Molecular Identification, Proximate Composition and Functional Properties of *Lentinus* Species in Keana Local Government Area, Nasarawa State, Nigeria

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DOI: https://dx.doi.org/10.51244/IJRSI.2026.1315PH00002

Received: 19 December 2025; Accepted: 27 December 2025; Published: 07 January 2026

ABSTRACT

Despite the extensive study on plant and animal biodiversity in Nasarawa state, our knowledge of microbial diversity in general and macrofungal diversity in particular is very limited. *Lentinus* species collected from Keana Local Government Area, Identification was based on morphological characteristics and ITS region rRNA sequences. Based on the phylogenetic analyses the *Lentinus* species collected was *Lentinus squarrosulus*. The proximate composition, phytochemical and functional properties were investigated using standard analytical techniques. The nitrogen from soil were *Lentinus squarrosulus* was harvested ranging from 10.1114 ± 2.01 mg/g, phosphorus 8.9562 ±1.11 mg/g, potassium2.1202 ±0.20 mg/g), calcium 1.6423 ±0.13 mg/g and magnesium 0.3415 ±0.21 mg/g. The nutritional compositions of *Lentinus squarrosulus* were moisture 7.54 %, ash 4.15 %, fat 2.52 %, crude protein 21.61 %, crude fiber 5.22 % and carbohydrate 58.96 %. The functional properties were foaming capacity 131.6 %, foaming stability 56.1 %, water absorption 262.0 %, oil absorption 460.2 %, oil emulsion 61.3 mLg^{-1}, Oil emulsion stability 41.0 mLg^{-1} gelation concentration 14.0 % and bulk density 413.5 gL^{-1}. The phytochemical components were flavonoids 12.67mg/g, saponins 0.95mg/g, proteins 9.41 mg/g, alkaloids 7.57 mg/g, tannins 6.55 mg/g, steroids (1.43 mg/g), glycosides 2.99 mg/g, terpenoids 3.11 mg/g, phenols 6.11 mg/g, carbohydrates 8.78 mg/g, inorganic acid 1.34mg/g, and organic acid 9.41 mg/g. The results showed that these nutrients rich mushroom studied may prove useful in the formulation of different food products.

Key words: Phytochemical and functional properties, morphological characteristics, nutrients

INTRODUCTION

Mushrooms are the fleshy spore-bearing fruiting bodies of fungi, typically produced above ground on soil or on its food source (substrate). Based on standard morphology, the word "mushroom" would be mostly used to describe those fungi that have a stem (stipe), a cap (pileus), and gills (lamellae) or pores on the underside of the cap e.g. (*Basidiomycota* and *Agaricomycetes),* However, it generally refers to a variety of gilled fungi, with or without stems [1]. Mushrooms are also described as macro-fungi with a distinctive fruiting body, which can be either epigeous or hypogeous and large enough to be seen with the naked eyes and to be picked by hand. Only fruiting body of the mushroom can be seen whereas the rest of the mushroom remains underground as mycelium [2].

During the early days of civilization, mushrooms would be consumed mainly for their palatability and unique flavors. However, several research works on the chemical composition of mushrooms have revealed that mushrooms can be used as a diet to combat diseases making the present use of mushrooms to be totally different from the traditional use [3]. Many research reports have described the nutritional compositions of mushrooms as attractive, being good sources of dietary protein, carbohydrate, fats, vitamins, fiber and minerals [4, 5]

Edible mushrooms are consumed by humans for their nutritional and medicinal values. Mushrooms consumed for health reasons are known as medicinal mushrooms. While hallucinogenic mushrooms (e.g., Psilocybin mushrooms) are occasionally consumed for recreational or religious purposes, they can produce severe nausea and disorientation, and are therefore not commonly considered edible mushrooms [6].

Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivatable and common wild mushrooms are often available in markets, and those that are more difficult to obtain (such as the prized truffle and matsutake) may be collected on a smaller scale by private gatherers. Some preparations may render certain poisonous mushrooms fit for consumption [7].

Mushrooms are also easily preserved, and historically have provided additional nutrition over winter. Many cultures around the world have either used or continue to use psilocybin mushrooms for spiritual purposes as well as medicinal mushrooms in folk medicine. Some fungi consumed by humans are currently cultivated and sold commercially. Commercial cultivation is important ecologically, as there have been concerns of depletion of larger fungi such as chanterelles in Europe, possibly because the group has grown so popular, yet remains a challenge for cultivation [7].

MATERIAL AND METHODS

Methods

Study Area

This was carried out in keana**,** Keana Local Government Area is situated in the town of Keana, is well known for its Salt Village which is located 100 kilometers away from Lafia**.** keana has an area of 1,048.1 km^2 and a population of about 80,000. t is home to Federal Government Girls College, Keana. [8].

Field Collection and Morphology identification of *Lentinus squarrosulus* samples

Lentinus squarrosulus were collected from Keana Local Government Area. The various species of *Lentinus squarrosulus* collected from Keana Local Government Area, Nasarawa State was transported to the Department of Microbiology, Nasarawa state university Keffi for physical identification and Microscopical identification was made from specimens mounted in 5% KOH and stained with 1% aqueous Melzer`s reagent and Congo red. Sections of pileus, lamellae and context was prepared with a razor blade and then observed under light microscope. At least twenty basidiospores was measured from each specimen.

physiochemical analysis of the soil

Determination of Potassium (K) and Sodium (Na)

The soil samples were weighed in an appropriate amount in a beaker and 1m ammonium acetate was added into the beaker and stirred gently. The soil-extraction solution mixture was allowed to stand for 30 minutes to extract the K and Na using filter paper. The concentration of K and Na in the filtered extract was measured using flame photometry [9].

Determination of Calcium (Ca) and Magnesium (Mg)

The prepared soil samples were weighed in an appropriate amount in a beaker. 1m ammonium acetate was added into the beaker and stirred gently. The soil-extraction solution mixture was allowed to stand for 30 minutes to extract the Ca and Mg using filter paper. The concentration of Ca and Mg in the filtered extract was measured using atomic absorption spectroscopy [9, 10].

Determination of Phosphate (P)

Phosphate was determined using portions of the soil samples which were weighed into a beaker. A 0.5m of NaHCO3 extraction solution was added to the beaker and stirred gently. The soil-extraction solution mixture was allowed for 30 minutes to extract the P which was filtered using a filter paper. The concentration of P in the filtered extract was measured using colorimetry [11].

Phytochemical analysis of the

The extract was subjected to qualitative phytochemical analysis to identify bioactive compounds in the sample using standard procedure described by [12].

Test for carbohydrates

a. General test (Molisch's) few drops of Molisch reagent was added to the 1 ml of the extract solution, followed by addition of 1 ml of conc. sulphuric acid down the side of the test tube. The mixture was allowed to stand for 2 minutes and diluted with 5 ml of distilled water. The appearance of red color at the interphase of two layers, confirm the presence of carbohydrate.

b. Barfoed's test (test for sugar)

The ethanol extract (0.5 g) was dissolved in distilled water in a test tube and 1 ml of distilled water containing in a test tube, 1 ml of Barfoed's reagent was added. The mixture was heated on a water bath for 2 minutes. A red precipitate of Cu2O was observed which indicate the presence of sugars.

c. Test for reducing sugar (Fehling test) the ethanol extract (0.5 g) was in dissolved water in a test tube, equal volume (5 ml) of Fehling solution A and B was added and heat on a water bath. Red precipitate of Cu2O observed indicate the presence of reducing sugar.

d. Benedict's test the extract was treated with 2ml of benedicts reagent and was gently heated. the formation of an orange-red precipitate indicated the presence of reducing sugar.

Test for phenols

Ferric chloride tests the extract (0.5 g) was dissolved in ethanol in a test tube and few drops of 10 % ferric chloride (FeCl3) solution was added and boiled on a water bath. Violet colored was observed which indicated the presence of a phenolic hydroxyl group.

Lead acetate test the extract was mixed with a few drops of 10% lead acetate reagent. The presence of white precipitate was observed which indicated the presence of phenolic compounds.

Test for flavonoids

A method by [13] was adopted for ferric chloride test and alkaline reagent test.

Ferric chloride test 3ml of the extract was mixed with a few drops of 55vferric chloride. The presence of a dark green color indicated the presence of flavonoids.

Alkaline reagent tests a few drops of sodium hydroxide were added to the extract afterwards a dark yellow color was formed the a few drops of dilute acetic acid was added after which it became colorless which indicated the presence of flavonoids.

Test for tannins

Approximately (2 g) of ethanol extract was boiled in 10 ml of distilled water in a test tube on a water bath and cooled, the mixture was filtered and the filtrate was use for the following test. (A). To 1 ml of filtrate in first test tube, few drops of lead acetate was added to it in test tube, a white precipitate was observed which indicate the presence of tannins. (B). To 1 ml of the filtrate in second test tube, three drops of 1 % of ferric chloride (FeCl3) solution was added and the blue–black, green or blue-green precipitate was observed to show the presence of tannins. (c). Approximately (2 ml) of Ethanoic acid and 2 ml of 10 % lead acetate each was added to 5 ml of filtrate in third test tube and a white precipitate was observed which indicates the presence of tannins (d). Approximately 3 drops of 10 % HCl and 1 drop of methanol was added to 2 ml of filtrate in fourth test tube and mixture was boiled on a water bath. A red precipitate was observed which indicate the presence of tannins [13].

Test for alkaloids

Sample of methanol extract (1 g) was dissolved 5 ml of 1 % aqueous hydrochloric acid (HCl) in a test tube and the mixture was stirred on the water bath and filtered, 3 ml of the filtrate was taken and divided into 3 portions 1 ml each.

a. Dragendoff' s reagent to the 1 ml of the first portion of filtrate in a test tube Dragendoff' s reagent was added and orange red precipitate was observed which show the presence of alkaloid. b. Mayer's reagent to the 1 ml of the second portion of filtrate, Mayer's reagent was added and a buff coloration was observed which indicate the presence of alkaloid c. Wagner's reagent to the 1 ml of the third portion of filtrate, Wagner's reagent was added and a dark brown precipitate was observed. The colors changed observed in a, b and c, confirmed the presence of alkaloid

Test for steroids

Libermann Burchard test Approximately (0.5 g) of extract was dissolved in 2 ml of chloroform in a test tube and filtered. Acetic acid (2 ml) was added to the filtrate and the mixture was cooled in iced. Concentrated tetraoxosulphate (VI) acid was added to the mixture down the side of the test tube in iced and violet colored changed to bluish green was observed which indicate the presence of steroidal nucleus (i.e aglycone portion of the cardiac glycoside as described by Sofowora, [14]

Test for Glycosides

General test Two grammes (2 g) of ethanol extract was dissolved in 25 ml of 2.5 M tetraoxosulphate (VI) acid in a beaker and boiled on a water bath, the mixture was neutralized with 20 % potassium hydroxide. 5 ml of equal volume of Fehling solution A and B was added to a neutralized mixture in beakers and boiled. Brick red precipitate was observed which indicated the presence of glycosides.

Keller – Killiani test Ethanol extract (0.5 g) was dissolved in distilled water in a test tube; 1 ml of glacial acetic acid and few drops of ferric chloride solution were added to the aqueous solution of the ethanol extract. 1 ml of concentrated sulphuric acid was added to the mixture at an angle 45 °C to the wall of the test tube. The mixture was allowed to stand for some minutes, and a purple ring color was observed at the interphase, which indicates the presence of glycoside.

Test for proteins

Ninhydrin test few drops of ninhydrin reagent was added to the extract. The presence of blue color indicated a positive result.

Millon's test a few drops of millon's reagent was added to 2ml of the extract. The presence of a white precipitate indicated the presence of protein.

Xanthoproteic test a few drops of concentrated nitric acid was added to the extract. Yellow coloration indicated the presence of protein.

Test for terpenoids

Salkowski test: Two (2) mL of the extracts were treated with 2 mL of chloroform and 3 mL of concentrated sulphuric acid, to form a layer. A reddish-brown coloration of the interface confirms the presence of terpenoids.

Test for Saponins

Foam test 1ml of the extract is boiled with 6ml distilled water and is Shaken rapidly. The formation of foam indicated the presence of saponins.

Test for Organic acid

Malic acid test two drops of 40% FeCl were added to the test solution. The presence of yellow color indicated the presence of organic acids.

Test for Inorganic acid

Carbonate test diluted HCL was added to test extract. Bubbles of CO indicated the presence of carbonate.

Molecular Identification of *Lentinus squarrosulus*

Fungal Genomic DNA Extraction

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by Inqaba South Africa. A heavy growth of the pure culture of the fungal isolates was suspended in 200 microlitre of isotonic buffer into a ZR Bashing Bead Lysis tubes, 750 microlitre of lysis solution was added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processedat maximum speed for 5 minutes. The ZR was washing bead lysis tube was centriguged at 10,000x*g* for 1 minute.

Four hundred (400) microlitres of suprnatant was transferred to a Zymo-Spin IV spin Filter (orange top) in a collection tube and centrufuged at 7000 x*g* for 1 minute. One thousand two hundred (1200) microlitres of fungal/bacterial DNA binding buffer wasadded to the filtrate in the collection tubes bringing the final volume to 1600 microlitres, 800 microlitres was then transferred to a Zymo-Spin IIC Colum in a collection tube and centrifuged at 10,000x*g* for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-spin and spun. Two hundred (200) microlitres of the DNA Pre-Was buffer was added to the Zymo-spin IIC in a new collection tube and spun at 10,000x*g* for 1 minute followed by the addition of 500 microlitres of fungal/bacterial DNA was washed Buffer and centrifuged at 10,000x*g* for 1 minute.

The Zymo-spin IIC colum was transferred to a clean 1.5 microlitre centrifuge tube, 100 microlitres of DNA elution buffer was added to the colum matrix and centrifuged at 10,000x*g* microlitre for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at -20 degree for other downstream reaction.

Amplification

Internal Transcribed Spacer (ITS) Amplification

The ITS region of the rRNA genes of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5'- TCCTCCGCTTATTGATATGC-3, primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95ºC for 5 minutes; denaturation, 95ºC for 30 seconds; anealing, 53ºC for 30 seconds; extension, 72ºC for 30 seconds for 35 cycles and final extention, 72ºC for 5 minutes.

The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator.

Sequencing

Sequencing was done using the Big Dye Terminator kit on a 3510 ABI sequencer by Inqaba Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25 ul Big Dye® terminator v1.1/v3.1, 2.25ul of 5 x Big Dye sequencing buffer, 10µM Primer PCR primer, and 2-

10ng PCR template per 100bp. The sequencing condition was as follows 32 cycles of 96°C for 10s, 55°C for 5s and 60°C for 4min.

Phylogenetic Analysis

Obtained sequences was edited using the bioinformatics algorithm Trace edit, similar sequences were downloaded from the National Center for Biotechnology Information (NCBI) data base using BLASTN. These sequences were aligned using ClustalX. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method.

The obtained ITS sequence from the fungal isolates produced an exact match during the megablast search for highly similar sequences from the NCBI non-redundant nucleotide (nr/nt) database.

RESULTS

Characterization of the mushroom

Table 1: Morphologic and microscopic attributes of *Lentinus squarrosulus*. The mushroom grows in a convex shape, later expanding broadly to nearly a shape flat, white to cream color, occasionally with pale brown or grayish tints in the Centre. Microscopic study shows that the spores are ellipsoid to oblong, relatively thick walled and no amyloid, the size ranges from 7-8.3µm.

Physicochemical parameters of the soil

The soil physicochemical parameters are as shown in Table 2. The highest physicochemical parameters detected from soil *Lentinus squarrosulus* were harvested. The nitrogen content (10.1114 ± 2.01 mg/g) followed by phosphorus (8.9562 ±1.11 mg/g), potassium (2.1202 ±0.20 mg/g), calcium (1.6423 ±0.13 mg/g) and the lowest was magnesium (0.3415 ±0.21 mg/g)

Nutritional composition of *Lentinus squarrosulus*

The nutritional compositions of *Lentinus squarrosulus* from Keana are as shown in Table 3. The moisture (7.54 ± 0.14 %), ash (4.15 ± 0.03%), fat (2.52± 0.09 %), crude protein (21.61± 2.01 %), crude fiber (5.22± 0.11 %) and carbohydrate (58.96± 2.43 %)

The molecular characterization

The Agarose gel electrophoresis showing the amplified ITS fragment is as shown in Plate 2. The amplified ITS fragment bands on Lane 1-2 represent the ITS bands at 500bp while lane L represent the 100bp DNA ladder.

The phylogenic tree of the *Lentinus squarrosulus* is as shown in Figure 1

Table 1: Morphology of the study mushroom from Keana Local Government Area

Physical morphology			**Microscopic morphology**		**Organism**
Shape	**Pigment**	**Size**	**Shape**	**Size**	
The mushroom grows convex, later expanding to broadly convex or nearly flat. Surface: Smooth, sometimes slightly sticky when moist	White to cream, occasionally with pale brown or grayish tints in the center	Ranges from 4-10 cm in diameter	Spores are ellipsoid to oblong, relatively thick walled and no amyloid. Chellocystidia are abundant, ventral and variable in shape Pileipllis having parallel hyphae	Ranges from 6-9.3µm	*Lentinus squarrosulus*

Plate 1 Picture showing *Lentinus squarrosulus* mushroom

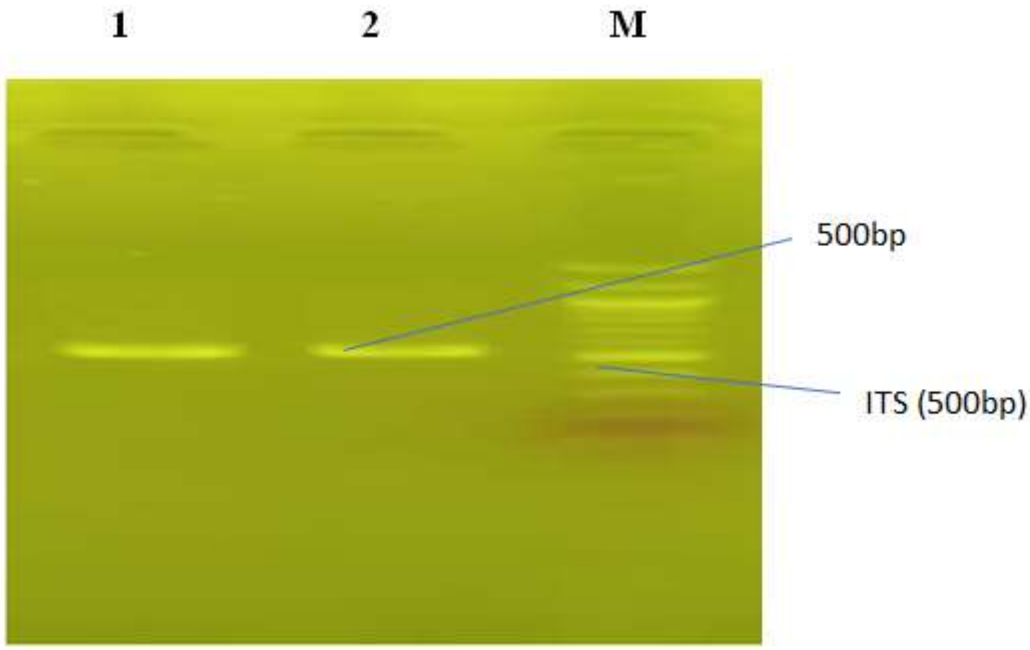

Plate 2: Agarose gel electrophoresis showing the amplified ITS fragment.

Lane 1-2 represents the ITS bands at 500bp while lane L represent the 100bp DNA ladder

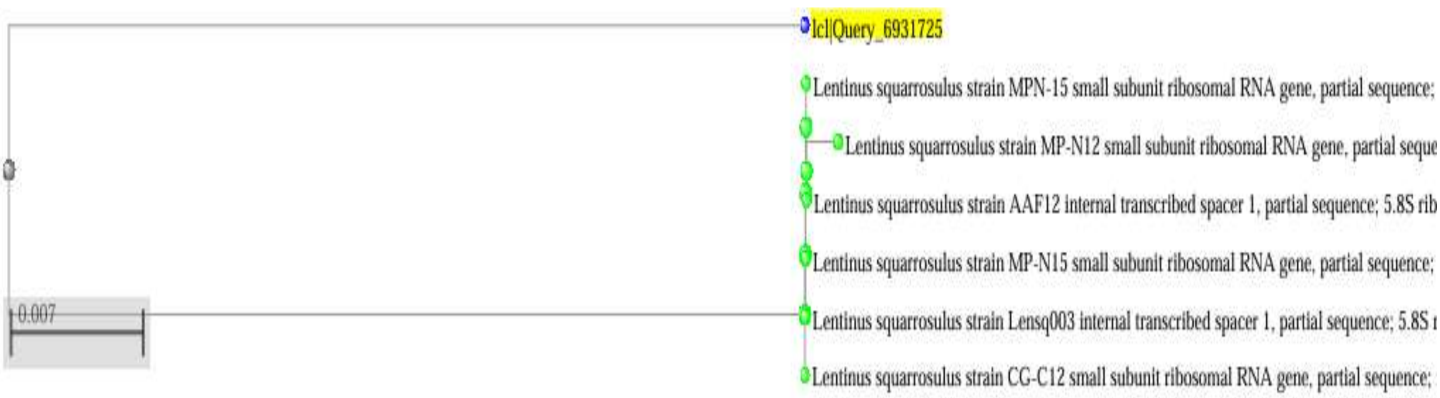

Figure 1 Phylogenetic trees showing the evolutionary distance between *Lentinus squarrosulus*

Table 2 Physicochemical parameters of the *Lentinus squarrosulus* soil growth Environment

Parameter	Concentration (mg/g)
Magnesium	0.3415 ±0.21
Calcium	1.6423 ±0.13
Nitrogen	10.1114 ±2.01
Potassium	2.1202 ±0.20
Phosphorus	8.9562 ±1.11

Table 3 Nutritional analysis of *Lentinus squarrosulus*

Parameters	Content (%)
Moisture	7.54 ± 0.14
Ash	4.15 ± 0.03
Fat	2.52± 0.09
Crude Protein	21.61± 2.01
Crude fiber	5.22± 0.11
Carbohydrate	58.96± 2.43

Functional properties of *Lentinus squarrosulus*

Some functional properties of *L. squarrosulus* sample are as shown in Table 4. Some of the functional properties were foaming capacity (131.6 ± 6.10%), foaming stability (56.1 ± 1.0 %), water absorption (262.0 ± 15.1 %), oil absorption (460.2 ± 11.12 %), oil emulsion (61.3 ± 5.01mLg^{-1}), Oil emulsion stability (41.0 ± 3.01 mLg^{-1}) gelation concentration (14.0 ± 0.05 %) and bulk density (413.5 ± 8.12 gL^{-1})

Phytochemical of *Lentinus squarrosulus*

The phytochemical compositions of *Lentinus squarrosulus* are as given in Table 5. The phytochemical components were flavonoids (12.67mg/g), saponins (0.95mg/g), proteins (9.41 mg/g), alkaloids (7.57 mg/g), tannins (6.55 mg/g), steroids (1.43 mg/g), glycosides (2.99 mg/g), terpenoids (3.11 mg/g), phenols (6.11 mg/g), carbohydrates (8.78 mg/g), inorganic acid (1.34mg/g), and organic acid (9.41 mg/g).

Table 4 Functional properties of *Lentinus squarrosulus*

Properties	**Contents**
Foaming capacity (%)	131.6 ± 6.10
Foaming stability (%)	56.1 ± 1.0
Water absorption (%)	262.0 ± 15.1
Oil absorption (%)	460.2 ± 11.12
Oil emulsion (mLg^{-1})	61.3 ± 5.01
Oil emulsion stability (mLg^{-1})	41.0 ± 3.01
Gelation concentration (%)	14.0 ± 0.05
Bulk density (gL^{-1})	413.5 ± 8.12

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XIII Issue XV January 2026 | Special Issue on Public Health

Table 5 Phytochemical qualitative contents of the *Lentinus squarrosulus*

Test	EtOH Extract	MeOH Extract
Test for Alkaloid		
a) Mayers test	Positive	Positive
b) Wagners test	Positive	Positive
c)Dragendroffs test	Negative	Positive
Test for terpenoids	Positive	Negative
Test for steroids	Negative	Positive
Test for Glycosides		
a) Libermann Burchards test	Positive	Negative
b) Keller-Killiani test	Positive	Positive
Test for Flavonoids		
a) Alkaline reagent test	Negative	Positive
b) Ferric chloride test	Positive	Positive
Test for Phenolics compounds		
a) ferric chloride test	Negative	Positive
b) lead acetate test	Negative	Positive
Test for carbohydrates		
a) fehlings test	Negative	Positive
b) benedict test	Positive	Positive
c) molischs test	Negative	Negative
d) barfoed test	Negative	Positive
Test for Amino acids		
a) ninhydrin test	Positive	Negative
b) millons test	Negative	Negative
c) xanthoproteic	Negative	Positive
Test for Saponins	Negative	Positive
a) froth test		
Test for tannin	Positive	Positive
a) ferric chloride test		
Test for organic acid	positive	Positive
a) malic acid test		
Test for inorganic acid		
a) carbonate test	positive	Positive

Table 6 Phytochemical contents of the *Lentinus squarrosulus*

Chemical	Contents (mg/g)
Flavonoids	12.67

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI | Volume XIII Issue XV January 2026 | Special Issue on Public Health

Saponins	0.95
Protein	9.41
Alkaloids	7.57
Tannins	6.55
Steroids	1.43
Glycosides	2.99
Terpenoids	3.11
Phenols	6.11
Carbohydrates	8.78
Inorganic acid	1.34
Organic acid	1.76

DISCUSSION

Historically, the local communities in Keana L.G.A have used *Lentinus squarrosulus* as a medicinal ingredient for healing Tonics for various infections and have incorporated in their diet as source of food. This study seeks to scientifically elucidate its properties and validates it traditional use. For thousands of years mushrooms are known as an important source of food and folk medicines [15, 16]. The *Lentinus squarrosulus* is one of mushroom genera representing a unique group of edible species which are superior in taste to every other edible mushroom [15].

Morphologically *Lentinus squarrosulus* exhibited the typical convex to broadly convex cap structure with smooth creamy-white pigmentation consistent with the description by Osemwegie et al. [17]

Phylogenetic analysis of partial ITS sequences collections using Distance, Parsimony measurements and Maximum Likelihood presented similar inferred trees that only had minor differences. Based on the partial ITS sequencing and phylogenetic analysis clustered the isolate within the *Lentinus squarrosulus* clade, which validates its identity as reported by Adebayo *et al.* [18]

The soil nutrient composition where the mushroom thrived in Keana revealed relatively high Nitrogen(10.11mg/g) and Phosphorus (8.95mg/g) levels. This nutrient -rich soil likely contributed to the robust growth of the mushroom and similar finding was reported by Gbolagade *et al.* [19] who emphasized the role of soil mineral composition in mushroom development. And this study showed that the soil was enrich with components from decay materials which is similar to study reported by Chandrawati *et al.*[20], which suggested that the minerals elements in the soil support the growth of *Lentinus squarrosulus.*

The nutritional composition found in this study showed that *L. squarrosulus* is a protein-rich (21.61%), carbohydrate-dense (58.96%), and low in fat (2.52%). These findings are consistent with previous reports by Adebayo *et al.,* [21]; Rashid *et al.* [22] highlighting mushroom as a “poor man’s meat “due to their protein and low lipid contents values, and the protein contents in this study were found to be higher compared to crude proteins present in protein-rich foods such as soybeans, cowpeas and pigeon pea and groundnut this is similar to study reported by Aremu *et al.* [23]. The high crude fiber contents (5.22%) further positioning *L. squarrosulus* as a functional food that could promote digestive health. the moisture content is an indication that *L. squarrosulus* are highly perishable because high moisture content encourages susceptibility to microbial growth and enzyme activity which hastens spoilage.

Some of functional properties of *L. squarrosulus* studied in this study indicated a high foaming capacity (131.6%) and oil absorption (460.25%), such properties are highly desirable in food processing, especially in bakery, confectionery, and meat industries, where mushroom can serve as natural binders, emulsifiers and texturizers [24]. The bulk density (413.5g/l) indicates suitability for formulation of composite flours, while the gelation concentration (14.0%) gives it potential for product structuring, similar to study carried out and reported by Bhaben *et al*. [25]. The oil absorption capacity is important since oil acts as a flavor retainer and increases the mouth feel of foods. It has been reported that variations in the presence of non-polar side chains, which might bind the hydrocarbon side chains of oil among the flours, explain differences in the oil binding capacity of the *L. squarrosulus* flours.

L. squarrosulus studied in this study contains significant amounts of flavonoids (12.67mg/g), alkaloids (7.57mg/g), phenols (6.11mg/g) and tannins (6.55mg/g). The secondary metabolites are well documented for their antioxidant, antimicrobial and anti-inflammatory properties as reported by (Oyetayo 2011). The qualitative test confirmed the presence of multiple bioactive groups; with both ethanol and methanol extract showing positive reactions for alkaloids, flavonoids, glycosides and amino acids which indicated that the extraction solvents play a role in phytochemical recovery as reported by Wasser [26]. The richness of phytochemicals may be clarify why the indigenous or people use it as medicinal fruits.

CONCLUSION

This study demonstrates that *Lentinus squarrosulus* is nutritionally valuable suggesting a promising application in food, nutraceutical industries. *Lentinus squarrosulus* is a good source of proteins and carbohydrates. Several functional properties have also been detected as favourable, making it potentially useful in many food formulations. These are foaming capacity and stability, water and oil absorption capacities, oil emulsion capacity and stability, least gelation concentration and bulk density. This study indicates that *Lentinus squarrosulus* may prove useful in the formulation of different food products where foaming, emulsification, retention of flavour and palatability as well as gel formation are required.

Compliance with ethical standards

Acknowledgments

With immensely thank all the staff in Department of Microbiology Laboratory Nasarawa State University Keffi for the help during the bench work and analysis.

Disclosure of conflict of interest

No conflict of interest among of the authors

Statement of informed consent

Informed consent was obtained from all individual participants included in this study

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