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Food Value and Safety Status of Raw (Unfermented) and Fermented Higher Basidiomycetes, *Lenzites quercina* (L) P. Karsten

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ABSTRACT: Food value and safety of a wild macrofungus, *Lenzites quercina* were investigated. The proximate and mineral composition of raw and fermented *L. quercina* were assessed using standard methods. Brine shrimp lethality bioassay was adopted for assessment of the toxicological property of the extracts obtained from raw and fermented *L. quercina*. The result of proximate composition revealed higher protein content (26.15%) in fermented *L. quercina*, while the value of carbohydrate (38.3%), crude fibre (27.6%), and ash contents (6.5%) were higher ($P<0.05$) in raw *L. quercina* when compared to fermented *L. quercina*. The macro and micro elements in the raw and fermented *L. quercina* were in decreasing order of Ca> K> Zn> Fe> Na> Mg> Pb> Cu with values ranging from 4.04 mg/g to 721.6 mg/g. The amino acids in raw and fermented *L. quercina* ranged from 0.05 mg/g to 23.78 mg/g, while the fatty acids ranged from 0.11% to 38.5%. The mortality rate of the *Artemia salina* against the extracts was from 8.0% to 38.0% with lethal dose at 50% of population within 49.11 and 250.50 μg/mL. The results from this study revealed that *L. quercina* possesses essential amino acids, fatty acids, and substantial micro elements, which may be useful in the formulation of functional foods and nutraceuticals.

Keywords: amino acids, fatty acids, medicinal mushrooms, brine shrimp lethality bioassay (BSLB)

INTRODUCTION

Mushrooms are fruiting bodies belonging to the division of Basidiomycota or Ascomycota. Some of these mushrooms are saprophytic microorganisms, owing to the presence of extracellular enzymes that break complex organic compounds into bioactive secondary metabolites (1). The biological activity of these compounds as dietary supplements may help to maintain stable physiological functions of the body (2). Hence, medicinal mushrooms can be utilized as nutraceuticals to replace low nutrient compounds found in some widely consumed foods.

Wild medicinal mushrooms are used for a variety of applications such as bioconversion of agricultural wastes into food and other valuable products (3). The toughness, lignolytic property of some wild medicinal macrofungi with concentrated biomolecules, have made them non-palatable. Polyphenolic, polysaccharides, protein, lectins, vitamins, minerals, and steroids are bound bioactive compounds in the cellular component of wild macrofungi (4). Hence, there is a need in expanding the knowledge on the chemical composition and bioactivity of these wild underutilized macrofungi. The use of advanced bioprocessing techniques to extract biologically active compounds from fungi (wild mushrooms) would increase the production of desired biomolecules (5,6), which can be used in the production of foods, cosmetics, and in mycotherapy. These are considered as means for providing functional foods with pharmacological properties and thus, improving the bioavailability of nutritional supplements to treat some dietary aliments.

Fermentation has been widely used in food biotechnology as a method of food processing that involved the conversion of complex organic molecules in substrates into simple byproduct compounds, with the help of various microorganisms. In this context, the chemical components of a substrate are broken down releasing additional bioactive metabolites. In some wild mushrooms, their bioactive substances are protected by a hard sporoderm (7), which can be completely broken by fermenta-

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tion in order to release bioactive substances within the spores (8).

Wild mushrooms accumulate high quantities of bioactive compounds that have health benefits (9). However, the reliance on bioactive compounds in wild and medicinal mushrooms as alternative therapies for health care requires scientific validation of their biosafety before general acceptance for prescription. This is due to the significant amounts of bioactive compounds in mushrooms that are beyond basic nutritional contents. *Lenzites quercina*, is one of the underutilized wild mushrooms with biologically active compounds. In this study, the food value and biosafety of unfermented and fermented wild macrofungus, *L. quercina* was assessed using a brine shrimp lethality bioassay (BSLB).

MATERIALS AND METHODS

Collection and identification of *L. quercina*

The fruiting body of *L. quercina* was collected from a farmland near The Federal University of Technology, Akure (FUTA), Nigeria. The fruit body was kept dried in a desiccator until it was needed for use. The mushroom sample, which had been tentatively identified as *Lenzites* spp., was confirmed with molecular tools using the internal transcribed spacer (ITS) region of the rDNA. Basic local alignment search tool analysis revealed the ITS sequence of the macrofungus to be 100% homologus to *L. quercina* with accession number of JF689829.1 on the NCBI GenBank (10). A voucher of the specimen was kept in the Department of Microbiology, FUTA.

Fermentation of *L. quercina*

The dried *L. quercina* was divided into two equal portions (500 g), raw (unfermented) and the other part was subjected to solid state fermentation in order to release bound bioactive compounds. Briefly, 200 mL of water was added to 500 g of *L. quercina* and fermented for 4 days. The temperature and pH during the fermentation process were monitored using a thermometer (HANNA HI 9828, Hanna Instruments, Woonsocket, RI, USA) and pH meter (HANNA 98161, Hanna Instruments), respectively. The fermented portion was thereafter dried in an oven [DHG 9053-A, Zenith Lab (Jiangsu) Co., Ltd., Jiangsu, China] at 30°C.

Proximate analysis of raw and fermented *L. quercina*

Proxaimate composition of raw and fermented *L. quercina* was determined using method of the Association of Official Analytical Chemists (11). The moisture contents of raw and fermented *L. quercina* was determined by drying mushroom sample (2.0 g) in an oven at 105°C for 3 h. The ash content of raw and fermented *L. quercina* was determined by incinerating the test samples in a muffle furnace (F62700, Barnstead Thermolyne Corporation, Dubuque, IA, USA) at 550°C for 24 h. The fat content was determined by extracting 2.0 g of the raw or fermented *L. quercina* with petroleum ether in a Soxhlet apparatus. The Soxhlet extractor was filled with a reflux condenser and heated gently. It was left to siphon for 5 h over a barrel. The condenser was detached, while the thimble was removed. The petroleum ether was distilled from the flask, and the fat residue was dried in an air oven at 100°C for 5 min and cooled in desiccator. Protein content was determined by Kjedahl method, briefly, 2 g of each of the samples were weighed into a 50 mL micro-Kjedahl flask containing 5 mL of concentrated H_2SO_4 with a half Kjedahl catalyst tablet and weighed. The mixture was heated until the sample was totally digested. The digested sample was then made up to 100 mL. Five millimeters of 2% boric acid were placed into a 100 mL conical flask, while 3 drops of indicator (0.198 g bromocresol green and 0.132 g methyl red in 200 mL ethanol) was added. The sample (10 mL) was then pipetted into the distillation unit plus 10 mL of NaOH (40% v/v). This was then distilled until 50 mL was obtained in the receiving flask. The distillate was later titrated with standard 0.01 M HCl, while a blank was also titrated using the same acid. The percentage of crude protein was calculated as crude protein (%)=$N_2\times4.38$. Crude fibre of the raw and fermented *L. quercina* was determined by dilute acid and alkali hydrolysis method. Carbohydrates in the mushroom samples was calculated as:

$$\%\ \text{Carbohydrates} = 100 - (\%\ \text{moisture} + \%\ \text{ash} + \%\ \text{protein} + \%\ \text{fat} + \%\ \text{crude fibre}).$$

Determination of mineral content of raw and fermented *L. quercina*

The mineral composition (calcium, magnesium, zinc, iron, and lead) of each sample was determined by the ashing method described by AOAC (11). Triplicates of 1.0 g from each sample was weighed into porcelain crucibles and placed in a muffle furnace. The temperature was raised gradually to 550°C for 6 h. After cooling to room temperature, the ash was dissolved in 1 mL of HNO_3 (0.5% v/v). The sample volume was brought to 100 mL with distilled water, and the levels of the minerals present were analyzed by atomic absorption spectrophotometer (201VGP, Buck Scientific Inc., Norwalk, CT, USA). A flame photometer (PFP 7, Jenway, Staffordshire, UK) was used to determine potassium (K) and sodium (Na) contents.

Determination of amino acids in raw and fermented *L. quercina*

Each of the raw and fermented samples (2.0 g) was

weighed into the extraction thimble. The fat was extracted with a chloroform-methanol mixture using a Soxhlet extraction apparatus. The extraction lasted for 6 h. The defatted samples (40 mg) were weighed into glass ampoules. Seven milliliters of 6 M HCl were added and oxygen was expelled by passing nitrogen gas into the ampoule to avoid possible oxidation of amino acids during hydrolysis. Each glass ampoule was then sealed with a Bunsen flame and put into an oven at 105±5°C for 22 h. The ampoule was allowed to cool before breaking it open at the tip, and the content was filtered to remove humins. The filtrate was then evaporated to dryness at 40°C under vacuum in a rotary evaporator. Each residue was dissolved with 5 mL of acetate buffer, stored in a plastic specimen bottle, and kept in a deep freezer.

Loading of hydrolysate into the technicon sequential multisample (TSM)

The amino acids in raw and fermented *L. quercina* were determined by ion exchange chromatography using the TSM amino acid analyzer (Technicon Instruments Corporation, New York, NY, USA). The amount of hydrolysate loaded was between 5~10 μL and dispensed into the cartridge of the TSM amino acid analyzer. The TSM separates and analyzes free acidic, neutral, and basic amino acids of the hydrolysate, and the period of the analysis lasted for 76 min at 60°C with a gas flow rate of 0.50 mL/min. The net height of each ion exchange chromatogram peak produced by the chart recorder of TSM (each representing an amino acid) was measured. The half-height of the peak on the chart and width of the peak on the half-height was accurately measured and recorded. The approximate area of each peak was then obtained by multiplying the height with the width at half-height (12).

Determination of fatty acid methyl esters (FAMEs) in raw and fermented *L. quercina*

The fatty acids were determined after a trans-esterification procedure described by Stojković et al. (13) with slight modifications. Briefly, 50 mg of fat extracted from raw and fermented *L. quercina* was esterified for 5 min at 95°C with 3.4 mL of 0.5 M KOH in dry methanol. The mixture was neutralized using 0.7 M HCl. Then, 3 mL of boron triflouride (14%) in methanol was added. The mixture was heated for 5 min at 90°C to achieve complete methylation. FAMEs were extracted from the mixture with redistilled *n*-hexane three times. Thereafter, the content was concentrated to 1 mL, and 1 μL was injected into the injection port of a gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan) with an auto injector, capillary column, analytical conditions of autosampler, injection port settings, column oven settings, and column information used for analysis of FAMEs. The quantification of the FAMEs was performed using a standard mixture (C_4~C_{24}, Sigma-Aldrich Co., St. Louis, MO, USA). The concentration and area of each peak of the FAMEs was computed using the GC post-run analysis software (Shimadzu).

Preparation of extracts from raw and fermented *L. quercina*

The dried samples of raw and fermented *L. quercina* were ground into powder using a mill machine (5657 HAAN 1 TYPE ZM1, Retsch GmbH, Haan, Germany). The raw and fermented powder (200 g) of *L. quercina* was sequentially extracted with different solvents of the same volume and concentration (1,000 mL of 95% v/v) of petroleum ether, followed by ethyl acetate and ethanol. After soaking for 72 h, the filtrate obtained through Whatman filter paper No 1 was concentrated in a rotary evaporator (RE-52A, Lanphan, Zhengzhou, Henan, China). The extracts were freeze dried and maintained in a refrigerator at 4°C. The extracts were coded as RPP: raw *L. quercina* extracted with petroleum ether, REA: raw *L. quercina* extracted with ethyl acetate, RET: raw *L. quercina* extracted with ethanol, FPP: fermented *L. quercina* extracted with petroleum ether, FEA: fermented *L. quercina* extracted with ethyl acetate, and FET: fermented *L. quercina* extracted with ethanol.

Toxicological assay of *L. quercina* extracts using *Artemia salina* larvae

The toxicity or safety of *L. quercina* extracts was determined using the method of Hamidi et al. (14). The brine shrimp eggs were obtained from the Department of Medicine and Nursing, University of Vicosa, Brazil. An artificial saline solution was prepared by dissolving 3.8% w/v marine salts in distilled water. The eggs were hatched in a rectangular tank (80 mm×60 mm) containing artificial sea water and incubated under light at 26±1°C for 36~48 h with adequate aeration. The newly hatched brine shrimps (nauplii) were separated from the shell, and the remaining cysts were transferred to fresh sea water with a Pasteur pipette and immediately used for the bioassay. Assays were performed in 12-well culture plates (Corning, New York, NY, USA). Each well contained 8~10 larvae in 1,000 μL of sea water. Five microliters of each dilution of extract were transferred to the wells. Dimethyl sulfoxide was used as the positive control, while the well without extract was used as the negative control. The experiment was carried out in triplicates. The percentage of larval mortality was determined after exposure to different concentrations of the extract for 24 h at 26±1°C. The mortality end point for this bioassay was defined as the absence of forward motion of shrimps.

Statistical analysis

All experiments were carried out in triplicates. Data ob-

Table 1. Changes in temperature and pH during the fermentation of *Lenzites quercina* for 4 days

Day	Temperature (°C)	pH
0	28.7±0.0[c]	6.8±0.0[d]
1	29.0±0.0[d]	6.7±0.0[c]
2	27.6±0.0[b]	6.7±0.1[c]
3	27.0±0.2[b]	6.6±0.0[b]
4	26.8±0.0[a]	6.5±0.0[a]

Values are mean±SD of triplicates (n=3).
Means with different letters within a column are significantly different by Duncan's test (P<0.05).

tained were analyzed by Oneway Analysis of Variance and means were compared by Duncan's new multiple range test and Student's independent *t*-test (SPSS version 21, SPSS Inc., Chicago, IL, USA). Differences were considered significant at $P<0.05$. The lethality dose required to kill 50% (LD_{50}) of the *A. salina* larvae was calculated from the dose-dependent curves generated by GraphPad Prism version 6.0 (GraphPad software Inc., San Diego, CA, USA).

RESULTS

Table 1 shows the changes in temperature and pH during fermentation of *L. quercina* for 4 days. There was a decreased in temperature from day 1 to day 4 and mean values were significantly different at (P<0.05) for each day. The pH value obtained during the fermentation of macrofungus ranged from 6.5 to 6.8 for 4 days. The lowest pH was observed at day 4 when compared to the pH obtained for other days. Fig. 1 shows the proximate composition of the raw (unfermented) and fermented *L. quercina*. The values of carbohydrate (38.3%), crude fibre (27.6%), ash (6.5%), and fat (5.1%) contents were higher in the raw sample (P<0.05), while moisture (9.8%) and protein contents (26.2%) increased in fermented *L. quercina*.

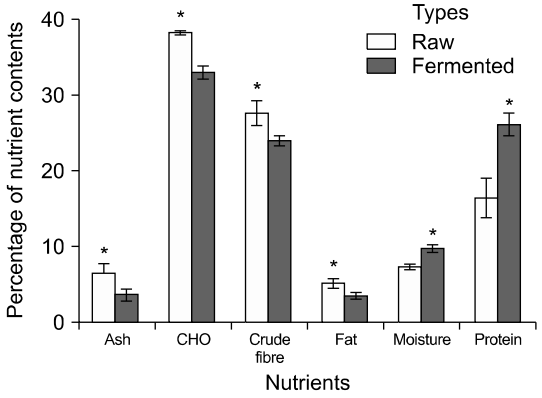

Fig. 1. Proximate composition of raw and fermented *Lenzites quercina*. *Significantly different for each nutrient by independent *t*-test at P<0.05. Error bar represents standard deviation. CHO, total carbohydrates.

Table 2. Mineral contents (mg/g) of the raw and fermented *Lenzites quercina*

Minerals	Raw	Fermented
Na	26.70±2.00*	18.10±9.10
Ca	721.60±8.00*	702.64±9.50
Fe	34.60±2.10	51.94±2.00*
Zn	71.90±2.90	91.90±1.45*
Cu	4.80±0.00	4.04±0.70
Pb	9.06±0.00*	7.38±0.00
K	678.98±17.10*	481.21±9.00
Mg	12.90±1.10*	8.43±0.94

Values are mean±SD of replicates (n=3).
*Significantly different within a row by independent *t*-test (P< 0.05).

quercina.

Table 2 shows the mineral contents of the raw and fermented *L. quercina*. The essential micro elements zinc and iron, were increased in fermented *L. quercina* with the value of 91.90 mg/g and 51.94 mg/g, respectively. The concentrations of other minerals like Ca, K, Na, Mg, and Pb were higher (P<0.05) in raw *L. quercina*, which also correlates with the higher ash content (6.5%) in the raw *L. quercina*. Amino acids in raw and fermented *L. quercina* are presented in Table 3. The value of essential amino acids like isoleucine (3.57 mg/g), leucine (8.83 mg/g), lysine (5.90 mg/g), tyrosine (2.05 mg/g), tryptophan (3.70 mg/g), and valine (5.04 mg/g) were increased in the fermented sample and significantly different from raw *L. quercina* (P<0.05). Oleic acid was the predominant fatty acid in both raw and fermented *L. quercina* with values of

Table 3. Amino acids content (mg/g) of the raw and fermented *Lenzites quercina*

Amino acids	Raw	Fermented
Alanine	3.98±0.00	6.54±0.00*
Arginine	8.86±0.00	12.93±0.40*
Aspartic	6.73±0.20*	3.98±0.00
Cystine	2.99±0.00	8.00±0.00*
Glutamic	0.06±0.00	0.05±0.00
Glycine	8.02±0.00	11.60±0.00*
Histidine	0.76±0.00*	0.49±0.00
Isoleucine	0.49±0.00	3.57±0.00*
Leucine	5.39±0.00	8.83±0.00*
Lysine	2.15±0.00	5.90±0.00*
Methionine	3.02±0.00	2.03±0.00
Phenylalanine	2.08±0.00	1.87±0.00
Proline	23.78±0.00*	21.29±0.00
Serine	2.20±0.00	7.97±0.00*
Threonine	0.86±0.00	0.76±0.00
Tyrosine	1.92±0.00	2.05±0.00*
Tryptophan	0.90±0.00	3.70±0.00*
Valine	3.61±0.00	5.04±0.00*

Values are mean±SD of replicates (n=3).
*Significantly different within a row by independent *t*-test (P< 0.05).

Table 4. Fatty acids content (%) of the raw and fermented *Lenzites quercina*

Fatty acids	Number of carbon	Raw	Fermented
Butyric acid	C4:0	0.95±0.00	0.81±0.00
Caporic acid	C6:0	0.16±0.00	0.23±0.00*
Caprylic acid	C8:0	4.05±0.00	4.61±0.00
Capric acid	C10:0	2.89±0.00*	2.19±0.00
Lauric acid	C12:0	22.70±0.10*	18.71±0.30
Myristic acid	C14:0	1.57±0.00*	1.18±0.00
Myristoleic	C14:1	6.84±0.00*	5.73±0.00
Palmitic acid	C16:0	4.40±0.00	4.11±0.00
Palmitoleic acid	C16:1	2.52±0.00	2.14±0.00
Margaric acid	C17:0	4.83±0.00	4.50±0.00
Stearic acid	C18:0	11.96±0.20	16.99±0.30
Oleic acid	C18:1	38.50±0.70	37.92±0.00
Linoleic	C18:2	7.91±0.00	12.48±0.40*
Linolenic	C18:3	17.40±0.00	22.50±0.00*
Behenic acid	C22:0	0.14±0.00	0.20±0.00*
Erucic acid	C22:1	0.11±0.00	0.20±0.00
Arachidonic acid	C22:4	1.80±0.00*	1.37±0.00
Lignoceric	C24:0	11.16±0.20	10.83±0.40

Values are mean±SD of triplicates (n=3).
*Significantly different within a row by independent *t*-test ($P<0.05$).

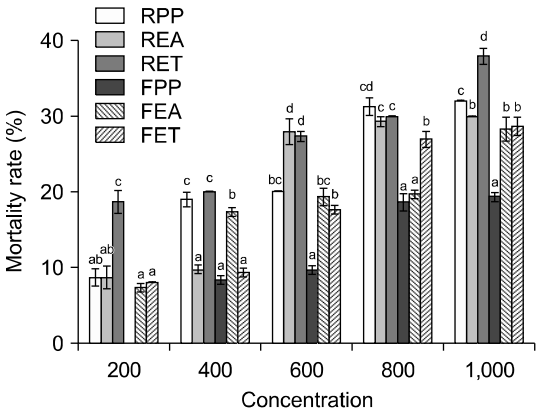

Fig. 2. Mortality rate of the *Artemia salina* larvae against extracts of *Lenzites quercina*. Value with different letters (a-d) at each concentration (200~1,000 μg/mL) are significantly different by Duncan's test ($P<0.05$). Error bar represents standard deviation. RPP, raw *L. quercina* extracted with petroleum ether; REA, raw *L. quercina* extracted with ethyl acetate; RET, raw *L. quercina* extracted with ethanol; FPP, fermented *L. quercina* extracted with petroleum ether; FEA, fermented *L. quercina* extracted with ethyl acetate; FET, fermented *L. quercina* extracted with ethanol.

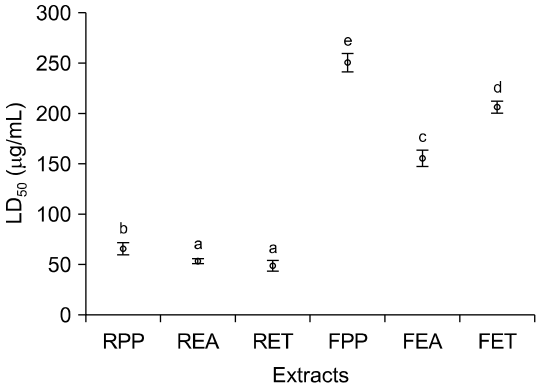

Fig. 3. Lethal dose of extracts (μg/mL) that killed 50% (LD_{50}) of *Artemia salina* larvae. Different letters (a-e) are significantly different from each other ($P<0.05$) by Duncan's new multiple range test. Error bar represents SD. RPP, raw *L. quercina* extracted with petroleum ether; REA, raw *L. quercina* extracted with ethyl acetate; RET, raw *L. quercina* extracted with ethanol; FPP, fermented *L. quercina* extracted with petroleum ether; FEA, fermented *L. quercina* extracted with ethyl acetate; FET, fermented *L. quercina* extracted with ethanol.

38.5% and 37.92%, respectively (Table 4). The essential fatty acids, linoleic (12.48%) and linolenic (22.50%), in fermented *L. quercina* increased and were significantly different ($P<0.05$) from raw *L. quercina*. The mortality rate of brine shrimp against extracts at different concentrations is shown in Fig. 2. The mortality rate ranged from 8.0% to 38.0%, which is proportional to the concentration of the extracts from raw and fermented *L. quercina*, that is, in a dose-dependent relationship. The value obtained for LD_{50} is within 49.11 and 250.50 μg/mL (Fig. 3).

DISCUSSION

The potential uses of microbial metabolites in food production is expanding with varying technical improvements to meet nutritional demands. This study assessed the food constituents of raw and fermented *L. quercina*, which can be used as functional ingredient to supplement food products. The crude fibre content in the raw and fermented *L. quercina* is similar to the findings of Di Anibal et al. (15) and Cheung (16). The researchers have revealed that wild and commercial edible mushrooms are source of crude fibre. Higher basidiomycetes have much insoluble dietary fiber bound with chitin, hemicellulose, mannans, glucans, glycogen, and trehalose in their cell wall (17). Mushrooms' cell wall components contain non-digestible carbohydrate and fiber that can be adequately utilized to augment the nutritional content of some low fibre foods. The findings of Cheung (16) and Fernandes et al. (18) reported the health benefits of dietary fibre, which include the following: constipation relief, prevention of colon disease, and hemorrhoids as well as maximizing the viscosity of the food matrix, slowdown of digestion, lower blood glucose, and strengthens immune system with antitumor activity. Hence, mushrooms are excellent source of dietary fiber that can be used for the enrichment of biopharmaceutical products.

In this study, there was a marked increase in protein but a reduction in carbohydrate content in fermented *L. quercina*. This could be as a result of the release of protein bound in polysaccharides in the cell wall of mush-

rooms, which can increase the protein content. Mushrooms are known to possess complexes of polysaccharides and proteins (19), which enhance innate and cell-mediated immune responses, and exhibit antitumor activities in animals and humans (20).

Essential metals such as Zn, Fe, Mn, and Cu are present in both raw and fermented mushrooms, and these minerals were reported in wild and medicinal mushrooms from Ghana (21). The researchers highlighted the importance of minerals as constituent of metalloenzymes, which are involved in biochemical processes such as haemoglobin synthesis and catalysis of metabolic growth. Calcium and potassium were abundantly present in raw and fermented *L. quercina*. These elements are known to regulate blood pressure and maintain cellular functions (22). The bioavailability of macro and micro elements in medicinal mushrooms could promote their uses for health benefits. Mushrooms are known to possess a very effective mechanism that enable them to readily take up some metals from the ecosystem (23) and can be supplemented into low mineral diets. The zinc and iron contents of fermented *L. quercina* were increased ($P<0.05$), which indicates a substantial change in the mineral composition of the mushrooms during fermentation improving the bioavailability of minerals. Fermentation had played a pivotal role in human food production, nutritional supplementation, and health promotion (24).

The raw and fermented *L. quercina* contain a considerable amount of essential and non-essential amino acids, similar amino acids have been reported in some edible and medicinal mushrooms (25). These amino acids are regarded as valuable components of functional foods (26). The findings of Wu (27) highlighted the usefulness of amino acids in humans and in the formulation of animal feeds. Amino acids are involved in the synthesis of melanin, serotonin, and transportation of ammonia into the liver and kidneys to aid the production of urea (28). Thus, amino acids are the building blocks of proteins as well as enzymes for the normal function during growth and convalescence.

The presence of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs) in raw and fermented *L. quercina* conformed to the findings of Obodai et al. (21), Bisen et al. (29), and Ribeiro et al. (30). The researchers revealed the food values of fatty acids in some wild and edible mushrooms. The higher content of oleic acid in raw and fermented *L. quercina* agreed with the findings of Ergonul et al. (31) who reported that PUFAs predominated over MUFAs and SFAs in mushrooms. The essential fatty acid linoleic acid is the precursor of 1-octen-3-ol, which has been the principal active compound that contributed to the aroma and flavour of mushrooms (32,33). The raw and fermented *L. quercina* contain essential amino acids and fatty acids such as isoleucine, leucine, lysine, tyrosine, tryptophan, valine, linoleic, and linolenic, which cannot be synthesized in the human body but required to be injected from external sources. Mushrooms are well-proportioned with required amounts of amino acids, fatty acids, minerals, and vitamins (34). Hence, the wide acceptance of wild and medicinal mushrooms in dietary management requires safety assessment.

The preliminary toxicological bioassay of *L. quercina* demonstrate lower mortality rate against larvae of *A. salina* (<40.00 %) at 1,000 μg/mL. The finding is similar to the study of Oyetayo et al. (35). The researchers revealed that ethanolic extract of *Lenzites betulina* and *Trametes versicolor*, respectively exhibited low mortality of 35.48% and 55.66% against larvae of *A. salina* at 1,000 μg/mL. The extracts of *L. quercina* have LD_{50} values less than 1,000 μg/mL. Low mortality at 1,000 μg/mL with LD_{50} values <300 μg/mL were obtained in this study. The extract obtained from fermented *L. quercina* exhibited the higher ($P<0.05$) lethality concentrations of 155.6 to 250.5 μg/mL when compared to the raw *L. quercina* (51.8 to 53.5 μg/mL), this showed that bioactive compounds in the fermented mushroom sample are safe since it can be easily utilized by brine shrimp larvae. Fermentation is a food processing method that reduces the toxic compounds of food products and enhances the availability of essential metabolic products. Therefore, food processing techniques may improve the extraction, yields, and quality of bioactive compounds (36).

Conclusively, food components such as amino acids, fatty acids, and minerals in the examined *L. quercina* can be exploited as food supplements and for the development of biopharmaceuticals, since it contained some biologically compounds that are safe.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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