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Original Research Article

Antioxidant Activities of Enriched Polysaccharide Fractions from Mycelia of *Amauroderma subresinosum*

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ABSTRACT

The polysaccharides found in *Amauroderma subresinosum* have been recognized, but their characterization has been limited. In this study, enriched polysaccharide fractions (ASF) were extracted from the mycelia of *Amauroderma subresinosum* using hot water, sodium hydroxide, and acetone. To determine these ASF structural characteristics, deproteination and precipitation with cold ethanol were performed to remove residual proteins. Fourier transform infrared (FTIR) spectroscopy was employed to assess the purity of the ASF, while gel permeation chromatography (GPC) was used to measure molecular weight, and high-performance liquid chromatography with precolumn derivatization using 1-phenyl-3-methyl-5-pyrazolone (PMP) reagent was utilized for monosaccharide analysis. The antioxidant activity of the ASF was evaluated through DPPH and ABTS radical scavenging assays. Seven enriched polysaccharide fractions, named ASF1–7, were isolated, and three main fractions were identified as ASF-1 (22.53 kDa, 6.16 ± 1.01%, w/w), ASF-3 (0.75 kDa, 16.46 ± 0.26%, w/w), and ASF-7 (35.73 kDa, 12.60 ± 1.48%, w/w). These fractions corresponded to the primary polysaccharide derived from hot water, alkaline solution, and insoluble residue. Monosaccharide analysis revealed that glucose was the predominant component in ASF-1 (70.24%), ASF-3 (84.11%), and ASF-7 (87.78%). FTIR spectroscopy confirmed the presence of carboxyl and hydroxyl groups, as well as pyranose rings in these ASFs. Additionally, ASF-1, ASF-3, and ASF-7 demonstrated significant DPPH and ABTS radical scavenging activities, with IC_{50} values ranging from 12.8 to 16.1 µg/mL and 8.5 to 12.6 µg/mL, respectively. This study provides the initial evidence that polysaccharides extracted from *Amauroderma subresinosum* may possess potential as functional food ingredients with potent antioxidant activity.

***Keywords*:** *Amauroderma subresinosum*; Ganodermataceae; polysaccharide fractions; mycelia; DPPH; ABTS.

Introduction

The Ganodermataceae family has held significant value for over two thousand years in Asian countries, where it has been utilized for improving health conditions and reversing aging.[1] Extracts derived from the basidiome (fruiting body) and mycelium (vegetative part) of certain Ganodermataceae species have been found to contain polysaccharides, triterpenoids, steroids, glycoproteins, and other bioactive compounds. These compounds have been extensively studied and applied in various fields. Polysaccharides, in particular, have garnered significant attention due to their remarkable properties, including antioxidant, antitumor, and immunomodulatory activities. These polysaccharides have shown the potential to scavenge free radicals, inhibit tumor growth, and modulate the immune system. As a result, they have become appealing candidates for incorporation into food supplements, cosmetics, and pharmaceuticals.[2,3]

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One of the notable advantages of Ganodermataceae polysaccharides is their relatively low toxicity, making them suitable for long-term use. Their natural origin and diverse biological activities have led to extensive research exploring their potential applications in health promotion and disease prevention.[1] In recent studies, researchers have focused on extracting polysaccharides from basidiocarps using various methods and optimizing extraction conditions to enhance polysaccharide production.[2,3] These extracted polysaccharides were then evaluated for their bioactivities. However, it is important to note that the polysaccharide content and other bioactive compounds in basidiomes can be influenced by cultivation conditions and their place of origin.[4] Therefore, the development of fermentation technology and a deeper understanding of the polysaccharide synthesis process could be beneficial for achieving mass production of fungal polysaccharides through submerged fermentation.[5] Several Ganodermataceae species, such as *G. amboinense*, *G. australe*, *G. applanatum*, *G. lucidum*, *G. lingzhi*, *G. sinense*, *G. tsugae*, and *A. rugosum*, have been cultured to collect polysaccharides from the mycelia.[6-11]

A. subresinosum is a species belonging to the *Amauroderma* genus in the Ganodermataceae family, which is classified under the Polyporales order. It is commonly found in tropical regions.[12-15] Previous research on Vietnamese *A. subresinosum* basidiocarps identified the presence of fatty acids, including unsaturated fatty acids such as lignoceric acid and 11-octadecaenoic acid. Esters of cholesta-7,22-dien-3β-ol with three fatty acids, namely 14-methylpentadecanoic acid, 8,11-octadecaenoic

acid, and 8-octadecenoic acid, were also identified.[16] Further chemical investigation of the fruiting bodies of *A. subresinosum* from China led to the isolation of epoxy ergosterol derivatives with inhibitory activity against acetylcholinesterase.[17] However, to the best of our knowledge, there are no available reports on the structure of polysaccharides derived from submerged cultured mycelia of *A. subresinosum*. Therefore, in this study, *A. subresinosum* obtained through submerged fermentation was purified, and the structural properties of the isolated polysaccharide were investigated. Additionally, the antioxidant properties of these enriched polysaccharide fractions were evaluated using DPPH and ABTS radical scavenging assays.

Materials and Methods

Materials

The fungal strain *Amauroderma subresinosum* was classified using morphological and phylogenetic methods based on the 5.8S ITS rDNA gene, and the corresponding GenBank locus was MZ2706942.[15] Potato dextrose agar (PDA) medium and potato dextrose broth (PDB) medium were obtained from Himedia (Mumbai, Maharashtra, India). Chemicals such as acetone ($(CH_3)2CO$, 99.8%), ethanol (C_2H_5OH, 99%), hydrochloric acid (HCl, 37%), n-butanol ($CH_3(CH_2)2OH$, 99.9%), chloroform ($CHCl_3$, 99.8%), and sodium hydroxide (NaOH, 97%) were purchased from Merck (KgaA, Darmstadt, Germany). Mannose ($C_6H_{12}O_6$, 99%), rhamnose ($C_6H_{12}O_5$, 99%), glucose ($C_6H_{12}O_6$, 99%), xylose ($C_5H_{10}O_5$, 99%), galactose ($C_6H_{12}O_6$, 99%), arabinose ($C_5H_{10}O_5$, 99%), and sucrose ($C_{12}H_{22}O_{11}$, 99.5%) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Distilled water from a water purification system (Water stills, Merit® W4000, Radnor, Pennsylvania, USA) was used throughout the experiments. PTFE syringe filters with a pore size of 0.22 µm were purchased from Membrane Solutions (Auburn, Washington, USA). All other chemicals used in this study were of analytical grade.

Fungal culture and collection of dried mycelia

The strain of *A. subresinosum* was preserved and maintained on potato dextrose agar (PDA) at 4°C. For breeding and fermentation, potato dextrose broth (PDB) medium was used. In submerged fermentation, square sections of mycelia from PDA were inoculated into the seed medium and cultured at 28 ± 2°C and 150 rpm for two weeks (Figure 1). The seed culture medium containing *A. subresinosum* mycelia was then transferred to 250 mL fermentation medium in a 500 mL Erlenmeyer flask. A 10% inoculum from the seed culture was transferred to a 2500 mL PDB medium in a 5000 mL Erlenmeyer flask. The mycelia were obtained by centrifugation to remove the culture broth. The collected mycelia were washed three times with sterile distilled water and then dried at 50°C in a heating/drying oven (Memmert UFE 600, Buechenbach, Germany) until a constant weight was achieved. The dried mycelia were stored at -20°C for further experiments.

Isolation of polysaccharides

The dried mycelia of *A. subresinosum* were pulverized using an electrical mill and passed through a 0.2 mm stainless steel mesh sieve.[17] The isolation process is illustrated in Figure 2. The powder was then subjected to multiple washings with 80% ethanol (1500 mL) at 75°C in a round-bottomed flask for 3 hours to remove lipids and small molecules. This washing step was repeated three times. The resulting residue was dried at 50°C until a constant weight was achieved. For further extraction, 20 g of the dried residue was mixed with 1500 mL of distilled water and subjected to extraction at 90°C for 4 hours. This water extraction step was performed three times. The water extract was separated from the solid by centrifugation at 8000 rpm for 15 minutes using a Hettich centrifuge. The water extract was then evaporated using an IKA RV-10 digital V-C vacuum rotary evaporator at 50°C, resulting in a volume of approximately 70 mL. The concentrated extract was precipitated by adding 96% ethanol in a ratio of 1:4 (v/v). The precipitated product was washed with 80% ethanol, 96% ethanol, and acetone, and then dried at 50°C, yielding fraction ASF-1. The insoluble portion from the previous step was dissolved in 300 mL of 2% NaOH at room temperature (25 ± 2°C) for 6 hours. The alkaline extraction mixture was separated by centrifugation at 8000 rpm for 15 minutes. The resulting alkaline extract was filtered through a 0.22 µm syringe filter and further centrifuged at 10,000 rpm using a Hermle refrigerated universal centrifuge for 15 minutes. The obtained precipitate was washed with 80% ethanol, 96% ethanol, and acetone, and then dried at 50°C, resulting in fraction ASF-2. The supernatant from the centrifugation step was evaporated and precipitated using the same method as described for fraction ASF-1, resulting in fraction ASF-3. The solid obtained after the extraction with 2% NaOH at room temperature was dissolved in 300 mL of 2% NaOH at 90°C for 2 hours. The hot alkaline extraction mixture was separated by centrifugation, and the resulting extract was evaporated and precipitated as described above for fraction ASF-1, yielding fraction ASF-4. The insoluble part remaining after the extraction with hot alkaline solution was washed three times with sterile distilled water, and then dissolved in 15% acetone at 60°C for 6 hours. The acetone extraction mixture was separated by centrifugation, and the resulting extract was adjusted to alkaline pH (pH = 10) using 0.1 M NaOH and filtered through a 0.22 µm syringe filter. The extraction process and precipitation were carried out as described for fractions ASF-2 and ASF-3, resulting in fractions ASF-5 and ASF-6, respectively. The insoluble part from the acetone extraction step was washed with 80% ethanol, 96% ethanol, and acetone, and then dried at 50°C, yielding fraction ASF-7 (Figure 2).

Removal of proteins and pigments

To remove free proteins from the crude polysaccharides, the Sevag method was employed.[18] Chloroform was added to the polysaccharide solution with a pH of 4-5 in a ratio of 1:5 (v/v). Alternatively, n-butanol was compared to chloroform using the same ratio. The mixture containing polysaccharide, chloroform, and n-butanol was vigorously shaken for 20 minutes and then subjected to centrifugation at 8000 rpm for 5 minutes. This Sevag method was repeated five times. The resulting precipitate was dried at 50°C. To decolorize dark-colored polysaccharide fractions, a 3% H_2O_2 solution was utilized. The polysaccharide was treated with the H_2O_2 solution in a weak alkaline solution with a pH of 8 to oxidize colorant pigments. Subsequently, 96% ethanol in a ratio of 1:3 (v/v) was added to precipitate the polysaccharide.[19] The resulting residue was neutralized, washed, and dried using the same process.

Phenol-Sufuric acid assay

Polysaccharides (carbohydrates) react in the presence of strong acid and heat to generate furan derivatives that condense with phenol to form stable yellow-gold compounds that can be measured spectrophotometrically.[20] 1 mL of each sample, 1 mL of 5% phenol solution and 5 mL of 96% sulfuric acid were added respectively to a glass tube. Each glass tube was shaken by a GFL Gesellschaft für Labortechnik mbH shaker (Burgwedel, Deutschland, Germany) and then stood for 20 minutes at room temperature. Then, the mixture was measured the absorbance at 490 nm on a 5100 UV-Vis spectrophotometer (Metash, Shanghai, China). Chromatograms, calibration curves (sucrose, 0 µg/mL – 70 µg/mL), and calculations were processed using Excel software (Microsoft, Redmond, Washington, USA).

Molecular weight determination

The molecular weight of polysaccharide fractions was detected with gel permeation chromatography-GPC (Agilent 1100) with Ultrahydrogel$_{TM}$ 250 (6 µm, 7.8 × 300 mm). The column was eluted by 0.1 M $NaNO_3$ at a flow rate of 1.0 mL/min.[21] Various dextrans with different molecular weights were used as standards, and the molecular weight of polysaccharide was calculated according to the equation.

Monosaccharide composition

Monosaccharide composition of polysaccharide was detected by high – performance liquid chromatography (Agilent 1100) with UV detector (HPLC-UV), a precolumn derivatization procedure with 1-phenyl-3-methyl-5-pyrazolone (PMP) reagent.[22] The samples was separated an ZORBAX Eclipse XDB-C18 HPLC column (5µm, 4.6 × 150 mm) at 30°C. Mobile phase composed of acetonitrile and 0.1M ammonium acetate buffer at flow rate 1.0 mL/min. Six monosugars (mannose,

rhamnose, glucose, xylose, galactose, arabinose) were used as internal standards.

Fourier-transform infrared spectra (FT-IR) analysis

FT-IR spectrum of polysaccharide fractions were measured on a Nicolet 6700 spectrometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) using potassium bromide (KBr) disc method in the wavelength of range 4000-400 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans.

DPPH activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity test was performed as protocol of Egharevba et al.[23] Initially, 6 mg of DPPH was dissolved in methanol through sonication and brought up to a total volume of 25 ml, resulting in a DPPH solution with a concentration of 0.6 mM. The test samples (ASF-1, 3, and 7) were dissolved in DMSO and then diluted with methanol to create a range of concentrations varying from 1 to 30 µg/mL. As a positive control, vitamin C was used at concentrations of 1.0 to 5.0 µg/mL. In each experiment, 0.5 mL of each ASF concentration was added to a clean, dry test tube. Subsequently, 0.5 mL of the DPPH solution (0.6 mM) and 4 mL of methanol were added to the test tube. The reaction mixture was then kept at room temperature in the dark for a duration of 30 minutes. Afterward, the absorbance of the test samples was measured at 517 nm. A blank sample was prepared using methanol in place of the test sample solution. The measurements were repeated three times, and an average result was calculated using the formula:

$$I(\%) = \left(\frac{A_0 - A_t}{A_0}\right) \times 100\%$$

In which: A_0 is the absorbance of the DPPH control sample, A_t is the absorbance of the test sample. From the values of antioxidant activity, a graph can be constructed to represent the correlation between I (%) and the sample concentration. The IC_{50} value was determined based on this equation.

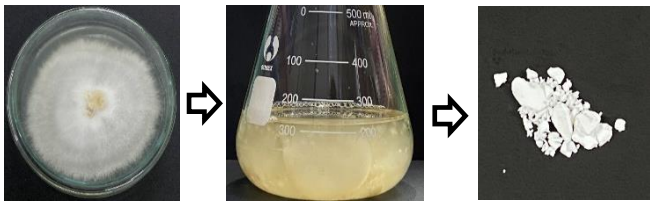

Figure 1: Mycelia of *A. subresinosum*: a. Hyphae on PDA plates (7 days old); b. Mycelia in broth culture (14 days old); c. ASF-1 polysaccharide fraction.

ABTS assay

The ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) experiment was performed following by the previous method.[24] In detail, ABTS solution (7 mM) was added to $K_2S_2O_8$ solution (2.4 mM) in equal volumes, and then the mixture was incubated in the dark at room temperature for 16 hours. The $ABTS^{\cdot+}$ solution was then diluted with methanol to obtain an absorbance of 0.706 ± 0.01 units at 734 nm. The test samples (ASF-1, 3, and 7) were dissolved in DMSO, and then diluted with methanol to a concentration range of 1-30 µg/mL. In this experiment, vitamin C (1-5 µg/mL) was used as a positive antioxidant agent. The reaction mixture consisted of 1.0 mL of the test solution and 1.0 mL of the diluted $ABTS^{+}$ solution. This was incubated for 7 minutes at room temperature in the dark, and then the absorbance was measured at 734 nm using a UV-Vis spectrophotometer.

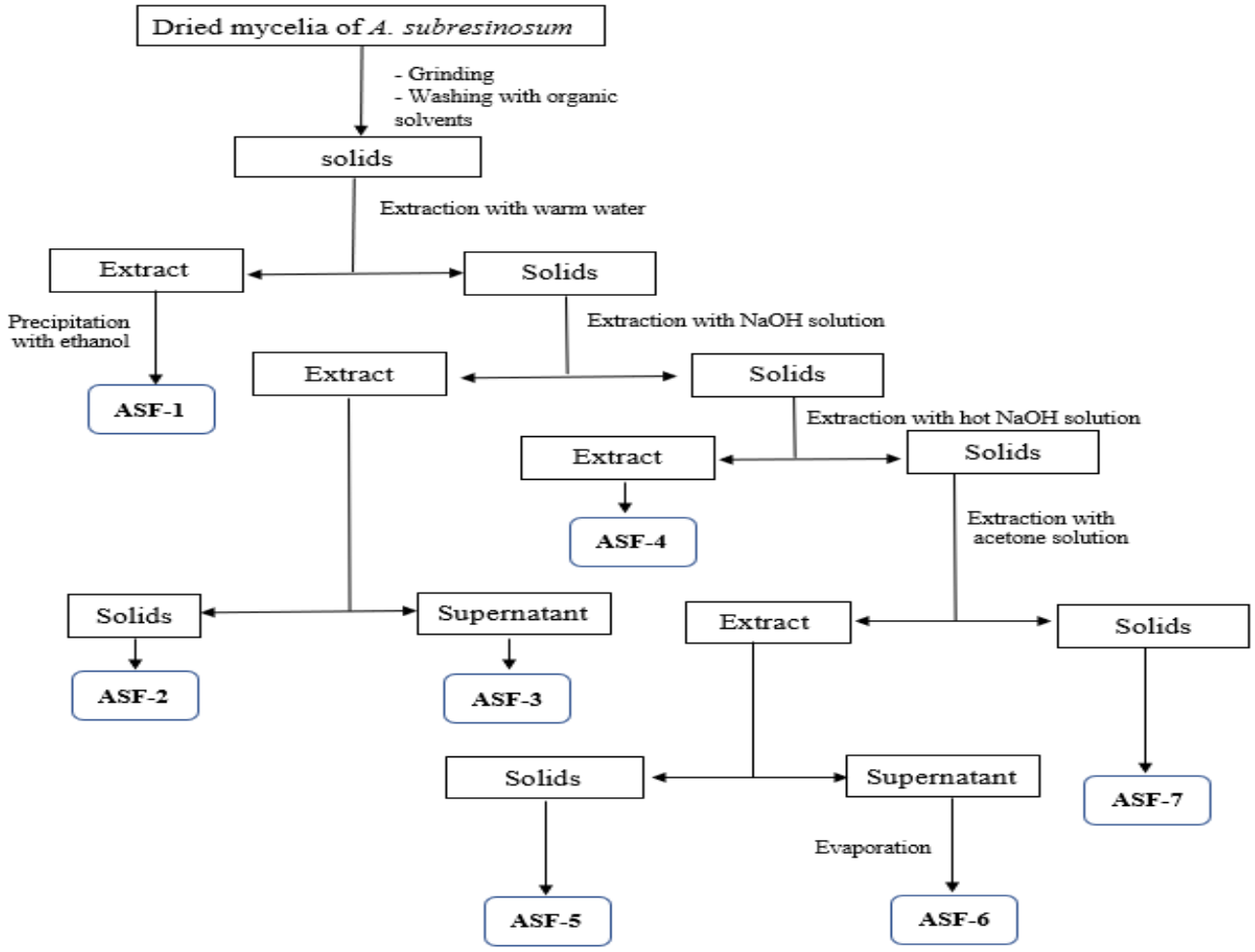

Figure 2: Isolation scheme of polysaccharide fractions from mycelia of *A. subresinosum*.

The control sample was prepared by replacing the test sample solution with methanol. The antioxidant activity of the sample is calculated according to the formula:

$$I(\%) = \left(\frac{A_0 - A_t}{A_0} \right) \times 100\%$$

In which: A_0 is the absorbance of the ABTS control sample, A_t is the absorbance of the test sample. From the values of antioxidant activity, a graph can be constructed to represent the correlation between I (%) and the sample concentration. The IC_{50} value was determined based on this equation.

Statistical analysis

Quantitative data were obtained by triplicate experiments and analysis was performed with one-way ANOVA followed by Duncan's multiple range test in Excel 2020.

Results and discussion

Yields of extraction

In Table 1, the mass yields, ASF-1 – ASF-7, of the different fractions obtained from the extraction process are summarized. Among them, the hot water extraction yielded ASF-1 with an efficiency of 6.16 ± 1.01% (w/w). This yield is higher than that reported for hot water fraction from *G. lucidum* mycelium (2.04%)[17] but lower than the polysaccharide extraction from *G. tsugae* mycelium using hot water (11.6%).[25] The alkaline extract fraction was filtered, and ASF-2 was obtained as the precipitate after centrifugation, while ASF-3 was the fraction obtained after passing the extract through a 0.22 μm membrane. The mass percentages of ASF-2 and ASF-3 were 0.18 ± 0.02% (w/w) and 16.46 ± 0.26%, respectively. The hot 2% NaOH solution was used to disrupt the cell wall, resulting in ASF-4 with a mass yield of 0.40 ± 0.04% (w/w). In addition, an acidic solution (15% acetone) was used to separate polysaccharides from the cell wall. ASF-5 and ASF-6 were obtained with mass percentages of 0.05 ± 0.02% (w/w) for ASF-5, and 0.48 ± 0.08% for ASF-6. Meanwhile, ASF-7 accounted for 12.60 ± 1.48% (w/w) of the mycelium weight, this fraction represents the residue left after the extraction process. The total yield of all the fractions after purification was calculated to be 39.5% ± 1.34% (w/w), which corresponds to more than a third of the initial mycelium mass. The yields obtained using hot water and alkaline solution for *A. subresinosum* polysaccharides from mycelium were higher than previously reported yields.[26-28] These results indicate that the highest extraction yield was achieved using an alkaline solution at room temperature, while the minimum yield was obtained using a 15% acetone solution as the solvent. The total yield of polysaccharides obtained from the mycelium extraction process was significantly higher compared to previous reports.[17,25] Additionally, among the seven fractions extracted using various solvents, the highest yields were observed for three fractions obtained from hot water (ASF-1), alkaline solution (ASF-3), and the residue (ASF-7). Therefore, these three fractions were selected for further experiments.

Molecular weight of polysaccharide fractions

In Table 2, the average molecular weights of the three main polysaccharide fractions (ASF1, 3 and 7) obtained from the extraction process are presented. The polysaccharide fraction obtained from hot water extraction (ASF-1) has an average molecular weight of 22.53 kDa. This molecular weight is consistent with the average molecular weights reported for polysaccharides in *Ganoderma* species extracted from hot water, which range from 10^3 to 10^4 Da. It is worth noting that ASF-1 was also found in the mycelia of *A. subresinosum*, a genus belonging to the Ganodermaceae family, and it shares similar molecular weight characteristics and medicinal effects.[26-28] The polysaccharide fraction extracted through alkaline extraction (ASF-3) has an average molecular weight of 750 Da. This fraction is lighter compared to other polysaccharide fractions extracted from *Ganoderma* species using alkaline methods. One possible reason for the lower molecular weight is that ASF-3 was derived from the supernatant of an alkaline solution after passing it through a 0.22 μm syringe filter. The insoluble residue fraction of the extraction process (ASF-7) has an average molecular weight of 35.73 kDa. This is higher than the molecular weights observed for other polysaccharide fractions. As ASF-7 represents the insoluble part, it may contain polysaccharides with larger molecular weights compared to the soluble fractions. These results demonstrate that the molecular weights of the main polysaccharide fractions obtained in this study vary depending on the extraction method and fraction type. ASF-1 from hot water extraction has a relatively high molecular weight, while ASF-3 obtained through alkaline extraction has a lighter molecular weight. ASF-7, the insoluble residue, has a higher average molecular weight, indicating the presence of larger polysaccharides.

Table 1: Yields and composition of the fractions

Fraction	Extraction medium	Yield (%, *w/w*)	Composition
ASF-1	Hot water	6.16 ± 1.01**	Polysaccharide, proteins
ASF-2	Solid of 2% NaOH	0.18 ± 0.02*	Polysaccharides
ASF-3	Supernatant of 2% NaOH	16.46 ± 0.26**	Polysaccharides
ASF-4	Hot 2% NaOH	0.40 ± 0.04*	Polysaccharides
ASF-5	Solid of 15% acetone	0.05 ± 0.02*	Polysacharides
ASF-6	Supernatant of 15% acetone	0.48 ± 0.08*	Polysacharides
ASF-7	Insoluble residues	12.60 ± 1.48**	Polysacharides
Total	All fractions	36.35 ± 1.34	Polysacharides, proteins

The statistical significance was accessed by Duncan's multiple range tests (* $p < 0.01$; ** $p < 0.05$).

Table 2: Molecular weights of polysaccharide fractions

Fraction	ASF-1	ASF-3	ASF-7
Mw[a)] (kDa)	22.53	0.75	35.73

[a]Mw: Molelucar weight (kDa-kiloDalton).

Monosaccharide composition

Table 3 presents the monosaccharide composition of different fractions obtained from *A. subresinosum* mycelia. Among these, the primary sugar detected in all fractions was glucose. This is consistent with previous reports on *Ganoderma* polysaccharides, where glucose is a major component. The culture medium used in this study (PDB) primarily contains glucose, and fungal cell walls are composed of glucose-rich structures. Glucose content exceeded 70% in the water-soluble fraction and reached almost 88% in the water-insoluble fraction. The high glucose content can be attributed to the carbon source in the culture medium and the structural composition of fungal cell walls. Interestingly, the rhamnose was not detected in any of the *A. subresinosum* fractions. Although rhamnose has been found in small percentages in the fruiting bodies and extracellular culture medium of *G. lucidum* and the fruiting bodies of *A. rugosum*,[25-28] it was absent in the fractions of *A. subresinosum* studied here. The fraction ASF-1 exhibited the highest proportions of mannose (16.73%) and xylose (10.73%). Arabinose (1.30%) and galactose (1.00%) were detected in

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equal percentages. These results suggest that the outer layer structure of *A. subresinosum* mycelia primarily consists of heteropolysaccharides present in the aqueous extract of fungal cell walls. Although some glucans may be present, arabinose and mannose were not detected in the ASF-3 fraction, which was obtained through alkaline extraction, indicating that the main glucan component was released in this fraction. In addition, the proportion of glucose increased with a decrease in the contributions of other sugars in the fractions. ASF-7 had the highest proportion of glucose (87.78%), while arabinose, galactose, and xylose were present in less than 5% quantities. These findings demonstrate that the monosaccharide composition of the *A. subresinosum* fractions is predominantly characterized by glucose, reflecting the carbon source in the culture medium and the composition of fungal cell walls. Mannose and xylose were found in higher proportions in the ASF-1 fraction, indicating their presence in the outer layer structure of the mycelia, while arabinose and galactose were detected at equal percentages. Rhamnose was not detected in any of the fractions analyzed.

FT-IR spectra analysis

The provided information further elaborates on the FTIR spectra of ASF-1, ASF-3, and ASF-7 fractions and their corresponding functional group assignments. The breakdown of the key observations is showed in Figure 3. All three fragments signaled strong peaks between 920 and 1300 cm^{-1}, indicating the majority of C-C and C-O stretching vibrations of pyranose rings, which indicate the predominant presence of polysaccharides in the fractions. In which, the spectrum of the ASF-1 segment (Figure 3A) shows the presence of a broad stretching peak at 3418.5 cm^{-1}, which indicates the presence of the hydroxyl group. The peak at 2925.2 cm^{-1} demonstrates C-H stretching vibration. Interestingly, the peak at 1382.5 cm^{-1} was presented for the pyranose ring, and the peak at 1071.1 cm-1 was assigned to β-glucan. The presence of a peak at 1653.1 cm^{-1} indicates C=O stretching and 1546.4 cm^{-1} indicates N-H bend, which implies that ASF-1 contains protein. About the ASF-3 spectrum (Figure 3B), the presence of a broad stretching peak at 3446.4 cm^{-1} indicates the presence of the hydroxyl group. Peaks at 1414.1, 1345.2, 1051.7, and 1019.8 cm^{-1} indicate pyranose C-O stretching. The peak at 926.8 cm^{-1} is a feature of β-anomeric unit configuration. The peak at 1563.4 cm^{-1} shows the presence of C=O stretching, which may suggest the presence of a chitin moiety with an N-acetylglucosamine, a derivative of glucose. About the ASF-7 spectrum, a broad stretching peak at 3446.4 cm^{-1} was observed which represented the hydroxyl group. The peak at 2923.7 cm^{-1} confirms the presence of C-H stretching vibration, 1379.5 cm^{-1} expresses a pyranose ring, and 1037.2 cm-1 assign for β-glucan moiety. Similar to the ASF-3, the peak at 1652.5 cm^{-1} was confirmed for a C=O stretching, which indicates the presence of a chitin group (Figure 3C). According to the spectra of three polysaccharide fractions, hydroxyl groups are revealed at 3418.5 cm^{-1} of ASF-1, 3446.4 cm^{-1} of ASF-3, and 3419.6 cm^{-1} of ASF-7. These results are the same as previous studies about polysaccharides of some *Ganoderma* species. They indicate that the typical major broad stretching peak is around 3400 cm^{-1} of hydroxyl groups.[4,19,29,30] Weak bands at 2923.7 and 2925.2 cm^{-1} can be represented by C-H stretching vibration. The bands at around 1653.1 cm^{-1} and 1345.2 cm^{-1}, were associated with the carboxyl group.[4] The FTIR spectra of ASF-1, ASF-3, and ASF-7 showed peaks at 1077.1, 1051.7, and 1037.2 cm^{-1}, respectively which indicated the presence of a pyranose ring.[4,19,29] Additionally, a weak band of ASF-3 at 926.8 cm^{-1} associated with the β configurations of glycosidic linkages confirmed that ASF-3's monosaccharide was mainly glucose.

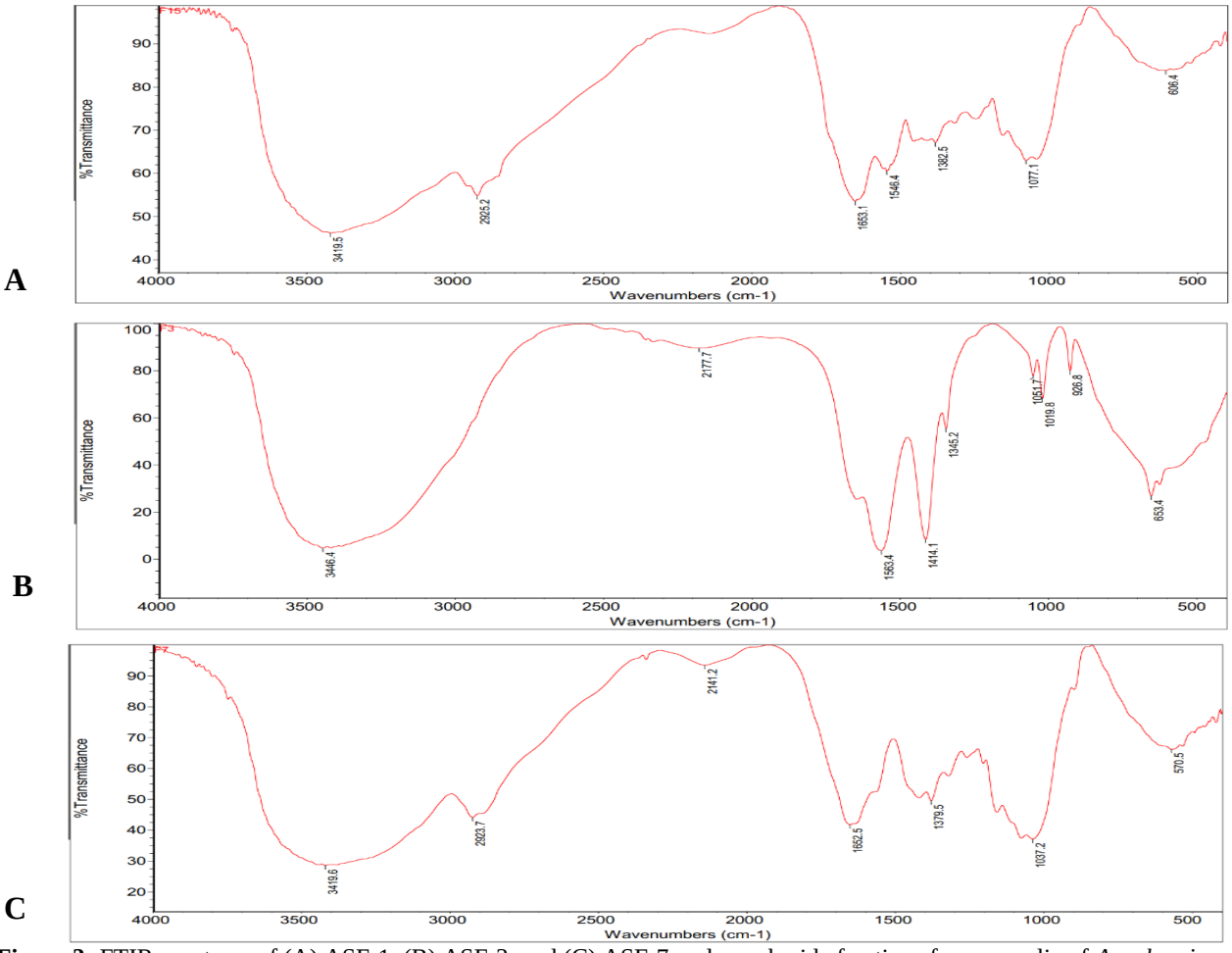

Figure 3: FTIR spectrum of (A) ASF-1, (B) ASF-3, and (C) ASF-7, polysaccharide fractions from mycelia of *A. subresinosum*. The analysis was measured the wave number on the infrared spectrum (between 4000 to 400 cm^{-1}) and %transmittance (the percentage of the absorption of infrared light).

Table 3: Weight ratio (%) of monosaccharides in different fractions obtained from mycelia of *A. subresinosum*

Fractions	Weight ratio (%)					
	Ara	Gal	Glc	Man	Rha	Xyl
ASF-1	1.30	1.00	70.24	16.73	ND	10.73
ASF-3	ND	8.61	84.11	ND	ND	7.28
ASF-7	0.24	1.22	87.78	6.04	ND	4.72

Ara: arabinose; Gal: galactose; Glc: glucose; Man: mannose; Rha: rhamnose; Xyl: xylose; ND: not detected.

Table 4: Scavenging activities of the enriched polysaccharide fractions from *A. subresinosum*

Compounds	Scavenging activity (µg/mL)[a)]	
	DPPH[b)]	ABTS[b)]
ASF-1	12.9 ± 1.5	10.7 ± 1.0
ASF-3	12.8 ± 2.0*	8.5 ± 1.0**
ASF-7	16.1 ± 1.8	12.6 ± 1.8
Ascorbic acid[c)]	4.1 ± 0.6	3.2 ± 0.4

[a] Results are expressed as IC_{50} in µg/mL, the scavenging effect was expressed as the mean ± standard deviation (SD) of three replicates; [b] DPPH: 2,2-diphenyl-1-picrylhydrazyl; $ABTS^{\cdot+}$: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{\cdot+}$). [c] Positive control (a common antioxidant). Statistical significance was accessed by Duncan's multiple range tests (* $p < 0.01$; ** $p < 0.05$).

Antioxidant assays

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical capable of absorbing molecular hydrogen and antioxidants. DPPH free radical was widely used to determine the free radical scavenging abilities of various natural compounds and other substances. As shown in Table 3, the DPPH radical scavenging activities of three enriched polysaccharide fractions, ASF-1, ASF-3, and ASF-7, were expressed by the IC_{50} values as 12.9 ± 1.5, 12.8 ± 2.0 and 16.1 ± 1.8 µg/mL, respectively. Within the test dosage range, ASF-3's IC_{50} value was found to be the most antioxidant in DPPH radical scavenging activity.

ABTS+ [2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate)] is a blue fluorescence stable free radical with a characteristic absorption wavelength of 734 nm. When an antioxidant compound is added, ABTS+ will be reduced to a colorless form leading to a decrease in the specific wavelength absorbance. This method became widely used for detecting the antioxidant activity of natural products. As shown in Table 4, ASF-1 exhibited ABTS radical scavenging activities in a dose-dependent manner, with an IC_{50} of 10.7 ± 1.0 µg/mL. ASF-7 was found to have an IC_{50} value of 12.6 ± 1.8 µg/mL. In contrast, ASF-3 showed the most ABTS scavenging activity with an IC_{50} value of 8.5 ± 1.0 µg/mL. In this experiment, ascorbic acid (a known antioxidant) was used as a positive control in the experiments, and it exhibited strong DPPH and ABTS radical scavenging activity, with lower IC_{50} values of 4.1 ± 0.6 and 3.2 ± 0.4 µg/mL, respectively, compared to the polysaccharide fractions.

Conclusion

In this study, polysaccharide fractions were isolated from dried mycelia of *A. subresinosum* for the first time. Hot water and alkaline solution extraction methods were used, resulting in significant yields. The three fractions (ASF-1, ASF-3, and ASF-7) had different molecular weights and contained glucose, xylose, and galactose in varying proportions. The polysaccharide fractions exhibited notable antioxidant activity, with IC_{50} values ranging from 12.8 to 16.1 µg/mL for DPPH and 8.5 to 12.6 µg/mL for ABTS. These findings suggest the potential application of *A. subresinosum* polysaccharides as natural antioxidants in various industries and health-promoting products.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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