled and weighed [43].

2.7.3. Determination of Phenols

To determine the percentage of phenol in the samples, the method described by AOAC [2] was followed. 2g of the sample was used. The absorbance of the solution was read at 505nm wavelength using a spectrophotometer.

2.7.4. Determination of Flavonoids

5g of the dry powdered sample was used to determine the flavonoids content according to AOAC [2], The sample was mixed with 100ml of 2M HCI at room temperature. The solution was then boiled for 30 min with water bath, cool and filtered. 20 ml of ethyl acetate was added to the filtrate and was filtered again with a weighed filter paper. The filter paper was then oven dried, cool and weighed again.

2.7.5. Determination of Tannins

Tannins were determined according to the method of Okeke and Elekwa (2002). 0.5g of the sample in 10ml of 2M HCI was shaken for 5mins and transferred into a volumetric flask and made up to 50ml. the mixture was then filtered and 5ml of the filtrate was introduced into a test tube. 3ml of 0.1N HCI and 3ml of 0.008m of potassium ferro-cynide ($K_3F[CN]3$) was added. The absorbance was read at 720nm within l0mins [43].

2.7.6. Anthocyanin Determination

This was done gravimetrically by the method described by AOAC [2]. 5g of each test sample was treated with ethyl acetate and allowed to separate into two layers. The ethyl acetate layer (extract) was then recorded, while the aqueous layer was discarded. The extract was separated to dryness in the crucible over a steam bath. The dried extract was then treated with concentrated amyl alcohol to extract the anthocyanins. The weight of anthocyanin was determined and expressed as percentage of the original sample.

2.7.7. Hydrogen Cyanide (HCN) Determination

Hydrogen cyanide was determined by alkaline pikrate colorimetric method described by AOAC [2]. 2g of the sample was dispersed in 50ml of distilled water in a conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks. The set up was incubated overnight and each of the pikrate paper was eluted or dipped into a 60 ml of distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solutions was then measured with colorimeter at 540nm wavelength with the reagent blank at zero.

2.7.8. Determination of Steroid

5g of the powdered sample was hydrolyzed by boiling in 50ml of hydrochloric acid solution for about 30 minutes. It was then filtered using what man filter paper. The steroid content of the samples *Hypsizygus ulmarius* was determined using the method described by Okeke and Elekwa (2002) [43].

2.8. Statistical Analysis

The values obtained from the various parameters were statistically analysed using analysis of variance and the mean were separated using Duncan's Multiple Range Test at 0.05 level of significance.

3. Results

3.1. Vitamin Composition

The result showed that there was variation in vitamin contents (Table 1). The highest vitamin B1 was obtained from the substrate SB (0.94±0.01 mg/100g) and MS (0.78±0.02 mg/100g) had the least. Vitamin B2 in the mushroom varied from 0.29±0.00 mg/100g in CF to 0.50±0.03 mg/100g in CF+MS. The substrate combination MS+SB produced the highest vitamin B3 Content (2.81±0.01 mg/100g) and the substrate CF gave the least vitamin B3 content (1.72±0.02 mg/100g). The substrate CF (8.33±0.11 mg/100g) had the highest vitamin A content while the least was observed in the substrate SB (5.77±0.11 mg/100g). Also, the substrate MS (8.64±0.00 mg/100g) had the highest vitamin C content while the least was observed in the substrate CF (7.57±0.13 mg/100g).

Table 1. Effect of substrates on the vitamin composition of *Hypsizygus ulmarius* (mg/100g)

Substrates	B1	B2	B3	A	C
CF	0.89^{b}±0.00	0.29^{d}±0.00	1.72^{g}±0.02	8.33^{a}±0.11	7.57^{d}±0.13
MS	0.78^{c}±0.02	0.40^{b}±0.02	2.16^{c}±0.00	7.40^{c}±0.00	8.64^{a}±0.00
SB	0.94^{a}±0.01	0.31^{cd}±0.01	1.78^{f}±0.00	5.77^{f}±0.11	8.02^{c}±0.11
CF+MS	0.81^{c}±0.01	0.50^{a}±0.03	2.03^{d}±0.03	7.90^{b}±0.00	8.33^{b}±0.18
CF+SB	0.93^{a}±0.01	0.34^{c}±0.00	1.86^{e}±0.06	6.92^{d}±0.09	7.62^{d}±0.00
MS+SB	0.88^{b}±0.01	0.35^{c}±0.01	2.81^{a}±0.01	6.33^{e}±0.18	8.30^{b}±0.07
CF+MS+SB	0.91^{ab}±0.02	0.35^{c}±0.02	2.33^{b}±0.00	7.55^{c}±0.10	8.28^{b}±0.13

Values are mean ± SD. Means on the same column with different superscripts are significantly different ($p<0.05$).

3.2. Mineral Composition

The effect of substrates on the mineral composition of *Hypsizygus ulmarius* (Table 2) showed that the substrate CF (290.51±0.48 mg/100g) produced the highest calcium content and the least was observed by the substrate MS (198.36±0.00 mg/100g). Calcium content indicated that there was significant difference at $p<0.05$ in fruit-bodies harvested from each substrate. Magnesium content varied with the highest magnesium content (144.44±0.40 mg/100g) produced by the substrate SB while the least magnesium content was observed in the substrate MS (72.00±0.00 mg/100g). The sodium content varied from 6.72±0.52 mg/100g (MS) - 9.36±0.16 mg/100g (CF). The highest potassium content was observed in the substrate CF (395.63±1.20 mg/100g) while the least was observed in the substrate combination CF+SB (314.41±1.16 mg/100g). Furthermore, the phosphorus content in the mushroom showed that the substrate CF (195.23±1.21 mg/100g) had the highest and the least phosphorus content was observed in the substrate SB (152.17±1.53 mg/100g).

Table 2. Effect of substrates on the mineral composition of *Hypsizygus ulmarius* (mg/100g)

Substrates	Calcium	Magnesium	Sodium	Potassium	Phosphorus
CF	290.51^{a}±0.48	108.73^{e}±0.48	9.36^{a}±0.16	395.63^{a}±1.20	195.23^{a}±1.21
MS	198.36^{g}±0.00	72.00^{g}±0.00	6.72^{d}±0.52	328.39^{e}±0.98	165.32^{e}±0.00
SB	272.97^{c}±0.95	144.44^{a}±0.40	7.28^{c}±0.00	318.04^{g}±0.59	152.17^{g}±1.53
CF+MS	203.69^{f}±1.71	100.89^{f}±1.11	7.62^{c}±0.00	323.11^{f}±0.00	157.54^{f}±0.22
CF+SB	280.93^{b}±1.07	116.41^{d}±0.73	8.26^{b}±0.09	314.41^{d}±1.16	171.80^{d}±0.66
MS+SB	234.66^{e}±0.00	128.80^{b}±0.00	9.02^{a}±0.00	370.10^{b}1.38	181.99^{b}±0.83
CF+MS+SB	240.30^{d}±1.10	124.38^{c}±0.00	8.84^{a}±0.11	366.75^{c}±0.37	177.20^{c}±0.27

Values are mean ± SD. Means on the same column with different superscripts are significantly different ($p<0.05$).

3.3. Heavy Metal Composition

The result of substrates effect on the heavy metal of *Hypsizygus ulmarius* fruit- bodies (Table 3) showed that the highest zinc concentration (2.40±0.14 mg/kg) was observed in the substrate CF while the least zinc concentration (1.13±0.11 mg/kg) was produced by the substrate MS. The iron content varied from 6.28±0.25 mg/kg in SB to 16.98±0.18 mg/kg in MS. Lead concentration ranged from 0.17±0.00 mg/kg in MS+SB to 0.25±0.01 mg/kg in CF. The substrate combination CF+SB had the highest cadmium concentration (0.15±0.01 mg/kg) while the least was produced by the substrate MS (0.09±0.00 mg/kg). Also, the substrate CF (0.21±0.03 mg/kg) recorded the highest copper concentration while the least was observed in the substrate MS (0.12±0.01 mg/kg).

Table 3. Effect of substrates on the heavy metal composition of *Hypsizygus ulmarius* (mg/kg)

Substrates	Zinc	Iron	Lead	Cadmium	Copper
CF	$2.40^{a}\pm0.14$	$7.25^{d}\pm0.00$	$0.25^{a}\pm0.01$	$0.13^{bc}\pm0.00$	$0.21^{a}\pm0.03$
MS	$1.13^{d}\pm0.11$	$16.98^{a}\pm0.18$	$0.23^{b}\pm0.01$	$0.09^{e}\pm0.00$	$0.12^{d}\pm0.01$
SB	$1.60^{c}\pm0.00$	$6.28^{e}\pm0.25$	$0.18^{cd}\pm0.01$	$0.14^{ab}\pm0.01$	$0.17^{bc}\pm0.00$
CF+MS	$1.85^{b}\pm0.07$	$8.60^{c}\pm23$	$0.24^{ab}\pm0.00$	$0.11^{d}\pm0.00$	$0.18^{ab}\pm0.00$
CF+SB	$1.78^{bc}\pm0.12$	$6.30^{e}\pm0.00$	$0.19^{c}\pm0.00$	$0.15^{a}\pm0.01$	$0.20^{a}\pm0.01$
MS+SB	$1.33^{d}\pm0.11$	$13.98^{b}\pm0.08$	$0.17^{e}\pm0.00$	$0.12^{cd}\pm0.00$	$0.14^{cd}\pm0.00$
CF+MS+SB	$2.00^{bc}\pm0.00$	$14.15^{b}\pm0.00$	$0.24^{ab}\pm0.00$	$0.12^{d}\pm0.00$	$0.18^{ab}\pm0.00$

Values are mean ± SD. Means on the same column with different superscripts are significantly different ($p<0.05$)

3.4. Proximate Composition

The result obtained from the proximate composition of the fruit- bodies of *Hypsizygus ulmarius* (Table 4) showed that there was variation in protein content. The highest protein content (24.50±0.00%) was observed in the substrate CF and SB (19.25±0.00%) had the least. The substrate MS and MS+SB had the highest fat content (4.20±0.00%) while the least was obtained from the substrate CF (3.80±0.00%). Fibre content of the mushroom varied from 14.98±0.32% in CF to 19.73±0.3% in SB. The moisture content ranges from 4.33±0.10% in CF+MS+SB to 6.28±0.07% in CF. The substrate CF+MS (43.25±0.00%) produced the highest carbohydrate content while the least was observed in the substrate MS (39.59 ± 0.06%).

Table 4. Effect of substrates on the proximate composition of *Hypsizygus ulmarius* (%)

Substrates	Protein	Fat	Fibre	Ash	Moisture	Carbohydrate
CF	$24.50^{a}\pm0.00$	$3.80^{d}\pm0.00$	$14.98^{f}\pm0.32$	$7.96^{e}\pm0.00$	$6.28^{a}\pm0.07$	$40.74^{d}\pm0.11$
MS	$22.05^{c}\pm0.21$	$4.20^{a}\pm0.00$	$18.79^{c}\pm0.16$	$10.50^{a}\pm0.00$	$4.88^{cd}\pm0.11$	$39.59^{e}\pm0.06$
SB	$19.25^{f}\pm0.00$	$4.00^{a}\pm0.00$	$19.73^{a}\pm0.39$	$10.00^{b}\pm0.00$	$5.44^{b}\pm0.00$	$41.65^{b}\pm0.32$
CF+MS	$22.83^{b}\pm0.11$	$4.10^{ab}\pm0.14$	$16.40^{e}\pm0.00$	$8.63^{d}\pm0.18$	$4.80^{cd}\pm0.14$	$43.25^{a}\pm0.00$
CF+SB	$21.37^{d}\pm0.16$	$3.85^{cd}\pm0.07$	$17.93^{d}\pm0.11$	$10.40^{a}\pm0.07$	$5.00^{c}\pm0.00$	$41.46^{bc}\pm0.20$
MS+SB	$20.40^{e}\pm0.00$	$4.20^{a}\pm0.00$	$19.33^{ab}\pm0.11$	$10.10^{b}\pm0.14$	$4.73^{d}\pm0.11$	$41.05^{cd}\pm0.35$
CF+MS+SB	$21.35^{d}\pm0.00$	$3.95^{bc}\pm0.07$	$18.90^{bc}\pm0.14$	$9.58^{c}\pm0.18$	$4.33^{e}\pm0.10$	$41.85^{b}\pm0.04$

Values are mean ± SD. Means on the same column with different superscripts are significantly different ($p<0.05$).

3.5. Bioactive Compounds

The phytochemical screening of *Hypsizygus ulmarius* fruit-bodies (Table 5) indicated that there were variations among the different bioactive compounds. The substrate combination MS+SB (0.74±0.00 mg/g) had the highest tannin content while the least was observed in the substrate combination CF+MS (0.56±0.01 mg/g). The alkaloid content ranged from 4.80+0.28 mg/g in SB to 7.90+0.14 mg/g in CF. Phenolic content varied from 2.82±0.00 mg/g in CF±MS to 4.55±0.07 mg/g in SB. The flavonoid varied with the substrate CF (4.30±0.14 mg/g) producing the highest and the least were observed by the substrate SB (3.80±0.00 mg/g). Also, the steroid content varied from 0.16±0.00 mg/g (MS) to 0.75±0.04 mg/g (CF). The hydrogen cyanide (HCN) content equally varies from 0.01±0.00 mg/g (SB) to 0.07±0.01 mg/g (CF+MS+SB). Anthocyanin content ranged from 0.22±0.04 mg/g (MS) to 0.35±0.00 mg/g (CF). The substrate MS (10.00±1.41 mg/g) produced the highest saponin concentration and the least was observed in the substrate SB (2.60±0.28 mg/g). Saponin content further indicated that there was a significant difference in MS when compared to other substrates.

Table 5. Effect of substrates on the Phytochemical Composition of *Hypsizygus ulmarius* (mg/g)

Substrates	Tannin	Alkaloid	Phenol	Flavonoid	Steriod	HCN	Anthocyanin	Saponin
CF	$0.67^{e}\pm0.00$	$7.90^{a}\pm0.14$	$4.26^{b}\pm0.00$	$4.30^{a}\pm0.14$	$0.75^{a}\pm0.04$	$0.03^{cd}\pm0.01$	$0.35^{a}\pm0.00$	$3.00^{c}\pm0.00$
MS	$0.64^{d}\pm0.00$	$5.00^{d}\pm0.28$	$3.06^{e}\pm0.00$	$4.05^{bc}\pm0.07$	$0.21^{e}\pm0.00$	$0.03^{bc}\pm0.00$	$0.22^{d}\pm0.04$	$10.00^{a}\pm1.41$
SB	$0.63^{d}\pm0.00$	$4.80^{d}\pm0.28$	$4.55^{a}\pm0.07$	$3.80^{d}\pm0.00$	$0.16^{f}\pm0.00$	$0.01^{e}\pm0.00$	$0.30^{bc}\pm0.00$	$2.60^{c}\pm0.28$
CF+MS	$0.56^{f}\pm0.01$	$6.00^{c}\pm0.00$	$2.82^{f}\pm0.00$	$3.90^{cd}\pm0.00$	$0.45^{c}\pm0.03$	$0.02^{de}\pm0.01$	$0.28^{bc}\pm0.01$	$4.00^{c}\pm0.28$
CF+SB	$0.71^{b}\pm0.01$	$7.00^{b}\pm0.14$	$3.30^{d}\pm0.14$	$4.15^{ab}\pm0.07$	$0.57^{b}\pm0.00$	$0.04^{b}\pm0.00$	$0.32^{a}\pm0.00$	$2.80^{c}\pm0.00$
MS+SB	$0.74^{a}\pm0.00$	$6.00^{c}\pm0.00$	$4.06^{bc}\pm0.05$	$4.00^{bc}\pm0.00$	$0.33^{d}\pm0.01$	$0.06^{a}\pm0.01$	$0.26^{c}\pm0.00$	$6.30^{b}\pm0.14$
CF+MS+SB	$0.59^{e}\pm0.00$	$7.30^{b}\pm0.14$	$2.94^{ef}\pm0.03$	$4.10^{b}\pm0.00$	$0.18^{ef}\pm0.00$	$0.07^{a}\pm0.01$	$0.31^{b}\pm0.02$	$5.55^{b}\pm0.14$

Values are mean ± SD. Means on the same column with different superscripts are significantly different ($p<0.05$).

4. Discussion

The mushroom *Hypsizygus ulmarius* exhibited a good profile of vitamins which include: B1 (thiamin), B2 (riboflavin), B3 (niacin), A (retinol) and C (ascorbic acid) cultivated on different substrates. This report agrees with Isikhuemhen *et al.*, 2000; Mattila *et al.*, 2002 and Okwulehie *et al.*, 2014

where they observed higher vitamin content in other mushroom species and reported that mushrooms are sources of thiamin, riboflavin, niacin, ascorbic acid and biotin [24, 36, 49]. These vitamins are essential in human diet and for the body and can be produced from easily available agro-wastes [51]. Vitamin B1 content (0.78 - 0.943mg/100g) was observed to be relatively higher than those obtained in vegetables (0.01 -0.12), eggs (trace - 0.04), cereal (0.29 - 0.33), fruit (0.02 - 0.07) [15]. The result obtained for vitamin B1 is in agreement with Nakalembe *et al*. (2015) where they observed vitamin B1 content ranging from 0.05 and 0.94 mg/l00g [38]. This mushroom (*Hypsizygus ulmarius*) can be a source of adequate vitamin B1 to all class of people except during pregnancy and lactation [16]. Riboflavin concentration (0.29-0.503mg/100g) was higher than that of fruits (0.01-0.05mg/100g), common vegetables (0.01-0.3mg/100g), and most common cereals (0.11-0.18 mg/100 g) [15]. Niacin (1.72-2.81mg/100g) is an important vitamin in human diet and it has been reported that 1-5g of niacin everyday helps to regulate blood cholesterol [29]. Again, the mushroom had a good profile for vitamin C and A concentration. It has been reported that vitamin C possess both antioxidant and therapeutic properties [4]. It acts on cicatrizing wounds, collagen synthesis, skin lightener [20, 30]. On the other hand, deficiency in Vitamin A causes night blindness, rough and peeling skin, dry mucous membranes, growth inhibition, reduced resistance to infections, defects in bone development and modulation [8].

Mushrooms are known to contain an appreciable amount of Calcium (Ca), Magnesium (Mg), Sodium (Na), Potassium (K) and Phosphorus (P) and they are essential in human nutrition [48, 49]. In human, these elements play important role in building up strong bone and teeth, repairing worn out tissues and maintains osmotic balance [65]. Calcium, Magnesium, Potassium and Phosphorus were abundant in this edible mushroom studied. Potassium was the predominate element among the minerals investigated. The high mineral content of *Hypsizygus ulmarius* can be linked to the nature of the substrate, their ability to utilize the nutrients in the different substrate, environmental condition [21, 41]. The mineral content observed in this mushroom were higher than those reported for other mushroom species [40, 45]. Also, low concentration of Sodium was observed thus making it useful for patients with certain kind of kidney and heart diseases as well as hypertensive patients [14, 57]. *Hypsizygus ulmarius* had a high concentration of Magnesium and has been reported to play essential role in tissue maintenance and lymphoid cells. It also acts as co-factor of enzymes in various metabolic activities and in innate and acquired immune response [32]. Phosphorus is required by all cells of the body for proper functioning. They are efficiently absorbed from the gastrointestinal tract and available in most foods, hence less important in diet planning [64].

The result further revealed that there was variation in heavy metal concentration of *Hypsizygus ulmarius* fruit bodies cultivated in different substrates. Zinc is an important heavy metal and are widely recognized and used in medicine for the treatment of dandruff, athlete's foot, rashes and acnes, food and in industries to prevent corrosion. It is an essential element required by the body mainly because they are associated with carbohydrate and protein food [48]. It has also been documented that zinc deficiency can result in excessive excretion, inadequate dietary intake and impaired absorption while in children; it can result in growth retardation, weakness, loss of appetite and low spirited [25]. Iron was found to be the most concentrated heavy metal in mushroom grown on all the substrates when compared to other heavy metal. Iron is essential for almost all living things (plants and animals) and involving in a wide range of metabolic processes. It has been reported that sufficient amount of iron leads to a decrease in the incidence of anemia [31]. Lead is considered to be detrimental and serves as a foremost compound in paints and drug discovery. Lead causes health problems to include high blood pressure, miscarriages, kidney infection and anemia, tiredness, sleeplessness, weight loss and hearing impairment [48, 67]. Low concentration of Cadmium was observed; which is an indication that the mushroom is suitable for consumption. It has been documented that cadmium is a very toxic element, its presence at high level (> 0.49mg/kg) in the soil or drinking water is a threat to human health as they give rise to pulmonary, skeletal, hepatic, renal and reproductive effect and cancer and they attack mainly the kidney, spleen and liver [27, 67]. Lead and cadmium has been reported to be the most toxic elements and affect many biological processes [48]. This heavy metal can be absorbed through water and dietary food and can be absorbed by both plants and animals [9]. Low concentration of Lead (0.17-0.25mg/kg) and cadmium (0.09-0.15mg/kg) were observed in the mushroom. However, the values obtained for these elements (Lead and Cadmium) falls within the FAO/WHO (1996) permissible limit; Lead (1.5-1.75mg/kg) and cadmium (0.42-0.49mg/kg) [13]. Copper plays a vital role in human body through the protection of the skeleton, cardiovascular and nervous system [38]. The copper content (0.14-0.21mg/kg) of the mushroom was quite low compare to the copper content of other mushroom in the literature which ranges from 12–181mg/kg [63], 13.4–50.6mg/kg [60] and 10.6–144.2mg/kg [66].

Proximate analyses indicated the presence of protein, fat, fibre, ash, moisture and carbohydrate in all the samples but in different levels and it shows that *Hypsizygus ulmarius* is a good source of nutrients. The protein content ranges from

19.25 to 24.50% and this result agrees with the protein content reported for another species which was found to be in the ranged of 18.41 to 25.81 [50]. Also, the carbohydrate content was high. Protein and carbohydrate are important and serves as source of life, energy and are suitable for diet formulation. Mushroom contains high quantity of protein and carbohydrate which is essentially demanded by both man and animal [48]. Low fat concentration was observed in the fruit bodies harvest from the different substrates. This is an indication that the mushroom can be recommended for people with cardiac problems [44, 47]. *Hypizygus ulmarius* had a good profile of fibre content and could be a good source of dietary fibre. There were variations in ash and moisture content; the variation in moisture content may be as a result of the environmental conditions during growth and storage and the amount of metabolic water produced during storage [35]. It was observed that all the substrates produce fruit- bodies that contained appreciable quantity of protein, carbohydrate, fibre and ash. This fact confirms the nutritional quality of other mushroom species [22, 50, 51].

A high level of alkaloids, phenols, flavonoids and saponins were observed among the phytochemicals and is a clear indication of the medicinal importance of the mushroom. The presence of tannin content in the mushroom shows astringent properties which help to hasten healing of wounds and inflamed mucous membrane [46]. Tannin also play a role in inhibiting pathogenic fungi [47, 52]. Alkaloids have been reported to have impacts in animal physiology and in pharmacological activities [12]. It has been report to have stimulating effect, acts as pains reliever, antipuretic action and topical anaesthetic in Ophthalmology [12]. Phenols have proven to be a useful constituent of most disinfectants and antiseptics. The antifungal, therapeutic and antiseptic properties of a mushroom species can be as a result of the presence of phenolic compound [17]. Hamzah *et al*. (2013) reported that phenols have a wide range of medicinal (anti-diabetic, anticancer and anti-inflammatory effects) and antioxidant properties [19]. Also, flavonoids when detected in a mushroom indicate its medicinal importance. It possesses antioxidant properties against free radical scavengers, antivirals, anti-inflammatory, antiallergenic, vasodilating actions and also has good anticancer properties [46, 55, 56]. The mushroom produced little quantity of hydrogen cyanide and anthocyanin, thus making it safe for consumption. The result is in agreement with Okwulehie *et al.* (2017), where they observed low concentration in hydrogen cyanide and anthocyanin [53]. High concentration of hydrogen cyanine in the blood of a patient with kidney diseases may be associated with encephalopathy [1]. The richness of saponin in a mushroom indicates its importance both in pharmaceutical and medicine industries; especially for it foaming nature that produces effect in the food industry [46]. It has been reported that saponin prevent disease invasion by parasitic fungi [47, 52]. In addition, saponin also has other effect which includes: anti-bacterial, antifungal, ichthyotoxic and antieoplastic activities [5, 23].

5. Conclusion

The result revealed that *Hypsizygus ulmarius* has a good profile for vitamins, minerals, proximate, heavy metals and bioactive compounds. Also, heavy metal such as cadmium and lead which are considered to be very toxic to human health were quite low thus, making the mushroom fit for consumption.

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