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RESEARCH ARTICLE

PRODUCTION AND CHARACTERIZATION OF BIOMASS AND EXOPOLYSACCHARIDES FROM THE EDIBLE MUSHROOM *LENTINUS SQUARROSULUS*

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Abstract: This study investigated the production and characterization of fungal biomass and exopolysaccharides (EPS) from the indigenous edible wild-type mushroom *Lentinus squarrosulus* (*L. squarrosulus*) grown in submerged fermentation. This mushroom is widely consumed in rural communities but remains underexplored for industrial development of bioproducts. Two nutrient media, Potato dextrose broth (PDB) and Yeast extract broth (YEB) were used for cultivation over 21 days. The biomass yield, EPS concentration and pH changes were measured at intervals of 3 days. The highest EPS (0.46 ± 0.01 mg/mL) and biomass (6.83 ± 0.043 mg/mL) production was achieved on day 9 in PDB compared to lesser production with YEB. The nature of EPS was determined by Fourier-transform infrared (FT-IR) spectroscopy, which indicated the presence of characteristic functional groups of polysaccharide β-glycosidic bonds. Functional analysis revealed high water solubility (54.55 ± 0.80%) and water absorbing capacity (91.40 ± 1.20%), antioxidant assay showed highly active radical scavenging properties (87.41 ± 1.05% equivalent to 0.415 ± 0.004 mg/mL gallic acid). Protein and carbohydrate profiles indicated that EPS was predominantly carbohydrate (62.26 ± 0.16%). These results show that EPS from *L. squarrosulus* possess favourable structural and bioactive attributes that strongly support its potential use in food-based applications.

Keywords: *Lentinus squarrosulus*, exopolysaccharides, submerged fermentation, antioxidant activity, fungal biomass

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RESEARCH ARTICLE

1. Introduction

Mushrooms are macrofungi that belong to the phyla Ascomycota and Basidiomycota, together forming the sub-kingdom *Dikarya* (Hibbett *et al*., 2007). Their rich enzymatic systems enable them to efficiently degrade complex lignocellulosic substrates (Kumla *et al*., 2020). Although only about 200 out of the estimated 140,000 mushroom species that exist globally are considered edible, and fewer than 25 have been widely cultivated commercially (Stabnikova *et al*., 2024). This disparity underscores the insufficient utilisation of many indigenous species, that have potential nutritional and industrial values.

Among valuable microbial metabolites, exopolysaccharides (EPS) have attracted considerable interest for industrial applications due to their hydrophilicity, non-toxicity, biocompatibility, and broad functional versatility (Kiran *et al*., 2024). Diverse biological activities, such as antioxidant, antibacterial, anticancer, and immunomodulatory properties of fungal EPS have been shown from numerous studies (Salimi and Farrokh, 2023) and are increasingly considered sustainable alternatives to plant- and seaweed-derived polysaccharides, especially for food, pharmaceutical and cosmetic industries (Abd-Alla *et al*., 2025).

Structurally, EPS may be categorised as homo-exopolysaccharides (HoEPS), which are made up of a single monosaccharide or hetero-exopolysaccharides (HeEPS), which contain multiple sugar residues (Abd-Alla *et al*., 2025). It is important to understand their biochemical structure because functional properties such as antioxidant activity, solubility, viscosity and gelling potential are strongly determined by their glycosidic linkages and monosaccharide composition (Wang *et al*., 2023). As a result, current efforts in research lay emphasis on identifying efficient microbial strains, optimisation of culture conditions and characterisation of the biological and structural attributes of EPS.

Despite the growing interest, most studies on fungal EPS have focused on common mushrooms like *Ganoderma lucidum*, *Lentinula edodes,* and *Pleurotus* spp. (Łysakowska *et al*., 2023). Conversely, many edible tropical mushrooms, including *Lentinus squarrosulus* (*L. squarrosulus*), remain sparsely investigated with respect to biomass composition, EPS yield and functional properties. This knowledge gap is more evident in biodiversity-rich regions such as Nigeria, where there is an abundance of edible mushrooms which are under-utilised due to

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limited scientific characterisation and dependence on imported bioactive ingredients (Asonibare *et al*., 2023).

The present study addresses these knowledge gaps by investigating biomass production and exopolysaccharide yield from *L. squarrosulus* cultivated under submerged fermentation.

This study aims to evaluate the production and characterisation of biomass and EPS from *L. squarrosulus* cultivated under submerged fermentation. The physicochemical and biological properties of the extracted EPS were assessed to determine its potential for food and pharmaceutical applications. It is hypothesised that *L. squarrosulus* produces EPS with favourable physicochemical characteristics and significant antioxidant activity suitable for industrial applications.

2. Materials and Methods

Culture maintenance

The macrofungal species *L. squarrosulus* used in this study is a wild-type, indigenous strain originally isolated from a natural habitat and preserved in the culture collection of the Department of Microbiology and Biotechnology, Abiola Ajimobi Technical University, Ibadan, Nigeria. Stock cultures were maintained on Potato dextrose agar (PDA) slants at 4°C.

Mycelial transfer and inoculum preparation

The stock culture of *L. squarrosulus* was subcultured onto freshly prepared PDA plates using a sterile inoculating needle under aseptic conditions. The inoculated plates were incubated at 28 ± 2°C for 5 days to obtain a pure culture.

Submerged cultivation of L. squarrosulus for EPS production

Actively growing mycelial regions from pure culture were excised using a sterile 1 mm cork borer. These mycelial plugs were then transferred into separate 250 mL Erlenmeyer flasks, each containing 30 mL fresh culture media, which included Yeast extract broth (YEB) and Potato dextrose broth (PDB). Flasks were incubated at 28 ± 2°C on a rotary shaker at 120 rpm for 21 days. Sampling was performed at intervals of 3 days by withdrawing one flask for each medium. For each sampling, the pH, EPS production, and biomass yield were evaluated using standard procedures. The quantity of the inoculation for each culture medium was 10% (*v/v*). Submerged fermentation was carried out in the Erlenmeyer flasks for 21 days at 28ºC in the two different

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liquid media under sterile conditions with shaking at 120 rpm (Ogidi *et al*., 2020). At intervals of every 3 days, one flask from each medium was sampled to monitor pH, EPS production, and biomass accumulation.

Samples were taken at 3-day intervals for 21 days, pH was determined, and the dry biomass of *L. squarrosulus* was determined by weighing the pellet after centrifugation and oven drying.

Determination of biomass yield

Biomass of *L. squarrosulus* was separated from the broth medium by centrifugation at 4000 × g for 15 min. The resulting mycelial pellet was dried in an oven at 60°C until a constant weight was achieved.

Determination of pH of culture medium

The pH of the culture supernatant was measured at every sampling interval using a calibrated digital pH meter.

Extraction of exopolysaccharides

Crude exopolysaccharide was precipitated by adding cold 95% ethanol to the culture supernatant in a 1:4 (*v/v*) ratio and incubating at 4ºC overnight to allow complete precipitation of EPS. Exopolysaccharide was collected as a pellet after centrifugation at 10000 × g for 20 min at 4ºC. The resulting pellet was washed with 75% ethanol to remove impurities and centrifuged again at 10000 × g for 20 min at 4ºC (Ogidi *et al*., 2020). The EPS pellet was dried in a hot air oven at 60ºC, and the weight was recorded as EPS yield in mg/mL.

Characterization of exopolysaccharides

Fourier Transform-Infrared (FT-IR) spectroscopy of exopolysaccharides

The functional groups present in the exopolysaccharide were identified using Fourier transform-infrared (FT-IR) spectroscopy. Two milligrams of EPS powder were ground with 200 mg of dry potassium bromide (KBr) of spectroscopic grade and compressed into a thin pellet. The FT-IR spectrum of the EPS pellet was recorded using a Shimadzu IR Affinity-1S FTIR spectrophotometer (Shimadzu Corporation, Japan) over the spectral range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} at room temperature. The resultant spectrum was analysed to determine the characteristic functional groups and linkages based on their corresponding molecular frequencies. Assigned peaks were cross-referenced with standard FT-IR spectral databases with known absorption patterns, including Sigma-Aldrich IR Spectrum Table version 2019.

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RESEARCH ARTICLE

Water solubility and water absorption capacity of EPS

The water solubility index (WSI) and water absorption capacity (WAC) of the crude EPS were determined. Exactly 1.1 mg of each EPS sample was weighed into a pre-weighed centrifuge tube. To this, 1 mL of sterile distilled water was added. The mixture was vortexed and continuously stirred for 30 min at 30°C in a water bath. The suspension was centrifuged at 3000 × g for 10 min to separate the soluble fraction (supernatant) from the insoluble residue (pellet). The supernatant was carefully decanted into a pre-weighed glass Petri dish and dried at 105°C for 4 h in a hot air oven to obtain the weight of dried soluble solids. The remaining wet residue in the centrifuge tube was also weighed (Reddy *et al*., 2013).

The WSI and WAC were calculated using the following equations:

$$\text{WSI (\%)} = \left(\frac{\text{weight of dry solid in supernatant}}{\text{weight of dry EPS sample}}\right) \times 100$$

$$\text{WAC (\%)} = \left(\frac{\text{weight of wet residue}}{\text{weight of dry EPS sample} - \text{weight of dry solid}}\right) \times 100$$

Antioxidant activity assay of EPS

The antioxidant activity of the EPS was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging procedure (Gyamfi *et al*., 1999), with slight modifications. Gallic acid was used as the standard antioxidant. The assay is based on the reduction of DPPH by antioxidants, which results in a change in colour from deep violet to light yellow monitored at 517 nm.

A 0.1 mM DPPH solution was prepared by dissolving it in methanol. Gallic acid standard solutions of different concentrations (1.00, 0.50, 0.25, 0.125 and 0.0313 mg/mL) were obtained by serial dilution in methanol. One milliliter of DPPH solution was mixed with 1 mL of 0.016 mg/mL Exopolysaccharide solution. A solution of methanol and the sample, without DPPH, served as a blank. The control solution was prepared by mixing methanol and the DPPH radical solution. The incubation of all mixtures was carried out for 30 min in the dark, and absorbance was measured at 517 nm using a UV-Visible spectrophotometer.

The percentage DPPH radical scavenging activity (AA%) was determined based on the expression:

RESEARCH ARTICLE

$$AA\% = \left(\frac{Abs_{control} - Abs_{Sample}}{Abs_{Control}}\right) \times 100$$

Where, AA% = antioxidant activity (%)

Abs_{Sample} = absorbance of the sample

$Abs_{Control}$ = absorbance of the control.

The standard curve was generated by plotting the percentage inhibition of gallic acid against concentration. The antioxidant capacity of the EPS was expressed as gallic acid antioxidant capacity (GAEAC) using the equation of the trendline obtained from the graph.

Determination of protein content of EPS

The protein content of the EPS was determined using the method described by Lowry *et al.* (1951). The assay is based on the reaction of peptide bonds with copper (II) ions under alkaline conditions (biuret reaction), followed by reduction of the Folin–Ciocalteu reagent by the copper–protein complex, producing a blue colour whose intensity is proportional to the protein concentration. Bovine serum albumin (BSA) was used as the standard protein. A series of standard solutions of BSA (0–100 µg/mL) was prepared by serial dilution in distilled water. One milliliter of EPS solution (or standard) was mixed with 5 mL of alkaline copper solution containing 2% Na_2CO_3 in 0.1 N NaOH, 0.5% $CuSO_4$, and 1% sodium potassium tartrate. The mixture was incubated at room temperature for 10 min, after which 0.5 mL of freshly diluted Folin–Ciocalteu reagent (1N) was rapidly added with mixing. The reaction mixtures were allowed to stand at room temperature for 30 min for colour development.

The absorbance of the developed colour was measured at 750 nm using a UV–Visible spectrophotometer against a reagent blank. A standard calibration curve was constructed by plotting absorbance against BSA concentration, and the protein content of EPS samples was determined from the curve and expressed as µg/mL.

Determination of carbohydrate content of EPS

The carbohydrate content of the exopolysaccharide (EPS) was estimated using the phenol–sulfuric acid method (Dubois *et al.*, 1956). The principle of this method is based on the dehydration of carbohydrate molecules by concentrated sulfuric acid, forming furfural derivatives that react with phenol to produce a coloured complex measurable at 490 nm. EPS was dissolved in distilled water to prepare the test solution. Glucose was used as the reference standard. One milliliter (1 mL) of EPS solution or standard glucose solution (0–100 µg/mL) was

RESEARCH ARTICLE

pipetted into a test tube. To this, 1 mL of 5% phenol solution was added, followed immediately by 5 mL of concentrated sulfuric acid. The tubes were mixed thoroughly and allowed to stand at room temperature for 30 min for colour development. The absorbance of the reaction mixtures was read at 490 nm against a reagent blank using a UV–Visible spectrophotometer. A calibration curve was constructed by plotting absorbance values against standard glucose concentrations. The carbohydrate content of the EPS was then calculated from the curve and expressed as µg/mL.

Biomass characterization

Determination of protein content of biomass

The protein content of the mycelial biomass was determined following the procedure (Lowry *et al*., 1951). The mycelial biomass was first homogenized and extracted in distilled water. The extract was centrifuged at 5,000 × g for 10 min to remove insoluble materials, and the supernatant was collected as the protein extract. Protein content in the mycelial biomass extract was expressed as µg/mL.

Determination of Carbohydrate Content of Biomass

The carbohydrate content of the mycelial biomass was determined using the phenol–sulfuric acid method (Dubois *et al*., 1956). Carbohydrate content in the biomass extract was expressed as µg/mL.

Statistical analysis

All experiments were conducted in triplicate, and results are expressed as mean ± standard error (SE). Statistical analysis was performed using two-way analysis of variance (ANOVA). Where significant effects were detected, Tukey's post hoc test was applied to identify differences among means. Statistical analyses were carried out using Microsoft Excel (version 2019, Microsoft Corporation, Redmond, WA, USA), and differences were considered statistically significant at $p < 0.05$.

3. Results and discussion

Growth and exopolysaccharide production of L. squarrosulus

The temporal patterns of exopolysaccharide (EPS) production and fungal biomass accumulation of *L. squarrosulus* cultured in Potato dextrose broth (PDB) and Yeast extract broth (YEB) over a 21-day fermentation period are presented in Figure 1a and 1b, respectively. In PDB, EPS

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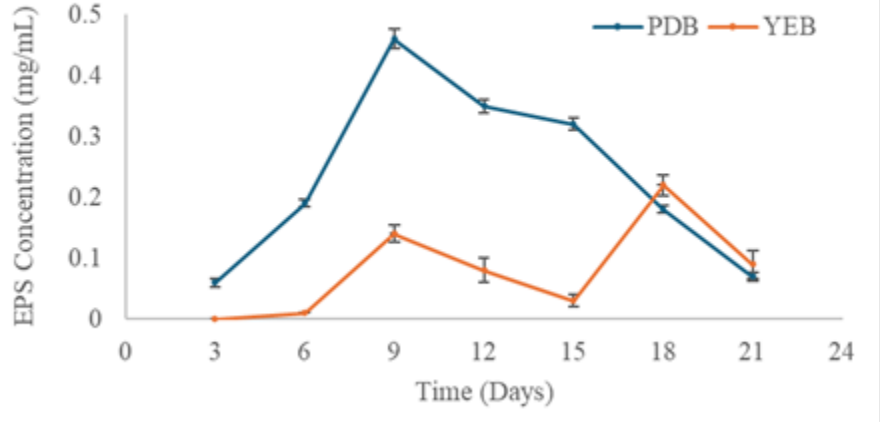
RESEARCH ARTICLE

concentration increased progressively up to day 9, reaching a maximum of 0.46 ± 0.01 mg/mL, followed by a gradual decline toward day 21. A similar trend was observed for biomass accumulation, which peaked at 6.83 ± 0.04 mg/mL on day 9 before decreasing thereafter. In contrast, YEB supported comparatively lower and more variable EPS and biomass production, characterized by an early increase up to day 9, a decline by day 15, and a delayed secondary peak on day 18 for both parameters.

Statistical analysis revealed that culture medium, fermentation time, and their interaction significantly influenced EPS production and biomass accumulation ($p < 0.05$), confirming distinct metabolic responses of *L. squarrosulus* in the two media. The higher EPS yield and biomass accumulation observed in PDB are likely attributable to its high carbohydrate content, which provides readily available carbon sources that support sustained mycelial growth and facilitate the redirection of excess carbon toward polysaccharide synthesis after the exponential growth phase (Kadam *et al*., 2022). Conversely, YEB is predominantly nitrogen-rich with comparatively lower fermentable carbohydrate content, resulting in fluctuating biomass formation and intermittent EPS accumulation. The delayed secondary peaks observed in YEB may reflect metabolic adaptation or utilization of alternative nutrient reserves during later stages of fermentation, as previously reported in submerged fungal cultures under nutrient-limited conditions (Yau *et al*., 2024).



a)

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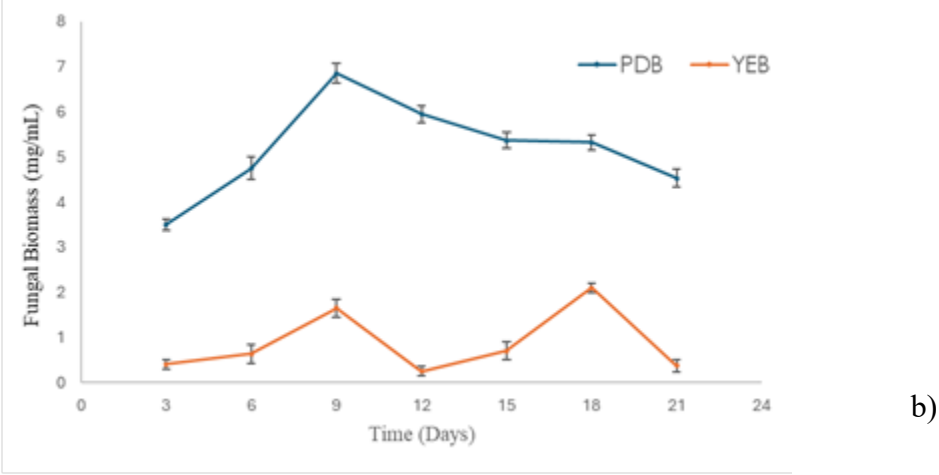

b)

Fig. 1. Growth and exopolysaccharide production of *L. squarrosulus* during submerged fermentation. (a) Exopolysaccharide (EPS) concentration (mg/mL) and (b) fungal biomass (mg/mL) measured over a 21-day fermentation period in Potato Dextrose Broth (PDB) and Yeast Extract Broth (YEB). Values represent mean ± standard error (n = 3). Two-way ANOVA showed significant effects of culture medium, fermentation time, and their interaction on EPS production and biomass accumulation ($p < 0.05$). PDB, Potato Dextrose Broth; YEB, Yeast Extract Broth.

pH dynamics in PDB and YEB media during 21-day fermentation

To evaluate the metabolic activity of *L. squarrosulus* during submerged fermentation, pH changes in PDB and YEB were monitored over a 21-day period (Figure 2). In PDB, the initial pH of 5.60 ± 0.02 at day 0 declined steadily to a minimum value of 2.83 ± 0.02 on day 18, followed by a slight increase to 2.90 ± 0.08 on day 21. In contrast, YEB exhibited greater pH variability, beginning with a higher initial pH of 6.31 ± 0.02, decreasing sharply to 4.50 ± 0.04 by day 3, increasing slightly to 5.09 ± 0.02 on day 6, and subsequently declining to its lowest pH of 3.00 ± 0.07 on day 18 before rising marginally to 3.14 ± 0.04 on day 21. Statistical analysis revealed that culture medium, fermentation time, and their interaction significantly influenced pH changes during fermentation ($p < 0.05$).

The progressive acidification observed in PDB suggests active carbohydrate metabolism by *L. squarrosulus*, where the utilization of dextrose and potato-derived sugars likely resulted in the accumulation of organic acids. This metabolic behavior is commonly associated with enhanced fungal activity and has been reported to accompany exopolysaccharide (EPS) production during submerged fermentation (Sepúlveda *et al*., 2025). Dynamic pH changes are known to influence

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fungal growth performance and metabolite synthesis, including antioxidant activity, which aligns with the trends observed in this study. In contrast, the fluctuating pH profile observed in YEB may be attributed to its nutrient composition, which is rich in amino acids, peptides, and vitamins but relatively low in fermentable carbohydrates. Additionally, the intrinsic buffering capacity of yeast extract may limit drastic pH shifts, thereby reducing acidogenic metabolism and contributing to the observed variability in pH changes (Suyoon *et al*., 2024).

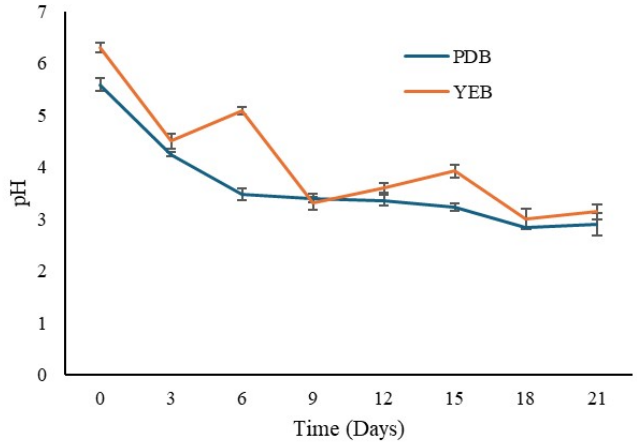

Fig. 2. pH profile of *L. squarrosulus* culture media during fermentation. This line graph illustrates the changes in pH levels measured over a 21-day period for *L. squarrosulus* cultures grown in Potato Dextrose Broth (PDB) and Yeast Extract Broth (YEB). Values represent mean ± standard error (n = 3). Statistical analysis was performed using two-way ANOVA, with significance set at $p < 0.05$. PDB, Potato Dextrose Broth; YEB, Yeast Extract Broth.

FT-IR profile of exopolysaccharide

The structural features of the extracted exopolysaccharide were examined using FT-IR spectroscopy, and the resulting FT-IR spectrum is presented in Figure 3. The spectrum indicated absorption bands at the following wavenumbers and corresponding absorbance strength: 3277.4 cm^{-1} (broad, intense), 2921.8 cm^{-1} (medium), 2852.4 cm^{-1} (medium), 1637.4 cm^{-1} (moderate), 1544.0 cm^{-1} (weak–moderate), 1368.5 cm^{-1} (medium), 1313.7 cm^{-1} (weak), 1204.0 cm^{-1} (medium), 1027.3 cm^{-1} (strong), and 893.4 cm^{-1} (sharp, weak). The FT-IR spectrum revealed multiple characteristic absorption bands across the mid-infrared region, providing the spectral fingerprint of the extracted exopolysaccharide. Peaks at 2921.8 cm^{-1} and 2852.4 cm^{-1} correspond to carbon-hydrogen (C-H) stretching vibrations of aliphatic methyl and methylene groups, which

RESEARCH ARTICLE

are indicative of sugar chains in exopolysaccharides (Patel *et al*., 2023). A broad peak observed at 3277.37 cm^{-1} is indicative of hydroxyl (O-H) stretching vibrations, which shows a high density of O-H bonds common to carbohydrate molecules and polyhydroxilic compounds contributing to their hydrophilic nature (Bhandary and Alagesan, 2024).

A moderate peak at 1637.40 cm^{-1} can be associated with carbonyl (C=O) stretching (Bhandary and Alagesan, 2024). This peak may also indicate bending vibrations of uronic acid groups commonly associated with hydrated microbial EPS (Chen *et al*., 2023).

The absorption band at 1368.5 cm^{-1} corresponds to C-H bending or symmetric stretching of COO^- groups, which is often present in acidic polysaccharides with a high content of uronic acid (Yang *et al*., 2024). The peaks at 1313.7 cm^{-1} and 1544 cm^{-1} may indicate amide II and amide I regions, indicating trace levels of proteinaceous components which might have been co-extracted with EPS (Sandt, 2024). The band at 893.4 cm^{-1} indicates the presence of β-glycosidic linkages between the monosaccharide units, a common configuration in exopolysaccharides (Chen *et al*., 2023). A notable strong band at 1027.25 cm^{-1} was observed, which corresponds to C–O stretching vibrations of glycosidic linkages and pyran ring configuration, which is a key structure and a characteristic peak of polysaccharide molecules (Huang *et al*., 2025).

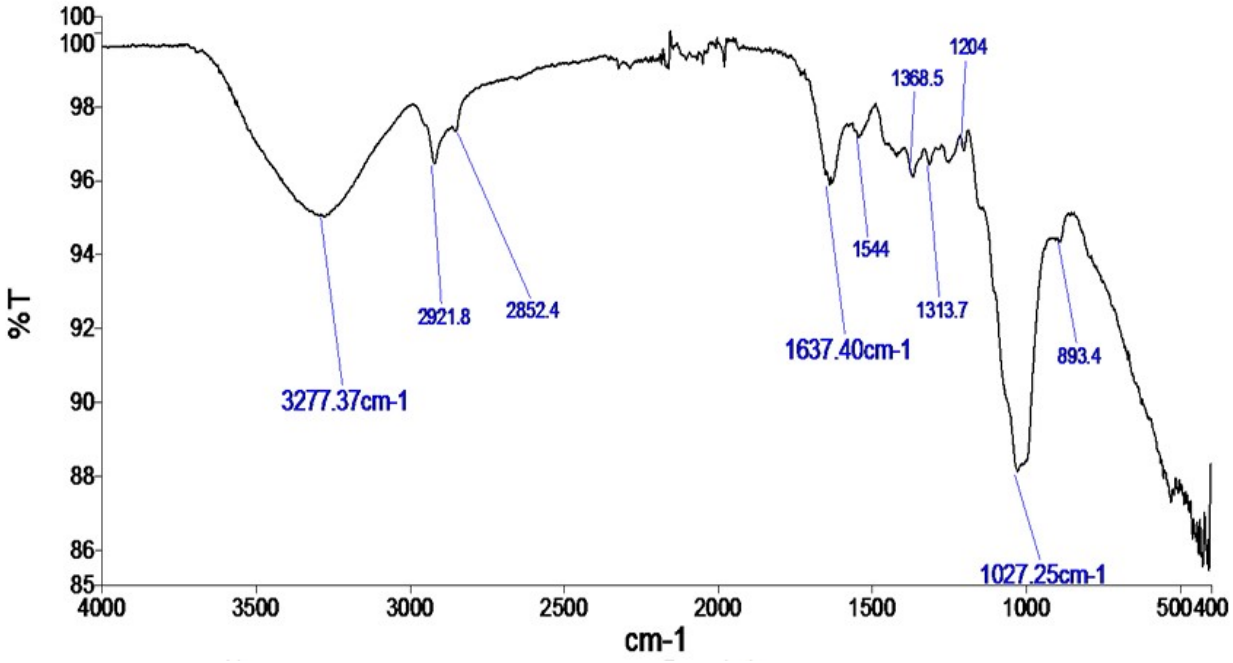

Fig. 3. FTIR Spectrum of exopolysaccharides (EPS) produced from *L. squarrosulus*. This spectrum illustrates the percentage transmittance (%T) across a range of wavenumbers (cm^{-1}) showing characteristic

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absorption bands corresponding to functional groups present in the EPS. Key peaks include 3277.37 cm^{-1} (O–H stretching), 2921.8 and 2852.4 cm^{-1} (C–H stretching), 1637.4 cm^{-1} (amide I, C=O stretching), 1544 cm^{-1} (amide II, N–H bending), 1368.5 cm^{-1} (C–H bending), 1313.7 cm^{-1} (C–O stretching), 1204 and 1027.25 cm^{-1} (C–O–C and C–O stretching of polysaccharides), and 893.4 cm^{-1} (anomeric region indicating β-glycosidic linkages).

Water solubility and water absorption capacity of exopolysaccharide

The water solubility index and water absorption capacity of the crude EPS are shown in Table 1. The crude EPS exhibited a WSI of 54.55 ± 0.80% and a WAC of 91.40 ± 1.20%. The extracted EPS showed moderate solubility in water and a high ability to absorb water. The water solubility of the exopolysaccharide (>40%) is consistent with previous reports for EPS produced (Ogunremi and Oriyomi, 2025) and aligns with the properties of fungal exopolysaccharides (EPS), which often consist of mannans and water-soluble *β*-glucans (Amanda *et al*., 2024). While the remaining insoluble fraction may be made up of complex or high-molecular-weight polysaccharides that are less likely to dissolve, the solubility indicates that a sizable amount of the EPS dissolves in water, most likely as a result of hydrophilic polysaccharide structures (Ben Akacha *et al*., 2023). The high-water absorption capacity (WAC) shows that the EPS can absorb water at a rate of 91.40% of its weight. Fungal polysaccharides, especially *β*-glucans, have a great ability to store water and create gel-like structures by hydrogen bonding (Ogunremi and Oriyomi, 2025). With respect to the WAC, the EPS possesses significant hydration qualities, which increase its potential for use in medicines, food, and cosmetics.

Table 1. Functional properties of exopolysaccharides from *L. squarrosulus*

Parameter	Value (%)
Water Solubility Index (WSI)	54.55 ± 0.80
Water Absorption Capacity (WAC)	91.40 ± 1.20

Values are mean ± standard error (n = 3)

Antioxidant activity of exopolysaccharide

The antioxidant capacity of the extracted EPS was determined using the DPPH radical scavenging assay, and the values are presented in Table 2. The EPS sample at 0.016 mg/mL gave an absorbance of 0.034, which corresponds to a percentage DPPH radical scavenging activity of 87.41 ± 1.05%. The antioxidant activity of the EPS sample was equivalent to 0.415 ± 0.004 mg/mL of gallic acid using the equation generated from the trendline of the gallic acid standard

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curve (y = 14.897x + 81.222). The EPS showed a high DPPH radical scavenging activity at low concentration, with equivalent antioxidant strength comparable to gallic acid.

The antioxidant activity of the EPS from this study demonstrated a strong free radical scavenging potential. At a concentration of 0.016 mg/mL, the EPS achieved an inhibition of 87.41%, which corresponds to a gallic acid equivalent of approximately 0.415 mg/mL. This level of inhibition is notably attained at a lower concentration than that required for gallic acid to produce a comparable effect. The observed antioxidant activity is possibly due to phenolic structures and hydroxyl groups that are able to donate hydrogen atoms or electrons (Chotmanee *et al*., 2025). Furthermore, the functional groups and monosaccharide compositions of EPS are prominently suggested to contribute to their antioxidant activity (Bouzaiene *et al*., 2024).

Table 2. Antioxidant activity of exopolysaccharides from *L. squarrosulus*

Sample	Concentration (mg/mL)	DPPH scavenging activity (%)	GAE (mg/mL)
EPS	0.016	87.41 ± 1.05	0.415 ± 0.004

Values are mean ± standard error (n = 3). GAE = gallic acid equivalent EPS = exopolysaccharides

Protein and carbohydrate content of mycelial biomass and exopolysaccharides

The protein and carbohydrate compositions of the mycelial biomass and EPS are shown in Table 3. While the exopolysaccharide showed 24.34 ± 0.44% and 62.26 ± 0.16% protein and carbohydrate content, respectively. YEB biomass gave a protein content of 42.91 ± 0.41% and carbohydrate content of 49.04 ± 0.84%. and PDB biomass showed a protein content of 17.03 ± 0.04% and carbohydrate content of 32.84 ± 1.02%. The EPS exhibited the highest carbohydrate proportion, whereas biomass produced in YEB contained the highest protein content.

The compositional analysis of cellular biomass and exopolysaccharides (EPS) revealed notable differences in protein and carbohydrate distribution depending on the growth medium. The EPS fraction was predominantly carbohydrate-rich, with 62.26% carbohydrate and 24.34% protein, consistent with the biochemical nature of EPS as high-molecular-weight sugar polymers often conjugated with small amounts of proteins or glycoproteins. This carbohydrate-rich profile of EPS aligns with other reports, which described EPS as predominantly polysaccharide in nature (Mouro *et al.,* 2024). In contrast, the cellular biomass composition varied substantially between media. Biomass derived from YEB medium exhibited higher protein content (42.91%) compared to carbohydrate (49.04%), reflecting the nitrogen-rich nature of YEB that promotes protein

RESEARCH ARTICLE

synthesis and structural macromolecule formation. Conversely, Potato Dextrose Broth (PDB) biomass contained lower protein levels (17.03%) and a more moderate carbohydrate fraction (32.84%), suggesting a metabolism directed primarily towards carbohydrate utilization and energy storage rather than protein accumulation.

Table 3. Protein and carbohydrate content of mycelial biomass and exopolysaccharide from *L. squarrosulus*

Sample	Protein Content (%)	Carbohydrate Content (%)
EPS	24.34 ± 0.44	62.26 ± 0.16
Biomass in YEB	42.91 ± 0.41	49.04 ± 0.84
Biomass in PDB	17.03 ± 0.04	32.84 ± 1.02

Values are mean ± standard error (n = 3). EPS = Exopolysaccharides, YEB=Yeast Extract Broth. PDB = Potato Dextrose Broth

Conclusions

This study demonstrated the successful production and characterisation of exopolysaccharides (EPS) and biomass from *L. squarrosulus* using Potatoes Dextrose Broth (PDB) and Yeast Extract Broth (YEB) under submerged fermentation. PDB supported higher EPS and biomass compared to YEB, with notable variations over the 21 days. FT-IR analysis confirmed the polysaccharide nature of the EPS. The crude EPS showed good water absorption capacity and strong antioxidant activity. These findings suggest *L. squarrosulus* EPS possesses desirable structural and functional properties with potential pharmaceutical applications.

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RESEARCH ARTICLE

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2026 by Galaţi University Press Received November 2025/Accepted December 2026

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SUPPLEMENTARY MATERIAL

Supplementary Table S1. FT-IR fingerprints of EPS from *L. squarrosulus*

Wavenumber (cm^{-1})	Transmittance (%T)	Functional Group / Vibration	Functional Property	Reference
893.4	~90%	β-Glycosidic linkage (C–H deformation)	Indicates β-configuration of sugar monomers	(Mouro *et al., 2024*)
1027.25	~88%	C–O stretching (pyranose ring)	Core polysaccharide structure / fingerprint region	(Mouro *et al., 2024*)
1204.0	~94%	C–O–H or C–O–C vibration	Glycosidic linkage in sugar rings	(Mouro *et al., 2024*)
1313.7	~94%	C–N stretch / CH_2 wag	Amine group, potential nitrogen-containing residues	(Chen *et al.*, 2023)
1368.5	~93%	C–H bending / COO^- symmetric stretch	Uronic acid presence / acidic polysaccharide marker	(Yang *et al.*, 2024)
1544.0	~93%	Amide II (N–H bending)	Possible protein residue or amine contamination	(Chen *et al.*, 2023)
1637.40	~92%	C=O stretching / O–H bending	Carboxylic groups, water content	(Chen *et al.*, 2023)

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2852.4	~91%	C–H symmetric stretching (–CH_3)	Structural aliphatic groups	(Mouro *et al., 2024*)
2921.8	~91%	C–H asymmetric stretching (–CH_2)	Backbone of sugar rings	(Mouro *et al., 2024*)
3277.37	~89% (estimate)	O–H stretching (broad)	Hydrogen bonding, hydrophilicity	(Mouro *et al*., 2024)