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Nutritional and bioactive potential of *Amanita ochracea*: Unlocking the health benefits of a wild edible mushroom

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Abstract
This study evaluates the proximate composition, mineral content, antioxidant activity, anti-nutrient levels, and phytochemical constituents of *Amanita ochracea*, a wild edible mushroom. The proximate composition revealed 6.82% ash, 89.447% carbohydrates, 1.204% protein, 2.533% fat, and 385.988 kcal/100g energy. Mineral content included 0.388 mg/g sodium, 44.6 mg/g potassium, and 42.267 mg/g calcium. Antioxidant tests demonstrated significant activity, with a total phenolic content (TPC) of 6.264 mg GAE/g, total flavonoid content (TFC) of 0.326 mg RE/g, DPPH radical scavenging activity of 9.262%, ABTS scavenging activity of 56.166%, and mineral chelation activity of 76.301%. The mushroom also contained anti-nutrients such as saponins (37.559 mg/g), tannins (9.983 mg/g), and oxalates. Phytochemical analysis revealed high levels of vitamin C (899.688 µg/100g), various B-vitamins, phenolic acids like gallic acid (197.582 µg/100g), and flavonoids such as catechin (7.329 µg/100g) and kaempferol (109.674 µg/100g). These findings suggest that *A. ochracea* sis a rich source of essential nutrients, antioxidants, and bioactive compounds, positioning it as a valuable candidate for further exploration as a functional food and nutraceutical with significant health benefits.

Keywords: *Amanita ochracea*, wild edible mushroom, antioxidant activity, phytochemicals, mineral content, functional food

Introduction

Wild edible mushrooms, often referred to as "forest meat," provide high-quality protein with essential amino acids and low-fat content, making them a vital protein source in impoverished areas where other protein-rich foods are scarce (Ambhore *et al*., 2024) [2]. These mushrooms also serve as sustainable, accessible alternatives to animal protein (Foht, 1990; Redzic *et al*., 2010) [14, 31]. Additionally, mushrooms are valued for their bioactive compounds, including organic acids, alkaloids, terpenoids, steroids, and phenolics, which contribute to antioxidant, immunomodulatory, anti-inflammatory, and cholesterol-lowering effects, enhancing their role as functional foods with disease-preventive properties (Kosanić *et al*., 2016) [18]. The current study highlights *Amanita ochracea* (Zhu L. Yang) Y.Y. Cui, Q. Cai & Zhu L. Yang, a wild edible mushroom from the Amanitaceae family. This ectomycorrhizal species thrives in temperate and subalpine coniferous forests of the Himalayan region. Notable for its large fruiting body, ochre-olive cap, free yellowish gills, and prominent basal bulb with a white volva, *A. ochracea* is easily recognized by foragers.

Field studies in West Bengal, Sikkim, and Himachal Pradesh confirm the abundance of *A. ochracea*, which serves as a key protein source for impoverished communities (Anand *et al*., 2024) [3]. Rich in bioactive compounds, vitamins, and minerals, it offers antioxidant and immunomodulatory benefits, positioning it as a functional food with potential to improve nutrition and health in resource-poor areas. Further research could unlock its role in addressing malnutrition and chronic diseases.

Materials and Methods

1. Plant material

The fresh *Amanita ochracea* samples were collected and identified by one of the authors (KD). The voucher specimens were preserved in our office. One portion of the samples were stored at -20^{0}C and processed for vitamin estimation. The other part was dried in shade, pulverized and stored in an airtight container to evaluate the antioxidant properties and quantitation of phenolics and polyphenolics by HPLC.

2. Proximate composition

The proximate composition of mushrooms was analyzed on a dry weight basis using standard methods (AOAC, 2000) [4]. Moisture, ash, crude protein (via Kjeldahl method), crude fat (via Soxhlet extraction with petroleum ether), and crude fiber were determined. Carbohydrate content was calculated by subtracting ash, protein, and fat from 100 g (Barros *et al*., 2007) [7]. Energy content was estimated using protein, fat, and carbohydrate values with factors of 4.00, 9.00, and 4.00, respectively (AOAC, 2000) [4].

3. Estimation of minerals

Minerals in dried mushrooms were analyzed by preparing sulphated ash through muffle furnace heating, followed by dissolution in 5% HCl. Elemental analysis was done using atomic absorption spectroscopy (AAS) with standard solutions (Indrayan *et al*., 2005) [17].

4. HPLC analysis for water soluble vitamins

HPLC analysis was performed using a Dionex Ultimate 3000 liquid chromatograph with a DAD and Chromeleon system (Seal *et al*., 2018) [32]. Samples were separated on a reversed-phase Acclaim C18 column (5 µm, 250 × 4.6 mm) at 22°C using acetonitrile (Solvent A) and 0.01% TFA (Solvent B) in a gradient elution. Freeze-dried mushroom extract (prepared in phosphate buffer) and standard vitamin solutions (C, B1, B2, B3, B5, B6, B9) were injected (20 µl). Vitamins were detected at 210, 245, 275, and 290 nm using retention times from standards (Seal *et al*., 2018) [32].

5. Antioxidant properties

5.1 *Preparation of plant extracts*

Powdered fruit body (10g) were extracted twice with 70 % aq. ethanol at room temperature, each time with agitation for 18–24 hours. The first and succeeding extractions' concentrates were mixed and concentrated in a rotary evaporator at reduced pressure to produce viscous extracts, which was dried with a freeze drier. The dried extracts (2.5g) from the solvent was stored at minus (-) 20°C. The weight of air-dried plant material was used to compute the percent yield.

5.2 Estimation of total phenolic content (TPC) and total flavonoid content (TFC)

The total phenolic content mushroom extract was determined according to Folin-Ciocalteu procedure (Singleton and Rossi, 1965) [38] and was calculated as gallic acid equivalent (GAE) in mg/g dry weight of extract. Total flavonoid content was estimated using the method of Ordonez *et al*. (2006) [25] and was calculated as rutin equivalent (RE) mg/ g dry weight of extract.

5.3 Determination of DPPH and ABTS free radical scavenging activity

The free radical scavenging activity of the plant samples was determined using the stable radical DPPH (1,1-diphenyl-2 picrylhydrazyl) (Blois, 1958) [8]. The 2,2'-azino-bis (3 ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation (ABTS. +)-scavenging activity was measured according to the method described by Re *et. al*. (1999) [30]. The capability to scavenge the DPPH/ ABTS radical was calculated, using the following equation: DPPH/ ABTS scavenged (%) = {(Ac – At)/Ac} × 100; where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the extracts.

5.4 Metal chelating activity

The process of Lin *et al*. (2009) [20] was followed for determination of metal chelating activity. The inhibition percentage of ferrozine–Fe^{+2} complex formation was calculated by using the formula given below: Chelating ability (%) = {(Ac – At)/Ac} × 100 Where Ac is the absorbance of the control reaction and at is the absorbance in presence of the sample of the extracts.

5.5 Estimation of phenolic acids, flavonoids using HPLC

Phenolic acids and flavonoids were quantified in plant extracts using HPLC as per Datta *et al*. (2019) [10]. Standard solutions (1 mg/ml) of phenolic acids and flavonoids were prepared in methanol, diluted with the mobile phase, and filtered through a 0.45 µm PVDF filter. Samples were analyzed on a Dionex Ultimate 3000 HPLC with a DAD, using a reversed-phase Acclaim C18 column (5 µm, 250 × 4.6 mm) at 25°C. Gradient elution employed methanol (Solvent A) and 0.5% acetic acid (Solvent B). Compounds were detected at 272, 280, and 310 nm, quantified via calibration curves, and results were validated.

6. Estimation of antinutritional composition:

Oxalate contents of edible plants were determined using the method described by Munro and Bassir (1969) [24]. Saponin was determined using the method of Hudson and El-Difrawi (1979) [16]. Tannins were assayed in accordance with the modified vanillin-HCl method of (Price *et. al*., 1978) [28] and catechin was used as the reference standard.

Result and Discussion

1. Proximate composition

The results presented in Table 1 show the percentage of ash, moisture, protein, crude fat, and carbohydrate content of the edible mushroom under investigation. Mushrooms are an excellent source of high-quality proteins, surpassing many fruits and vegetables in protein content (Rai and Arumuganathan, 2005) [29]. This study found a protein content of 1.204 ± 0.044% in the studied mushroom species, consistent with their nutritional Mushrooms are an excellent source of high-quality proteins, surpassing many fruits and vegetables in protein content (Rai and Arumuganathan, 2005) [29]. This study found a protein content of 1.204 ± 0.044% in the studied mushroom species, consistent with their nutritional potential. Wild edible mushrooms from Northeast India, such as *Pleurotus cystidiosus* (3.10%), *Lentinus cladopus* (2.36%), and *Pleurotus pulmonaris* (1.4%), exhibit diverse protein levels, with *Pleurotus cystidiosus* showing the highest (Atri *et al*., 2018) [6].

Compared to common fruits like apples (0.3%) and oranges (1.0%) or vegetables like potatoes (1.6%) and carrots (4%), *A. ochracea* offer superior protein content (FAO/WHO, 1989) [13]. This makes them a valuable plant-based protein source, particularly in regions where animal protein consumption is limited by cultural, economic, or environmental factors.

Table 1: Proximate composition, minerals, vitamins, antioxidant and antinutrients in *Amanita ochracea*

Proximate composition	**Ash (%)**	**Carbohydrate (%)**		**Protein (%)**		**Fat (%)**	**Energy (kcal/100g)**		
Amount	6.82 ± 0.095	89.45 ± 2.43		1.204 ± 0.026		2.533 ± 0.003	64.723 ± 0.088		
Minerals	**Sodium (Na)**	**Potassium (K)**	**Calcium (Ca)**						
Amount (mg/g)	0.388 ± 0.082	44.6 ± 0.208	42.267 ± 0.0.145						
Vitamin	**C**	**B1**	**B6**	**B5**	**B9**	**B2**		**B12**	
(µg/100g)	899.688 ± 0.007	161.933 ± 0.007	39.053 ± 0.012	82.545 ± 0.009	10.553 ± 0.0012	1.237 ± 0.009		36.025 ± 0.012	
Antioxidant profile	**TPC (mg GAE/g**	**TFC (mg RE/g)**	**% DPPH radical scavenging activity**		**% ABTS radical scavenging activity**		**% Metal chelating activity**		
Amount	6.264 ± 0.068	0.326 ± .009	9.262 ± 0.095		56.166 ± 0.245		76.301 ± 0.111		
Phenolic acids/flavonoids	**Gallic acid**	**Protocatechuic acid**	**Gentisic acid**	**p-Hydroxy benzoic acid**	**Chlorogenic acid**	**Vanilic acid**	**Caffeic acid**	**Syringic acid**	**p-Coumaric acid**

Amount (µg/100g)	197.582 ± 0.004	1.966 ± 0.012	0.049 ± 0.003	22.7382 ± 0.007	0.983 ± 0.008	0.078 ± 0.004	3.671 ± 0.007	13.012 ± 0.007	0.658 ± 0.005
Phenolic acids/flavonoids	**Ferulic acid**	**Sinapic acid**	**Catechin**	**Rutin**	**Myricetin**	**Quercetin**	**Apigenin**	**Kaempferol**	
Amount (µg/100g)	0.052 ± 0.001	0.719 ± 0.006	7.329 ± 0.003	0.009 ± 0.003	0.003 ± 0.002	39.085 ± 0.006	1.780 ± 0.005	109.674 ± 0.007	
Antinutrient profile	**Saponin**		**Tannin**		**Oxalate**				
Amount mg/g	37.559 ± 0.051		9.983 ± 0.727		1.35±0.27				

Each value is calculated as an average of three experiment and represented as Mean ± SEM

Mushrooms are valued for their low-fat content and favorable lipid profile, being rich in polyunsaturated fatty acids (PUFAs) and free from harmful trans fats, enhancing their appeal as a healthy food (Barros *et al*., 2007) [7]. This study found *A. ochracea* to have a fat content of 2.533 ± 0.003%, higher than some wild edible mushrooms like *Termitomyces eurrhizus* (1.8%) and *Pleurotus cystidiosus* (1.2%) from Northeast India but still classifying it as a low-fat food. Compared to other *Amanita* species like *A. muscaria* and *A. virosa*, *A. ochracea* exhibited relatively higher fat content, possibly due to species and environmental differences (Valverde *et al*., 2015; Sharma and Gautam, 2015) [37, 40]. Low-fat foods like *A. ochracea* reduce caloric density and help manage weight and chronic diseases such as cardiovascular issues and obesity.

Mushroom carbohydrate content varies by species, influencing dietary uses. High-carbohydrate content in studied mushrooms (89.45%), compared with other mushrooms like *Lentinus cladopus* (89.10%), and *Pleurotus cystidiosus* (85.86%), provide energy and β-glucans, offering gut health and immunity benefits. Lower-carb species like *Lactarius deliciosus* (60.30%) suit low-calorie diets, emphasizing species-specific nutritional profiling (Atri *et al*., 2018; Barros *et al*., 2007) [6, 7].

Ash content in mushrooms can reach up to 9% (Mattila *et al*., 2001) [22], influenced by season and developmental stage (Assemie & Abaya, 2022) [5]. The studied species showed 5.40 ± 0.025% ash and a calorific value of 385.988 ± 0.366 kcal/100g, aligning with the typical range of 325–770 kcal/100g (Valverde *et al*., 2015) [40].

Minerals estimation

The *Amanita* species studied shows a rich mineral profile, with notable levels of potassium (44.6 mg/g), calcium (42.267 mg/g), and sodium (0.388 mg/g). In comparison, other plants like *Pleurotus ostreatus* (Na: 1.36 mg/g, K: 26.82 mg/g, Ca: 23.5 mg/g) and *Pleurotus pulmonarius* (Na: 1.03 mg/g, K: 28.18 mg/g, Ca: 19.1 mg/g) (Manzi *et al*., 1999) [21] have lower potassium and calcium content. The high potassium and low sodium ratio in *A. ochracea* makes it beneficial for heart health, helping to lower blood pressure (Yusuf *et al*., 2007) [43]. Its calcium content also supports bone health and may reduce the risk of osteoporosis (Weaver, 2013) [42].

Estimation of vitamin

The HPLC chromatograms for the investigated mushroom (Fig. 1) revealed the presence of various water-soluble vitamins separated at 275 nm, as detailed in Table 1. HPLC analysis of the investigated mushroom, *A. ochracea*, revealed a range of essential water-soluble vitamins, including vitamin C (899 ± 0.007 µg/100g), vitamin B1 (161.933 ± 0.009 µg/100g), B3 (60.142 ± 0.009 µg/100g), and B5 (82.545 ± 0.009 µg/100g). Vitamin C is a potent antioxidant that boosts immunity, neutralizes free radicals, and aids collagen synthesis. Vitamin B1 is vital for energy production, carbohydrate metabolism, and nerve function, while B5 is key for fatty acid metabolism and energy production. Vitamin B3 supports DNA repair, maintains skin health, and regulates fat metabolism (FAO/WHO, 2001) [12]. Additionally, the presence of vitamin B12, typically found in animal-based foods, is significant as it supports amino acid metabolism, DNA synthesis, and neurological function (Watanabe *et al*., 2014) [41]. This makes the mushroom an excellent dietary source to combat vitamin B12 deficiency, especially among vegetarians and vegans. The presence of these vitamins enhances the mushroom's potential as a nutrient-dense, affordable food source for addressing malnutrition in underserved populations.

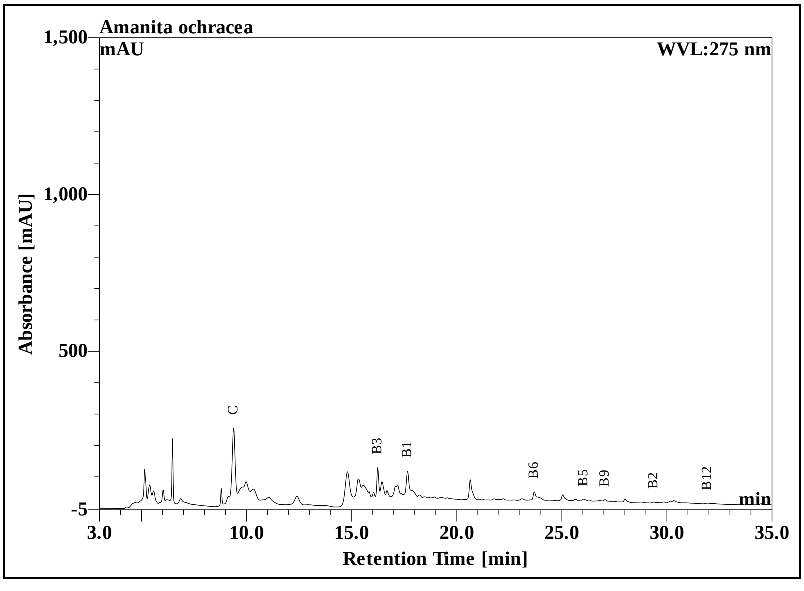

Fig 1: HPLC Chromatogram of showing water soluble vitamins in *A. ochracea*

Antioxidant activity

4.1. Total phenolic and flavonoid content

The results of antioxidant parameters studied are represented in Table 1. The antioxidant potential of *A. ochracea* was assessed by examining its phenolic and flavonoid content. The phenolic content of *A. ochracea* (6.238 ± 0.068 mg GAE/g) was significantly higher than other *Amanita* species, such as *A. calyptroderma* (0.83 mg GAE/g) and *A. princeps* (2.66 mg GAE/g) (Srikram & Supapvanich, 2016) [39], indicating strong antioxidant activity. This aligns with previous findings indicating a positive correlation between phenolic content and antioxidant activity (Pan *et al*., 2008) [27]. Flavonoid content in *A. ochracea* (0.326 ± 0.009 mg RE/g) was lower than *A. odoratus* (2.05 mg RE/g) but comparable to *A. calyptroderma* (0.37 mg RE/g) (Srikram & Supapvanich, 2016) [39]. Despite the lower flavonoid levels, the combined phenolic and flavonoid profile of *A. ochracea* suggests its significant antioxidant potential, supporting its use in functional foods and nutraceuticals (Datta *et al*., 2022) [10].

4.2 Radical scavenging activity and metal chelating

A. ochracea exhibited significant antioxidant activity, with 9.262 ± 0.095% scavenging for DPPH radicals and 56.165 ± 0.254% for ABTS radicals. This activity can be attributed to the high phenolic and flavonoid content of the species, both of which are known contributors to antioxidant capacity (Kumala *et al*., 2023) [19]. In addition to its radical scavenging ability, *A. ochracea* also demonstrated strong metal chelation, with a property of 76.301 ± 0.110%. This metal chelating ability is crucial as it helps prevent oxidative damage by binding to metal ions, particularly iron and copper, which are key initiators of lipid peroxidation and contribute to the formation of free radicals linked to diseases such as cancer and arthritis (Datta *et al*., 2019) [11]. These findings highlight *A. ochracea*'s potential as a natural source of antioxidants with important applications in health-promoting foods and nutraceutical development.

4.3 HPLC based identification and quantification of phenolic acids and flavonoids

The HPLC chromatograms for the investigated mushroom (Fig. 2) revealed the presence of 18 phenolic acids and flavonoids in *A. ochracea*, detected at 280 nm, as represented in Table 2. The diverse array of phenolic acids and flavonoids identified in this species plays a significant role in its antioxidant properties, contributing to its overall health benefits. Similar to other edible mushrooms such as *Boletus edulis*, *Cantharellus cibarius*, *Lactarius deliciosus*, *Pleurotus ostreatus*, *Agaricus bisporus*, and *Lentinus edodes*, which contain various phenolic acids like gallic acid, protocatechuic acid, p-hydroxybenzoic acid, p-coumaric acid, ferulic acid, sinapic acid, vanillic acid, and cinnamic acid as major phenolic compounds (Cheung *et al*., 2003; Ozen *et al*., 2011; Mattila *et al*., 2001) [9, 26, 22], *A. ochracea* also presents a rich profile of these bioactive compounds.

Among the identified compounds, gallic acid and kaempferol were the most dominant, followed by p-hydroxybenzoic acid and quercetin. Gallic acid, a potent antioxidant, is typically present in free or esterified forms and is known for its strong free radical scavenging activity, which contributes to its protective effects against oxidative stress (Seal *et al*., 2022) [34]. Kaempferol, another key antioxidant, is recognized for its ability to prevent the oxidation of low-density lipoprotein (LDL) proteins, suggesting its potential in the prevention of atherosclerosis, a major cardiovascular condition. p-Hydroxybenzoic acid is noted for its anti-mutagenic, anti-sickling, estrogenic, and antimicrobial properties, making it a versatile compound with various therapeutic applications. Quercetin, a widely studied flavonoid, is celebrated for its anti-cancer, anti-inflammatory, and anti-viral properties, further enhancing the medicinal value of the mushroom (Datta *et al*., 2019) [11].

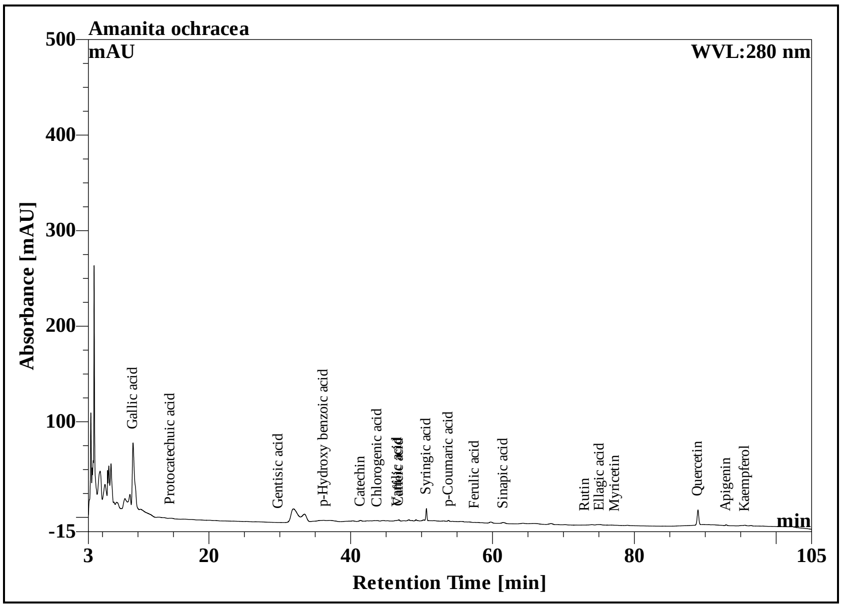

Fig 2: HPLC Chromatogram of phenolic acids and flavonoids in *A. ochracea*

The bioactive compounds in *A. ochracea*, including gallic acid, kaempferol, p-hydroxybenzoic acid, and quercetin, highlight its nutritional and medicinal value. These compounds contribute to its antioxidant, anti-inflammatory, and protective properties, positioning *A. ochracea* as a promising source for nutraceuticals and health-promoting food products.

Antinutrient composition

Tannins, cyanogenic glycosides, oxalates, and phytates are antinutrient components that can significantly hinder the bioavailability of essential nutrients, limiting their absorption and utilization in the body (Sengupta *et al*., 2023) [35]. The content of these antinutrients in the fruiting bodies of *A. ochracea* is presented in Table 1. *A. ochracea* contains several antinutrients, including oxalates (1.35 mg/g), tannins (9.983 ± 0.727 mg/g), and saponins (37.559 ± 0.051 mg/g), which can reduce the bioavailability of essential nutrients. Oxalates can interfere with calcium absorption by forming insoluble complexes (Muhammed *et al*., 2002; Sengupta *et al*., 2023) [23, 35], while tannins bind to proteins and inhibit iron absorption, which may affect individuals with iron-deficiency anemia (Abbas & Ahmad, 2018) [1]. Saponins, although inhibitory to digestive enzymes, also possess beneficial properties such as hypolipidemic, hypoglycemic, and antioxidant activities (Sharma *et al*., 2023) [36]. The FAO/WHO guidelines suggest a safe intake of saponins between 0 mg and 1 mg per kg of body weight daily, and the saponin content in *A. ochracea* falls within this safe range. Despite the presence of these antinutrients, the relatively low concentrations and the therapeutic benefits of saponins make *A. ochracea* a balanced food source when consumed in moderation, offering a range of potential health benefits (Sengupta *et al*., 2023) [35].

Conclusion

Edible mushrooms like *A. ochracea* offer nutritional benefits, including protein, carbohydrates, and fatty acids, along with medicinal properties from secondary metabolites, making them potential functional foods. However, accurately identifying edible species remains challenging and requires expertise and traditional knowledge to ensure safe consumption.

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