# Constituents, nutrient content, *in vitro* antioxidant and anti-inflammatory activity of *Tricholomopsis aurea* (Tricholomataceae)

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**ABSTRACT:** *Tricholomopsis aurea* is used as food in different parts of the world, but has not been investigated for its nutrients, metabolites, and biological potentials like other edible mushrooms. This work aimed to quantify the metabolic and nutrient content of *T. aurea* and evaluate the antioxidant and anti-inflammatory activities of the extract and isolated compounds. The method employed involves chromatographic, spectroscopic, Bovine Serum Albumin microplate, analytical and standard assays. Oleic, elaidic, petroselinic acids, ergosterol, ergosterol peroxide, 22E724R)-24-ethylcholesta-5,7,22-trien-3β-ol, and adenosine were isolated and identified using 1D and 2D-NMR spectroscopy and spectrometric data. The metabolic content revealed high phenolics (799.62 µgGaE/g of dry matter (DM)), low flavonoids (24.54 µgQE/g DM), alkaloids (32.92 µgQiE/g DM), and saponins (88.00 µgQSE/g DM). The nutrients content was made up of proteins (4.79%), lipids (10.43%), fibers (16.01%), ashes (15.96%), carbohydrates (8.74%), dry matter (85.93%), and moisture (14.07%) with energy value of 362.89 kcal. In mg/100g, the minerals were phosphorus (283.97%), calcium (817.25%), potassium (67.10%), magnesium (94.42%), iron (57.27%), and sodium (74.4%). The extract displayed the antioxidant activity against TAC and FRAP (100-1000 µg/mL), DPPH ($SC_{50}$ of 248.95 µg/mL) and ABTS ($SC_{50}$ of 180.7 µg/mL), while the test compounds were not active. The extract, adenosine, ergosterol peroxide, and ergosterol showed anti-inflammatory activity with $IC_{50}$ of 49.19 µg/mL, 4.91 µg/mL, 6.85 µg/mL, and 29.51 µg/mL, respectively. Conclusively, this study will help to promote the application of *T. aurea* in traditional dishes and functional or nutraceutical foods.

**KEY WORDS:** *Tricholomopsis aurea*, mushroom, constituents, nutrients, food, antioxidant, anti-inflammatory

**ABBREVIATIONS**: **NMR,** Nuclear Magnetic Resonance, **HEE,** hydro-ethanolic extract, **MS,** mass spectrometry, **TLC,** thin layer chromatography, **Rf,** retention factor, **UV,** ultra violet, **BSA,** bovine serum albumin

## I. INTRODUCTION

Edible mushrooms are incorporated into functional foods because they contain dietary supplements or phytochemicals that have important beneficial effects on health.[1,2] The phytochemical content of mushrooms include organic acids, tocopherols, phenols, saponins, alkaloids, tannins, flavonoids, and glycosides.[3,4] And the supplements encompass vitamins, amino acids, proteins, carbohydrates, dietary fibers, and essential minerals.[5,6] Apart from antioxidant properties, edible and medicinal mushroom species have been reported to exhibit anti-inflammatory, analgesic, antibacterial, and antitumor activities.[7,8,9] As concerns the genus *Tricholomopsis*, previous works on the fruiting body of *T. rutilans* resorted to the identification of myristic acid, palmitoleic acid, oleic acid, linoleic acid, palmitic acid, stearic acid, pentadecanoic acid, L-3- (3-carboxy-4-furyl) alanine; 20, 3β,5α-dihydroxy-(22E,24R)-ergosta-22-en-7-one-6β-yl oleate, and 3β,5α-dihydroxy-(22E,24R)-ergosta-7,22-dien-6β-yl oleate.[10,11] The analysis of its nutritional content proved the presence of Copper (Cu), Iron (Fe), Magnesium (Mg), Manganese (Mn), Zinc (Zn), and Sodium (Na).[12] The evaluation of its methanolic extract against Gram positive bacteria demonstrated minimal or no antibacterial effects.[13] The species *Tricholomopsis aurea* (Beeli) Desjardin & B.A. Perry is a wild edible fungus that is scarce in Cameroon but common in the Democratic Republic of Congo, Burundi, Tanzania, Zimbabwe, São Tomé, Togo, Uganda, Martinique, Guadeloupe, and Trinidad.[14,15] Though *T. aurea* is used as food in different parts of the world, there is lack of literature data on its consumption in Cameroon. In addition, *T. aurea* has not been investigated for its nutrients, metabolites, and biological potentials like it is with other edible mushrooms. To add to the knowledge on *Tricholomopsis* genus, the nutritive value and biological potentials of this species is important to support its recommendation as food ingredient in various dishes;[16] and its inclusion in functional food or nutraceutical food. Therefore, this study aims to quantify the metabolic and nutrient contents of *T. aurea.* In addition, isolate and identify constituents from the extract using chromatographic separation methods, and nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), then evaluate the antioxidant and anti-inflammatory potential of the extract and some of the compounds by standard assays.

## II. Materials and methods

### A. Sample collection and description

## 1. Sample collection

*T. aurea* was collected on a dead cypress stump on 10th April 2022, in Bambui (Northwestern region of Cameroon) and identified in the Mycological Laboratory at the Department of Plant Biology and Physiology, University of Yaoundé 1 in Cameroon, by comparing the macro- and micromorphological features of the samples with those described in the literature by Desjardin and Perry.[15] The sample was preserved in the Mycological Herbarium of the University of Yaoundé 1 under voucher specimen number HUY1-DM 1937.

## 2. Description of sample

The specimens of *T. aurea* collected in Cameroon are characterized by a bright to sulphur yellow basidiomata, measuring 25 – 53 mm diameter, the conspicuously rather flattened, fistulose to hollow and centrally depressed (from top to bottom) stipe measuring 35 – 55 mm long and 6.0 – 10 mm diameter, thin flesh, white spore-prints, the basidia of dominantly four (4) and two (2), but also one (1) sterigmata, the presence of cheilo- and absence of pleurocystidia, and cutis type pileipellis with erected hyphae which are subclylindrical to subclavate at the apex, some of which incrusted. This description fully matches that of Desjardin and Perry.[15]

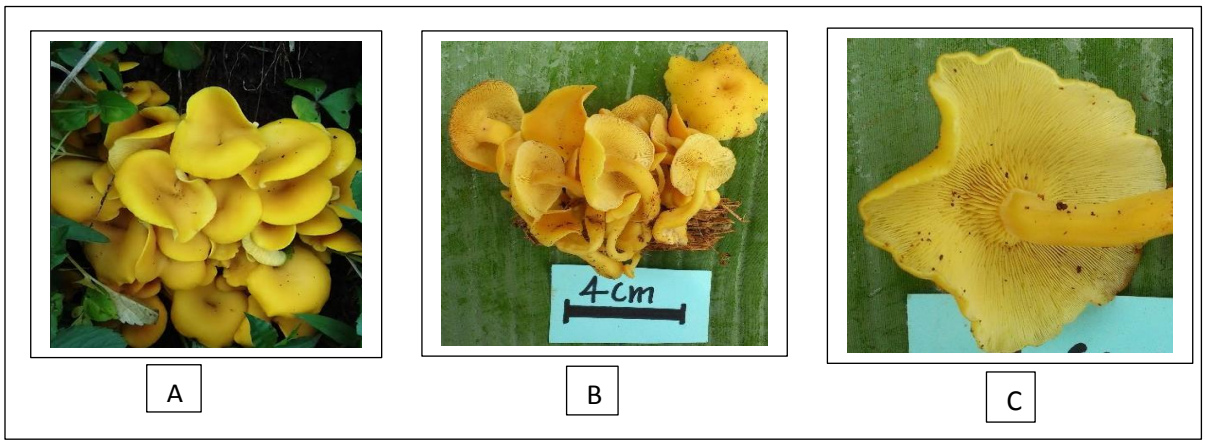


**Figure 1.** *Tricholomopsis aurea*: **A)** Grouped growth mode of the fruiting body on substrate (dead cypress tree), **B)** Upper and lower surface of the sample, **C)** Hymenophore with lamellae and tight lamellae and method of insertion of the lamellae on the stipe.

## 3. Extraction

The mushroom sample was washed to remove all sort of waste before carrying out the different analysis. For the isolation, the dried fruiting body (250 g) was blended and extracted with the mixture (EtOH-$H_2O$, 7:3 v/v) for 72 hours. The filtrate obtained was evaporated at reduced pressure affording a brown paste (25 g). Of these, 16 g was purified over silica gel column chromatography (length 74.0cm, diameter 3.8cm) eluted with n-hexane (n-Hex) and ethyl acetate (EtOAc) of increasing polarity and methanol (MeOH). 157 fractions of 100 mL each were collected as follows: n-Hex 100% (1-20); n-Hex-EtOAc (93:7 (21-29); 95:5 (30-36); 88:12(37-40); 82:18 (41-47); 76:24 (48-60); 72:28 (51-65); 68:32 (66-80); 60:40 (81-88); 50:50 (89-96)); EtOAc 100% (97-117); and EtOAc-MeOH (95:5 ( 118-125), 90:10 (126-135), 80:20 (135-157)). These fractions were matched based on their thin layer chromatography (TLC) profiles. The precipitates from fractions F48-49 (n-Hex-EtOAc, 74:26), F50-60 (n-Hex-EtOAc, 76:24), and F69-72 (n-Hex-EtOAc, 68:32) were washed with a mixture of n-Hex-EtOAc 3% to yield (22E724R)-24-ethylcholesta-5,7,22-trien-3β-ol (6.5 mg, Rf 0.31), ergosterol (18.5 mg, Rf: 0.30), and ergosterol peroxide (8 mg, Rf: 0.25), respectively. Fractions F30-37 (50 mg) was rechromatographed over a silica gel column (length 42.0cm, diameter 2.0cm) using an increasing gradient system of n-Hex-EtOAc (97:3, 94:6, 88:12, 85:15, 82:18, 80:20, 70:30) to yield 54 subfractions of 10 mL each. The subfraction SF12-14 (n-Hex-EtOAc 88:12) and SF15-18 (n-Hex-EtOAc 85:15) afforded petroselinic acid (5 mg, Rf: 0.60) and elaidic acid (15mg, Rf: 0.60), respectively. And subfraction SF20-23(82:18) yielded (Z)-oleic acid (12 mg, Rf: 0.73). A portion of fraction F136-138 (22 mg) was purified on a silica gel column (length 50.0cm, diameter 1.5cm) using an isocratic system of EtOAc-MeOH-$H_2O$-pyridine (10:4:1:0.5). 20 fractions of 10 mL each were collected and subfraction SF6-8, afforded adenosine (12 mg, Rf: 0.36).

## 4. Instruments used for isolation, analysis and characterization of compounds

The mass spectra were registered on a Waters ZQ-200 in electrospray ionization mode. NMR spectra were recorded on an AVANCE-NEO Bruker spectrometer equipped with a magnetic field of 14T operating at a proton frequency of 600 MHz. Low-resolution electrospray ionization mass spectrometry (ESI-MS) experiments were carried out on a Micromass Quattro Micro mass spectrometer. Column chromatography was performed on silica gel (0.04-0.063 nm; 230-400 mesh, ASTM; Merck, Germany). Thin layer chromatography (TLC) was performed on a silica gel

60 F254 (0.1 mm thick; Merck) with a size of 20 × 20 cm, and spots were detected by fluorescence at 254 nm or 366 nm on a UV-85/L basis. The samples were sprayed with 10% $H_2SO_4$, and then heated at 70°C. The solvents used were of analytical grade.

**B. Determination of total polyphenolic content (TPC), total flavonoid content (TFC), alkaloid content (AC) and total saponin content (TSC)**

The TPC was determined by the Folin–Ciocalteu method as described by Singleton and Rossi[17], while TFC of *Tricholomopsis aurea* HEE was quantified using the colorimetric method described by Aiyegoro and Okoh.[18] Alkaloids were estimated using the method of Singh et al.[19] whereas the TSC was quantified as reported in literature by Hiai et al.[20] All analysis were done in triplicates.

**C. Determination of nutrient content of mushroom**

The dried basidiocarp of *T. aurea* was milled to powder using a blender to afford a mass of 300 g, which was stored in airtight bottles at 4°C until use. The mushroom sample was analyzed for moisture content, crude fibers, lipids, proteins, carbohydrate, dry matter, and ashes. The moisture content expressed in mg/100g of dry matter (DM), was obtained by weighing after drying 10 g of the sample in an oven at $105^0$C until a constant weight was achieved.[21] Total lipids were extracted with hexane in a Soxhlet extractor as described by Bourely.[22] The total nitrogen obtained was converted to protein content by multiplying with a conversion factor of 5.7.[23] The ash content was obtained after incineration of a known weight of the dry sample in a furnace at 550$^o$C for 48 h.[24] The Crude fibers was obtained after hydrolysis of the dry sample in sulphuric acid (0.26 N $H_2SO_4$) and potassium hydroxide (0.23 N KOH) and incineration of the digest in a furnace at 550$^o$C for 3 h.[25] The dry matter content was determined via the oven drying method as described by Ache et al.[26] Carbohydrates content was through the difference between the weight of the dry sample and the sum of the crude fibers, lipids, proteins, and ashes weights.[25] The energy value was estimated using the Italian law of 1993 for proteins (x 4), carbohydrates (x 4) and fats (x 9). All the parameters (moisture content, proteins, lipids, carbohydrates, ash, crude fiber, and energy) were expressed in mg/100g of dry matter. The minerals include phosphorus, sodium, potassium, magnesium, calcium, and iron. The mushroom sample was heated at 450°C. The ash obtained was extracted with nitric acid (1N), followed by standardizing with respective elements. The values of the minerals were read on atomic absorption spectrophotometer (AAS). The limits of detection

(LOD) and limits of quantification (LOQ) for all analytes were calculated according to IUPAC recommendations. The analyses were done in triplicate.

### D. Assessment of antioxidant activity of extract and isolated compounds

The total antioxidant capacity (TAC) was determined by the phosphomolybdenum method as described by Prieto et al.[27] whilst the ferric iron ($Fe^{3+}$) reducing power (FRAP) of the extract was determined according to the method described by Oyaizu.[28] The antiradical DPPH (2,2-diphenyl-1-picrylhydrazyl) was performed using the method described in literature by Bassene.[29] And 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of the hydro-ethanolic extract was determined by the method of Khan et al.[30]

### E. Evaluation of *in vitro* anti-inflammatory activity of extract and compounds

The *in vitro* anti-inflammatory activity of *T. aurea* extract and compounds was carried out using the BSA denaturation based-assay following the protocol described by Prasad et al.[31]

### F. Statistical analysis

The data are expressed as the means ± SDs of three experiments using Microsoft excel 2013. Graphs were developed using STATGRPHICS centurion. The scavenging 50% reducing concentrations and the $IC_{50}$ values were determined using GraphPad prism 8.0.1(124).

## III. RESULTS AND DISCUSSION

### 1. Identification of isolated compounds

Seven known compounds were isolated from the fruiting body of *T. aurea by* chromatographic separation techniques. Their structures were determined by comparison of their NMR and Mass data with previous literature report as (Z)-oleic acid (1), petroselinic acid (2), elaidic acid (3), (22E724R)-24-ethylcholesta-5,7,22-trien-3β-ol (4), Ergosterol (5), Ergosterol 5α,8α-endoperoxide (6) and adenosine (7).[32,33,34,35,36,37] See Figure 2 and Supplementary material.

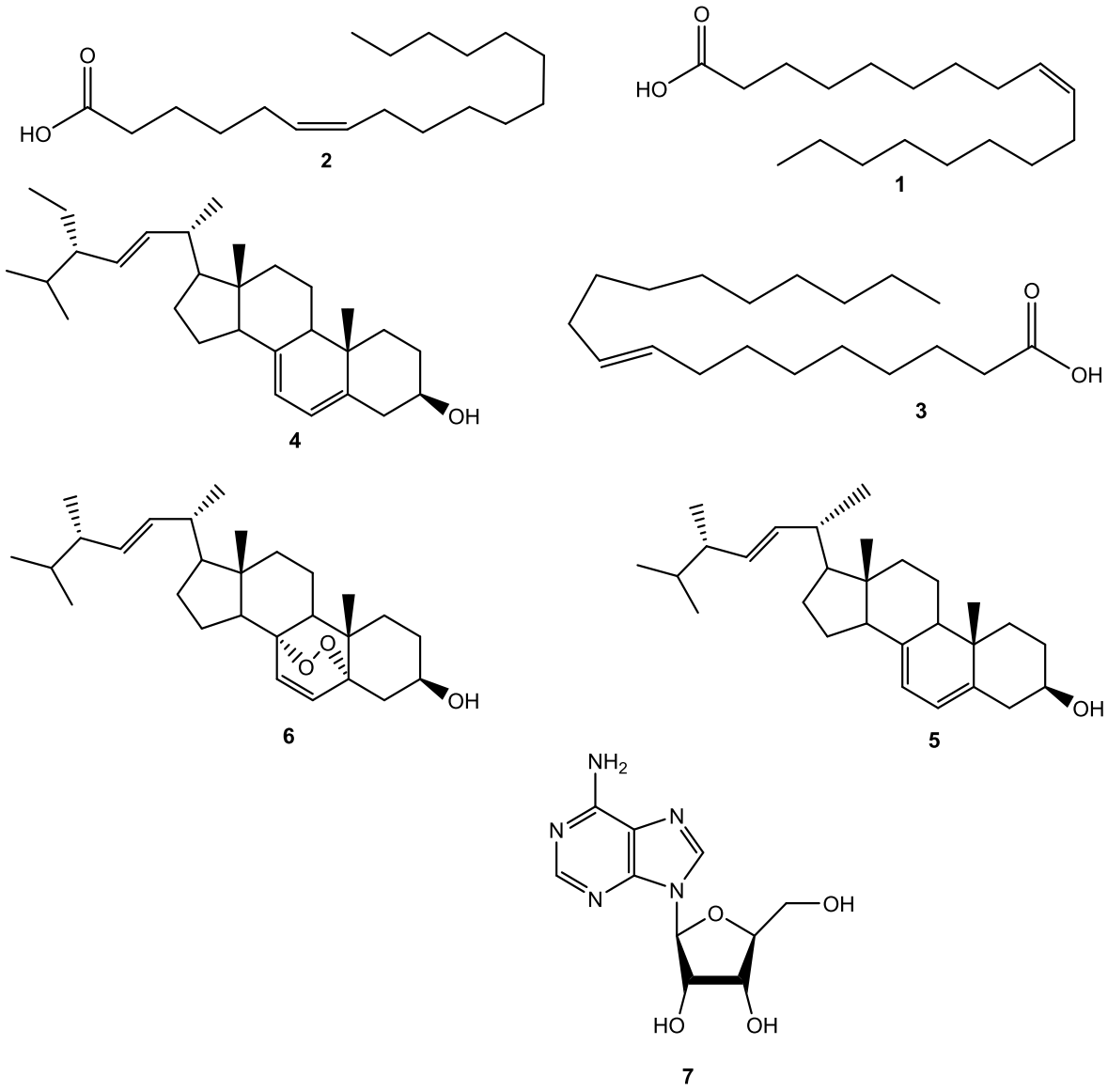


**Figure 2.** The constituents isolated from *T. aurea* hydro-ethanolic extract

### A. Phytochemical content

The present studies led to the isolation of one nucleoside and six lipids. However, literature findings revealed that mushrooms are a rich source of alkaloids, phenolics, saponins, flavonoids and other classes of compounds.[3,4] Thus, further investigation was done to quantitatively determine the presence of other metabolites in *T. aurea* as shown in Table 1.

**Table 1:** The phytochemical content of the hydro-ethanolic extract of *T. aurea*

| Sample | Phytochemical class | *T. aurea* |
|---|---|---|
| HEE | Polyphenols (µgGaE/g DM) | 799.62 ± 35.93 |
| | Flavonoids (µgQE/g DM) | 24.54 ± 6.57 |
| | Alkaloids (µgQuiE/g DM) | 32.92 ± 2.44 |
| | Saponins (µgQSE/g DM) | 88.00 ± 0.00 |

Legend: HEE: *Tricholomopsis aurea* hydro-ethanolic extract; µgGaE: microgram Gallic acid Equivalent; µg QE: microgram Quercetin Equivalent; µgQuiE: microgram Quinine Equivalent; µgQSE: microgram Quillaja Saponin Equivalent; DM: Dry Matter

### B. Total phenolic content (TPC)

The TPC was expressed in micrograms of gallic acid equivalent per gram of dry matter (µg GaE/g DM) using the following equation obtained from the calibration curve: $Y = 0.0014x-0.0058$, $R^2 = 0.9982$, where x is the gallic acid equivalent (GaE) and Y is the optical density. The findings demonstrated that the TPC in the HEE of *T. aurea* (799.62 ± 35.93 µgGaE/g DM), was lower than that in the 80% ethanolic extract of *Boletus edulis* (35.56 mgGaE/g), *Pleurotus ostreatus* (6.27 mg GaE/g), and *Lentinula edodes* (4.94 mgGaE/g).[38] The presence of phenolic compounds in mushrooms as well as plants results in good scavenging ability, and the free hydroxyl groups of these compounds directly contribute to the antioxidative effects that are significant in stabilizing lipid peroxidation.[39,40,41]

### C. Total Flavonoid Content (TFC)

The TFC was determined in micrograms of quercetin equivalent per gram of dry matter (µgQE/g DM) using the following equation based on the calibration curve: $Y = 0.0032x - 0.0572$, $R^2 = 0.9901$, where x is the quercetin equivalent (QE) and Y is the optical density. Flavonoids often act as free radical scavengers, thus terminating the radical chain reactions that occur during the oxidation of triglycerides in the food system.[42] The TFC of *T. aurea* HEE reported herein was 24.54 ± 6.57 µgQE/g DM extract (Table 1). Comparatively, the value of our TFC was higher than that of the 50% ethanolic extracts of *Lentinus edodes* (1.64 ± 0.36 mgQE/g dw), *Volvariella volvacea* (9.05 ± 0.89 mgQE/g dw), and *Pleurotus eous* (1.51 ± 0.98 mgQE/g dw).[43]

**D. Alkaloid content (AC) and Saponin content (SC)**

As shown in Table 1, the AC was expressed in micrograms per quinine equivalent (µgQuiE/g DM) extract, by the following equation based on the calibration curve: $Y = 0.001x - 0.0062$, $R^2 = 0.9226$, where x is the quinine equivalent (QE) and Y is the optical density. The results indicated that the AC treatment (32.92 ± 2.44 µgQuiE/g DM) was higher than that of *Russula cyanoxantha* (15.1 mg/kg), *Amanita rubescens* (26.6 mg/kg), and *Boletus edulis* (92.4 mg/kg), on dry weight basis.[44] The SC was expressed in micrograms Quillaja saponin equivalent (µgQSE/g DM), using the following equation based on the calibration curve: $Y = 0.002x - 0.09$, $R^2 = 0.9157$, where x is the Quillaja saponin equivalent (QSE) and Y is the optical density. The concentration of SC (88.00 ± 0.00 µgQSE/g) DM was lower than that of *Agaricus bisporus* (0.121 ± 0.01 g/100g).[45] The detection of these compounds indicates *T. aurea* also contain flavonoids, phenolics, alkaloid, and saponins.

**2. Nutrient content of *Tricholomopsis aurea***

With respect to dry matter, as shown in Table 2, *T. aurea* had a value of 85.93 ± 0.60%, which was far higher than that reported in *Boletus edulis* (33.1%), *Marasmius oreades* (52.8%), *Polyporus dictyopus* (17.77%), and *Termitomyces microcarpus* (15.56%).[26,46] This difference might have been influenced by factors such as relative amount of metabolic water produced during storage and environmental factors during growth and storage.[47] The moisture content (14.07 ± 0.60%) for *T. aurea* was higher than that of *Pleurotus sajo-caju* Fr. (6.61 ± 0.10%) and *Lentinus squarosulus Mont*. (9.77 ± 0.01%).[48] However, our value was lower than that of *Termitomyces letestui* (92.76%) and *T. aurantiacus* (94.34%).[16] The low water content of *T. aurea* observed indicates it could easily be dried and preserved for a longer period.

The carbohydrate content in *T. aurea* (38.74 ± 2.66%) was higher than that of *Lactarius deliciosus* (6.26%) and *Tricholoma portentosum* (3.64%), and lower than in *Termitomyces letestui* (53.83 ± 0.64%), and *Psathyrella tuberculate* (46.25 ± 0.59%).[49,50] The differences in content between *T. aurea* and those observed by the latter could be due to the variable proportions of carbohydrate compounds present in the form of monosaccharides, disaccharides, and polysaccharides of different sizes.[51] However, the carbohydrate value observed in this studies indicates the mushroom is a good source of energy.

**Table 2:** Nutrient content of *Tricholomopsis aurea*

| **Parameters** | *T. aurea* |
|---|---|
| Ashes | 4.79 ± 0.09% |
| Lipids | 16.01 ± 0.46% |
| Dry matter | 85.93 ± 0.60% |
| Moisture content | 14.07 ± 0.60% |
| Crude proteins | 15.96 ± 2.10% |
| Carbohydrates | 38.74 ± 2.66% |
| Crude fibers | 10.43 ± 0.61% |
| Energy (Kcal/100g) | 362.89 ± 23.18 |
| **Elements** | |
| **P** (mg/100g DM) | 283.97 ± 1.53 |
| **Ca** (mg/100g DM) | 817.25 ± 1.5 |
| **K** (mg/100g DM) | 67.1 ± 0.1 |
| **Mg** (mg/100g DM) | 94.42 ± 0.24 |
| **Fe** (mg/100g DM) | 57.27 ± 0.41 |
| **Na** (mg/100g DM) | 74.4 ± 0.6 |

Legend: P: Phosphorus; Ca: Calcium; K: Potassium; Mg: Magnesium; Fe: Iron; Na: Sodium **DM**: Dry Matter; **g**: gram; **mg**: milligram.

*T. aurea* crude protein proportion (15.96 ± 2.10%) was substantially equal to that reported in *Psathyrella tuberculata* (15.95 ± 0.21%) and *Volvariella volvacea* (15.73 ± 0.11%).[50] However, our value was much lower than those of *Termitomyces heimii* (28.54 ± 0.09%) and *Termitomyces microcarpus* (31.05 ± 0.58%) and higher than that of *Agaricus bisporus* (9.1 ± 0.44%).[45,52] Therefore, mushrooms, including *T. aurea* as a protein source, could supplement low-protein diets. *T. aurea* lipid (crude fat) content (16.01 ± 0.46%) is far above the reported lipid proportions of mushroom species such as *Agaricus bisporus* (1.73 ± 0.33%), *Termitomyces eurrhizus* (6.27 ± 0.57%), and *Leucopaxillus giganteus* (3.4%).[45,49,52] The observed differences between our sample and the later could be due to the abundance of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs).[12,53] Also, the MUFAs isolated in the present studies corroborates the lipid content, thus indicating the mushroom is a food source with high calorific value. The crude fiber content (10.43 ± 0.61%) obtained from *T. aurea* was closer

to that of *Schizophyllum commune* (11.09 ± 5.13%) and lower than that of *Pleurotus djamor* (25.63 ± 3.15%).[52] Mushrooms are rich in fibers and play a major role in human health by facilitating digestion and the excretion of waste and toxins from the body.[16,54]

The ash content or mineral salts of *T. aurea* (4.79 ± 0.09%) was greater than that of *Agaricus arvensis* (0.18%) and *Lactarius deliciosus* (0.51%).[49] However, our value was lower than that of *Lentinus squarrosulus* (7.45 ± 1.09%) and *Termitomyces microcarpus* (17.09 ± 1.17%).[52] The major minerals in mushrooms are Na, Mg, Ca, P, and K along with minor components such as cadmium, molybdenum, iron, copper, and zinc. The mineral content are often influenced by type of specie, mushroom part, level of maturity and growth substrate.[55] The energy value of *T. aurea* (362.89 ± 23.18 kcal/100g) was closer to that of starch (363 kcal), and greater than that of whole grain of barely (327 kcal), *Termitomyces letestui* (323.15 ± 2.61 kcal/100 g), and breast meat (137.75 ± 4.71 kcal/100g).[50,56,57] However, our value was lower than that of *Termitomyces eurrhizus* (1138.70 ±5.33 kcal/100g) and raw pig meat (611.5 - 648.1 kcal/100g).[52,58] Based on the predicted calorific value, *T. aurea* could be considered an energy-rich food and could be integrated into low-calorie diets.

The presence of mineral salts such as Na, K, Mg, Ca, P, and Fe could help in the enrichment of traditional dishes. Table 2 shows the analysis of the macromineral content. Calcium had the highest content, and potassium had the lowest. The calcium content in *T. aurea* was 817.25 mg/100g DM, which is far above the recorded values of 214 ± 2.6 mg/100g DM in *Coprinus cinereus* and 38.45 mg/100g DM in *Termitomyces microcarpus.*[59,60] Calcium ion ($Ca^{2+}$) intake reduces fracture incidence in life and boost patients' dietary quality.[61]

On one hand, the potassium content was 67.1 mg/100g DM. This value is greater than those of *Pleurotus pulmonarius* (7.25 mg/100g) and *Volvariella volvacea* (1.58 mg/100g DM). However, our value is lower than that of *L. sulphurous* (433.62 ± 4.28 mg/100g) and *C. cinereus* (3232 ± 2.6 mg/100g).[26,59,60] K is an intracellular cation in the human body vital in membrane potential and electrical excitation of both nerve and muscle cells and acid-base regulation.[62]

On the other hand, the concentration of iron was 57.27 mg/100g DM, which is lower than the value of *Volvariella volvacea* (426 ± 2.4 mg/100g). Nevertheless, our value was greater than that of *T. rutilans* (55.729 mg/kg dw).[12,60] Fe is essential for the formation of hemoglobin and transport of oxygen.[63] For phosphorus, *T. aurea* concentration was 283.97 mg/100g DM. Our value was 3-4-

fold lower than the reported values of *Termitomyces microcarpus* (898.17±3.44 mg/100g DM) and *Coprinus cinereus* (1142 ± 3.0 mg/100g DM).[26,60] Phosphorus is a crucial plant nutrient for early growth and an essential component of signal transduction, bone mineralization, phospholipids, DNA, ATP, RNA, and creatine phosphate.[64,65,66]

The magnesium content was 94.42 mg/100g DM and was found to be equivalent to that of *Polyporus tenuiculus* (94.48 ± 4.14 mg/100g DM).[26] However, our value was greater than that of *T. rutilans* (754.605 mg/kg dw).[12] Magnesium is important for cellular processes such as DNA replication and repair, cell proliferation and signal transduction, potassium and calcium ion transport, and intermediary metabolism.[66] *T. aurea* sodium concentration was 74.4 mg/100g DM. This value is greater than that reported in *T. rutilans* (126.895 mg/kg dw) and *Termitomyces striatus* (2.31 ± 0.33 mg/100g DM).[12,26] Nonetheless, our value was lower than that of *Pleurotus flabellatus* (686 ± 3.0 mg/100g DM).[59] Sodium is highly involved in the maintenance of normal cellular homeostasis, the regulation of fluid and electrolyte balance, and blood pressure.[67]

### 3. Antioxidant activity of *T. aurea*

### A. Total antioxidant capacity (TAC)

TAC depends on the ability of an extract to reduce Mo (VI) to Mo (V) by the formation of a green phosphate/Mo (V) complex at acidic pH4.8. In the present study, Figure 3 shows that, the TAC ranged from 0.19 to 0.68 µg AAE/g DM and exhibited a concentration-dependent activity.

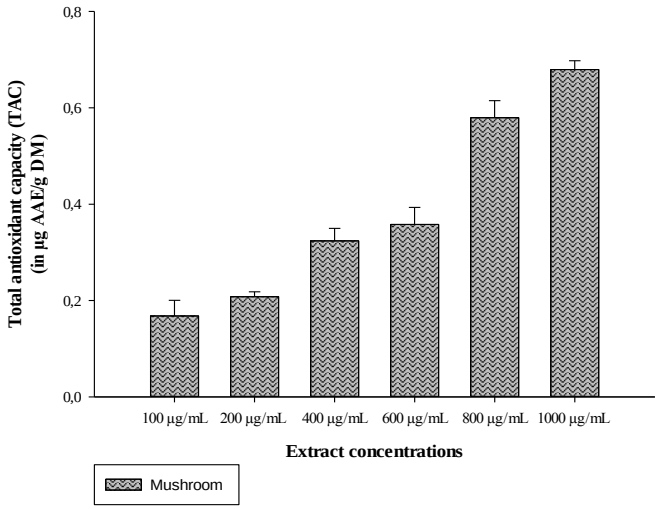


**Figure 3.** The Total Antioxidant Capacity of *T. aurea*

The highest activity was observed for the HEE of *T. aurea* at concentration 1000 µg/mL, followed by 800 µg/mL. Notably the activities at concentrations of 600 and 400 µg/mL, were statistically comparable ($P > 0.05$). Similarly, the extracts activity at 100 and 200 µg/mL, showed no significant difference ($P > 0.05$). The antioxidant capacity observed herein could be attributed to the total phenolic content of the mushroom extract. Therefore, these findings highlights the antioxidant potential of the mushroom room extract which may be beneficial in managing oxidative stress.

**B. Ferric ion reducing antioxidant power (FRAP)**

The FRAP depends on the ability of an extract to reduce ferric ($Fe^{3+}$) to its more active ferrous ($Fe^{2+}$) form by the donation of an electron. Here, a given antioxidant donates electrons to reactive free radical species to promote the termination of free radical chain reactions.[68] The results from the FRAP assay illustrated in Figure 4, indicates a concentration-dependent activity with values ranging from 1.0 to 2.4 µg AAE/g DM.

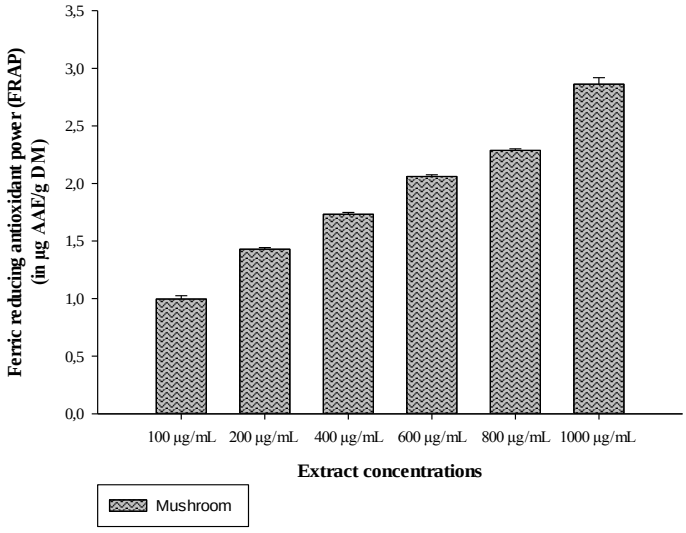


**Figure 4:** The Ferric Reducing antioxidant power of *T. aurea*

The extract demonstrated the highest activity at concentration 1000 µg/mL, followed closely by 800 µg/mL. The isolated compounds could not reduce ferric ions to ferrous ions at the tested concentrations. Previous reports on mushroom extracts, demonstrated that, the detection of phenolics and flavonoids possessing free hydroxyl groups could scavenge free radicals, thus

preventing lipid oxidation.[41,42] Therefore, we could suggest that, the antioxidant potential observed in this study might be associated to the synergistic effects of the phenolics and flavonoids detected in *T. aurea* extract.

**C. ABTS scavenging activity**

The ABTS radical scavenging activity of extracts indicates their ability to scavenge free radicals, thus preventing lipid oxidation through a chain breaking reaction,[68] while DPPH accepts an electron or free hydrogen radical to become a stable diamagnetic molecule that is purple in methanol solution and changes to pale upon reaction. This method of scavenging 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals has been used to evaluate the antioxidant activity of extracts or compounds.[34] In this study, the extracts and isolated compounds were evaluated for their ability to donate protons which stabilize the free radical DPPH and ABTS, as shown in Table 3.

**Table 3:** The antioxidant activity of the hydro-ethanolic extract and compounds of *T. aurea*

| **Samples** | **DPPH** | | **ABTS** | |
|---|---|---|---|---|
| | $SC_{50}$ (µg/mL) | SD | $SC_{50}$ (µg/mL) | SD |
| **HEE** | 180.75 | ± 28.07 | 248.95 | ± 48.01 |
| **Erg** | > 500 | ND | > 500 | ND |
| **Erge** | > 500 | ND | > 500 | ND |
| **Ade** | > 500 | ND | > 500 | ND |
| **Ecs** | > 500 | ND | > 500 | ND |
| **Gallic acid** | 1,83 | 0,05 | 1,63 | 0,12 |

Legend: $SC_{50}$: 50% scavenging concentration which means concentration necessary to trap 50% of radicals. **Erg:** Ergosterol; **Ade :** Adenosine ; **Erge :** Ergosterol peroxide; **Ecs :** (22E724R)-24-ethylcholesta-5,7,22-trien-3β-ol ; *HEE***:** *Tricholomopsis aurea* hydro-ethanolic extract *;* ND: Not determined.

The HEE of *T. aurea* effectively stabilize the free radicals DPPH and ABTS with concentrations ($S_{C50}$) of 180.75 µg/mL and 248.95 µg/mL, respectively. The isolated compounds did not exhibit any activity at concentrations of 500 µg/mL, suggesting that they may not be responsible for the extracts activity. The extracts effectiveness is likely due to other metabolites such as flavonoids and phenolics, which are well known for their antioxidant properties.[41,43] These findings indicates that *T. aurea* extract could be beneficial in combatting oxidative stress by donating protons and preventing lipid peroxidation.

## 4. Anti-inflammatory activity

As shown in table 4, the anti-inflammatory activity of adenosine, ergosterol, and ergostrol peroxide were stronger than that of the HEE of *T. aurea.*

**Table 4:** The anti-inflammatory of activity of *T. aurea* hydro-ethanolic extract and compounds by protein denaturation (BSA)

| | **Inhibitory Concentration 50** |
|---|---|
| **Samples** | **$IC_{50}$ (±)SD** (µg/mL) |
| **HEE** | 49.19(±) 0.50 |
| **Ecs** | ND |
| **Erge** | 6.85 (±) 0.11 |
| **Ade** | 4.91 (±) 0.08 |
| **Erg** | 29.51(±) 0.19 |
| **Diclofenac sodium** | 1.16 (±) 0.19 |

Legend: ND: Not Determined ; >100: Not active at tested concentration, **Erg:** Ergosterol; **Ade :** Adenosine ; **Erge :** Ergosterol peroxide; **Ecs :** (22E724R)-24-ethylcholesta-5,7,22-trien-3β-ol ; *HEE***:** *Tricholomopsis aurea hydroethanolic* extract

The HEE extract exhibited potent anti-inflammatory activity ($IC_{50}$ = 49.19 ± 0.50 µg/mL), while for the compounds significant activity was revealed for adenosine ($IC_{50}$ = 4.91 ± 0.08 µg/mL) and ergosterol peroxide ($IC_{50}$ = 6.81 ± 0.11 µg/mL) and good activity for ergosterol ($IC_{50}$ = 29.51 ± 0.19 µg/mL). The $IC_{50}$ value of the reference diclofenac sodium was 1.16 ± 0.19 µg/mL, while (22E, 24R)-24-ethylcholesta-5,7,22-trien-3β-ol was not active at the tested concentrations (> 100µg/mL). Nonetheless, the reference diclofenac sodium was more active than ergosterol, ergosterol peroxide, adenosine, and the extract. Previous literature studies using egg albumin denaturation assay demonstrated that, ergosterol interaction with BSA is by primarily binding to the tryptophan (Trp-212/Trp-214) residue of BSA within site I (subdomain II A).[70] As concerns adenosine it is known to bind with serum transport proteins which is vital in the context of its delivery to different sites of activity in the body.[71] Other studies on inflammation revealed that ergosterol peroxide and ergosterol inhibits lipopolysaccharide-induced inflammation, extracellular signal-regulated protein kinase (ERK), along with the phosphorylation of p38, c-Jun amino-terminal kinase (JNK), and mitogen-activated protein kinases (MAPKs). [72] Also, studies on the extracts of edible mushrooms such as *Pleurotus ostreatus* and *Pleurotus*. *pulmonarius*, were found

to possess anti-inflammatory activities.[73,74] Therefore, in the content of the BSA denaturation assay, *T. aurea* possess constituents with promising anti-inflammatory activity.

## IV. CONCLUSIONS AND FUTURE PERSPECTIVES

### A. Conclusions

The present investigation constitutes the first report on constituents, nutrient contents, and biological potentials of *T. aurea*. The active constituents: ergosterol, ergosterol peroxide, and adenosine may be independently acting for the anti-inflammatory activity of the mushroom, while the detected phenolics and flavonoids could be responsible for the weak antioxidant activity of the mushroom extract. The activities of *T. aurea* extract and test compounds suggests the mushroom could be administered as relief treatment during some painful conditions. The high content in lipids, crude fiber, low protein and high energy value together with the mineral content such as phosphorus, calcium, magnesium, and sodium indicates the mushroom is essential for the maintenance of life and for growth, thus could be added to traditional dishes. In regards to the phytochemicals or supplements identified in present studies, we recommend the seasonal mushroom be cultivated and disseminated at all time by mushroom farmers in Cameroon and other countries for inclusion in functional or nutraceutical food to support human wellbeing.

### B. Future perspectives

The future perspective would require studies on more mineral content such as Cu, Zn, and Mn and identification of other classes of compounds in the food matrix through Gas chromatography and UHPLC-ESI-MS/MS spectrometry.

Still in future prospects, about *Tricholomopsis aurea* which is the biological organism investigated in this study, considering on the one hand that its identification was based on close macro- and micromorphological comparison with the specimens described and ITSrDNA-sequenced by Desjardin and Perry,[15] and on the other hand that the material examined by these authors was made of two specimens collected only in the Island of Sao-Tomé and Principe, further studies could include the sequencing of more gymnopoid fungi originating from other tropical areas and particularly those belonging to the family of Tricholomataceae with the genera *Tricholomopsis* as center of interest, the main aim of these additional studies being to complete the genomic DNA extracted by Desjardin and Perry[15] and thereby also enlarge the basis of comparison of their

biogeographical origins in more larger phylogenetic trees. In this prospect, the additional specimens could be either obtained from fresh collections in other tropical countries within the known areas of distribution of *Tricholomopsis aurea* in particular including the DR Congo, Kenya, Tanzania, Togo, Uganda, the Caribbean, Gadeloupe, Martinique and Trinidad & Tobago, or by borrowing exsicatta from mycological herbaria in the countries where these herbaria are set up and functional.

**ACKNOWLEDGEMENTS**

The authors are thankful to the bioprofiling platform, supported by the European Regional Development Fund and the Walloon Region Belgium, as well as to YaBiNaPA project in Cameroon for the facilities that enabled the realization of this work.

## REFERENCES


1. Hasler CM. Functional food: The Western perspective. Nutri Rev. 1996;54(11):6.
2. Chang R. Functional Properties of Edible Mushrooms. Nutri Rev. 1996;54(11):S91.
3. Jayakumar T, Thomas PA, Sheu JR, Geraldine P. In-vitro and in-vivo antioxidant effects of the oyster mushroom (Pleurotus ostreatus). Food Res Int. 2011;44(4):851–61.
4. Unekwu HR, Audu JA, Makun MH, Chidi EE. Phytochemical screening and antioxidant activity of methanolic extract of selected wild edible Nigerian mushrooms. Asian Pac J Trop Dis. 2014;4:S153–37.
5. Colak A, Faiz O, Sesli E. Nutritional composition of some wild edible mushrooms. Turk J Biochem. 2009;34(1): 25–31.
6. Kalaˇc P. A review of chemical composition and nutritional value of wild-growing and cultivated mushrooms. J Sci Food Agric. 2013;93(2):209–18.
7. Lu YY, Ao ZH, Lu ZM, Xu HY, Zhang XM, Dou WF, Xu ZH. Analgesic and anti-inflammatory effects of the dry matter of culture broth of Termitomyces albuminosus and its extracts. J Ethnopharmacol. 2008;120(3):432–6.
8. Wasser SP. Medicinal mushroom science: history, current status, future trends, and unsolved problems. Int J Med mushrooms. 2010;12(1).
9. Morel S, Vitou M, Masnou A, Jumas-Bilak E, Rapior S, Licznar-Fajardo P. Antibacterial activity of wild mushrooms from France. Int J Med Mushrooms. 2021;23(1).
10. Hatanaka SI, Niimura Y. L-3-(3-carboxy-4-furyl) alanine from Tricholomopsis rutilans. Phytochemistry. 1975;14(5-6):1436.
11. Wang F, Liu JK. Two new steryl esters from the basidiomycete (Tricholomopsis rutilans). Steroids. 2005;70(2):127–30.
12. Işık H, Türkekul İ, Bengü AŞ, Yılmaz HÇ. Determination of fatty acid profile and mineral contents of Tricholomopsis rutilans collected from Yozgat. Anatolian J Bot. 2019;3(2):64–8.
13. Janeš D, Kreft S, Jurc M, Seme K, Štrukelj B. Antibacterial activity in higher fungi (mushrooms) and endophytic fungi from Slovenia. Pharm Biol. 2007;45(9):700–6.
14. Ndong HE, Degreef J, De Kesel A. Champignons comestibles des forêts denses d'Afrique centrale. Taxonomie et identification. ABC Taxa. 2011;10:253-671.
15. Desjardin DE, Perry BA. The gymnopoid fungi (Basidiomycota, Agaricales) from the Republic of São Tomé and Príncipe, West Africa. Mycosphere. 2017;8(9):1317–91.
16. Kansci G, Mossebo DC, Selatsa AB, Fotso M. Nutrient content of some species of the genus Termitomyces consumed in Cameroon. Food/Nahrung. 2003;47(3):213–6.
17. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am J Enology Viticult. 1965;16(3):144–58.
18. Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and in vitro antioxidant activities of the aqueous extract of (Helichrysum longifolium) DC. BMC Compl Alternat Med. 2010;10:21.
19. Singh DK, Srivastva B, Sahu A. Spectrophotometric determination of Rauwolfia alkaloids, estimation of reserpine in pharmaceuticals. Analytical Sci. 2004;20:571–3.
20. Hiai S, Oura H, Nakajima T. Color reaction of some sapogenins and saponins with vanillin and sulfuric acid. Planta Med. 1976;29(02):116–22.
21. AOAC: Official Methods of Analysis, Washington, DC; 1980.

22. Bourely J. Observation sur le dosage de l'huile des graines de cotonnier. Coton et Fibres Tropicales. 1982;37(2):183–96.
23. A.O.A.C., Official Method of Analysis (13th ed.), Association of Official Analytical Chemists. Washington, DC USA; 2000.
24. Bergeret B. La chimie analytique dans un laboratoire d'outre-mer. Organisation du laboratoire: Étude des techniques d'analyses employées au laboratoire de la section l'IECAM-ORSTOM. IRCAM, Paris, 1955; 56.
25. A.O.A.C., Official Methods of Analysis, Horwitz W, 1918-(13th ed.), Washington, DC; 1970.
26. Ache NT, Manju EB, Lawrence MN, Tonjock RK. Nutrient and Mineral Contents of Wild Edible Mushrooms from the Kilum-IjimForest, Cameroon. Nutri Food Sci J. 2020;3(2):128.
27. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269(2):337–41.
28. Oyaizu M. Studies on products of browning reactions: antioxidant activities of products of browning reaction prepared from glucoseamine. Jpn J Nutr Diet.1986;44(6):307–15.
29. Bassene E. Initiation à la recherche sur les substances naturelles: Extraction-Analyse-Essais Biologiques. Presses Universitaires de Dakar; 2012.
30. Khan M, Yi F. Rasul A, Li T, Wang N, Gao H, Gao R, Ma T. Alantolactone induces apoptosis in glioblastoma cells via GSH depletion, ROS generation, and mitochondrial dysfunction. IUBMB Life. 2012;64(9):783–94.
31. Prasad SB, Yashwant AV. In vitro anti-inflammatory activity of Raupya (Silver) Bhasma. J Chem Pharma Res. 2013;5:194-7.
32. Harvanová J, Maľučká UL, Uhrinová A, Vilková M, Vavra M, Pavlík M, Rajtar M, Furmaníková A. NMR and IR analysis of natural substances isolated from Cordyceps medicinal mushrooms. Čes slov Farm. 2018;67(5):200–4.
33. Takashima S, Toyoshi K, Yamamoto T, Shimozawa N. Positional determination of the carbon–carbon double bonds in unsaturated fatty acids mediated by solvent plasmatization using LC–MS. Sci Rep. 2020;10(1):12988.
34. Gebauer SK, Psota TL, Kris-Etherton PM. The diversity of health effects of individual trans fatty acid isomers. Lipids. 2007;42(9):787–99.
35. Wright JL. The occurrence of ergosterol and (22 E, 24 R)-24-ethylcholesta-5, 7, 22-trien-3β-ol in the unicellular chlorophyte Dunaliella tertiolecta. Can J Chem. 1979;57(19):2569–71.
36. Krzyczkowski W, Malinowska E, Suchocki P, Kleps J, Olejnik M, Herold F. Isolation and quantitative determination of ergosterol peroxide in various edible mushroom species. Food Chem. 2009;113(1):351–5.
37. Domondon DL, He W, De Kimpe N, Höfte M, Poppe J. β-Adenosine, a bioactive compound in grass chaff stimulating mushroom production. Phytochem. 2004;65(2): 181–7.
38. Alispahić A, Šapčanin A, Salihović M, Ramić E, Dedić A, Pazalja M. Phenolic content and antioxidant activity of mushroom extracts from Bosnian market. Bulletin of the Chemists and Technologists of Bosnia and Herzegovina. 2015;44(2): 5–8.
39. Duh PD, Tu YY, Yen GC. Antioxidant activity of water extract of harn jyur (Chyrsanthemum morifolium Ramat). LWT - Food Sci Technol. 1999;32(5):269–77.

40. Elmastas M, Isildak O, Turkekul I, Temur N. Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. J Food Compost Anal. 2007;20(3-4):337–45.
41. Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K. Antioxidant and antimicrobial activities of Laetiporus sulphureus (Bull.) Murrill. Food Chem. 2007;101:267–73.
42. Barros L, Cruz T, Baptista P, Estevinho LM, Ferreira IC. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol. 2008;46(8):2742–7
43. Boonsong S, Klaypradit W, Wilaipun P. Antioxidant activities of extracts from five edible mushrooms using different extractants. Agric Nat Res. 2016;50(2):89–97.
44. Ribeiro B, Lopes R, Andrade PB, Seabra RM, Gonçalves RF, Baptista P, Quelhas I. Comparative study of phytochemicals and antioxidant potential of wild edible mushroom caps and stipes. Food Chem. 2008;110(1):47–56.
45. Krishnamoorthi R, Srinivash M, Mahalingam PU, Malaikozhundan B. Dietary nutrients in edible mushroom, Agaricus bisporus and their radical scavenging, antibacterial, and antifungal effects. Process Biochem. 2022;121:10–7.
Vetter J. Chemical composition of eight edible mushrooms. Zeitschrift für Lebensmittel-Untersuchung und Forschung. 1993;196(3):224–7.
46. Mattila P, Könkö K, Eurola M, Pihlava JM, Astola J, Vahteristo L, Hietaniemi V, Kumpulainen J, Valtonen M, Piironen V. Contents of vitamins, mineral elements, and some phenolic compounds in cultivated mushrooms. J Agric Food Chem. 2001;49(5):2343–8.
47. Olayinka AA. Evaluation of the Nutritional Status of Two Edible Mushroom Species in Ekiti State, Nigeria. Food Sci Qual Manag. 2016;51:32–6.
48. Barros L, Baptista P, Correia DM, Casal S, Oliveira B, Ferreira IC. Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. Food Chem. 2007;105(1):140–5.
49. Kouame KB, Koko AC, Diomande M, Konate I, Assidjo NE. Caractérisation physicochimique de trois espèces de champignons sauvages comestibles couramment rencontrées dans la région du Haut-Sassandra (Côte d'Ivoire). J Appl Biosci. 2018;121:12110–20.
50. Vetter J. The Mushroom Glucans: Molecules of High Biological and Medicinal Importance. Foods. 2023;12(5):1009.
51. Gunasekara N, Nanayakkara C, Karunarathana S, Wijesundera R. Nutritional aspects of three Termitomyces and four other wild edible mushroom species from Sri Lanka. Chiang Mai J Sci. 2021;48(5):1236–46.
52. Ribeiro B, de Pinho PG, Andrade PB, Baptista P, Valentão P. Fatty acid composition of wild edible mushrooms species: A comparative study. Microchem J. 2009;93(1):29–35.
53. Nwagu LU, Obiakor-Okeke PN. Nutritional Profile of Three Different Mushroom Varieties Consumed in Amaifeke, Orlu Local Government Area, Imo State, Nigeria. Food Sci Qual Manag. 2014;31:70–7.
54. Sadli S, Saleha S, Fiana D, Misrahanum M. The formulation of white oyster mushroom (Pleurotus ostreatus (Jacq.) P. Kumm) as natural flavoring and the quality test in temperature and drying time variations. In IOP Conference Series: Earth Environ Sci. 2021;922(1): p.012054.
55. Food and Agriculture Organization. Food composition Table for use in East Asia. US Department Of Health, Education, And Welfare and FAO, Food Policy and Nutrition Division FAO Rome; 1972.

56. Hailemariam A, Esatu W, Abegaz S, Urge M, Assefa G, Dessie T. Nutritional composition and sensory characteristics of breast meat from different chickens. Appl Food Res. 2022; 2(2):10023.
57. Waszkiewicz-Robak B, Szterk A, Rogalski M, Rambuszek M, Kruk M, Rokowska E. Nutritional value of raw pork depending on the fat type contents in pigs feed. Acta Sci Pol Technol Aliment. 2015;14(2):153–63.
58. Adejumo TO, Coker ME, Akinmoladun VO. Identification and evaluation of nutritional status of some edible and medicinal mushrooms in Akoko Area, Ondo State, Nigeria. Int J Curr Microbiol Appl Sci. 2015;4(4):1011–28.
59. Mshandete AM, Cuff J. Proximate and nutrient composition of three types of indigenous edible wild mushrooms grown in Tanzania and their utilization prospects. African J Food Agric Nutri Develop. 2007;7(6):1–16.
60. Power ML, Heaney RP, Kalkwarf HJ, Pitkin RM, Repke JT, Tsang RC, Schulkin J. The role of calcium in health and disease. Am J Obstet Gynecol. 1999;1(6):1560–9.
61. Lanham-New SA, Lambert H, Frassetto L. Potassium. Adv Nutri. 2012;3(6):820–1.
62. Underwood EJ, Suttle NF. The mineral nutrition of livestock. 3rd ed. Wallingford: CABI International Publishing. 1999; 614pp.
63. Grant CA, Flaten DN, Tomasiewicz DJ, Sheppard SC. The importance of early season phosphorus nutrition. Can J Plant Sci. 2001;81(2):211–24.
64. Fukumoto S. Phosphate metabolism and vitamin d. BoneKEy Rep. 2014;3.
65. Kher K, Schnaper HW, Greenbaum LA. Clinical Pediatric Nephrology. 3rd ed. CRC Press; Boca Raton, FL, USA; 2016.
66. Blaszczyk U, Duda-Chodak A. Magnessium: Its role in nutrition and carcinogenesis. Rocz Panstw Zakl Hig. 2013;64(3):165–71.
67. Seldin DW, Giebisch G. The regulation of sodium and chloride balance. New York: Raven Press; 1990.
68. Govindan S, Sabapathy V, Rajan BIS, Ponnusamy L. Antioxidant Activity of Various Extracts from an Edible Mushroom (Pleurotus eous). Food Sci Biotechnol. 2012;21(3):661–8.
69. Cheng Z. Interaction of ergosterol with bovine serum albumin and human serum albumin by spectroscopic analysis. Mol Biol Rep. 2012;39:9493–508.
70. Takeda S, Miyauchi S, Nakayama H, Kamo N. Adenosine 5′-triphosphate binding to bovine serum albumin. Biophysic Chem. 1997;69(2-3):175-83.
71. Ming B, Yang BB, Hu L. Natural Bioactive Sterol 5α,8α- endoperoxides as Drug Lead Compounds. Med chem. 2014;4:709–16.
72. Yoo MS, Shin JS, Choi HE, Cho YW, Bang MH, Baek NI, Lee KT. Fucosterol isolated from Undaria pinnatifida inhibits lipopolysaccharide-induced production of nitric oxide and pro-inflammatory cytokines via the inactivation of nuclear factor-κB and p38 mitogen-activated protein kinase in RAW264. 7 macrophages. Food Chem. 2012 Dec 1;135(3):967-75.
73. Jayasuriya WB, Handunnetti SM, Wanigatunge CA, Fernando GH, Abeytunga DT, Suresh TS. Anti-inflammatory activity of Pleurotus ostreatus, a culinary medicinal mushroom, in Wistar rats. Evidence-Based Complement Alternat Med. 2020;2020(1):6845383.
74. Jose N, Ajith TA, Janardhanan KK. Antioxidant, anti-inflammatory, and antitumor activities of culinary-medicinal mushroom Pleurotus pufmonanus (Fr.) Quel. (Agaricomycetideae). Int J Med mushrooms. 2002;4(4).