

STUDY ON THE PHYSIOCHEMICAL PROPERTIES OF *PLEUROTUS OSTREATUS* POLYSACCHARIDES

Tasneema Khanam Dila* and Gurbanov Geldimyrat*

School of Food and Biological Engineering, Hafei University of Technology, Hefei 230009, China.

Corresponding Author: Tasneema Khanam Dila

School of Food and Biological Engineering, Hafei University of Technology, Hefei 230009, China.

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ABSTRACT

Aims and objectives: The current research project was designed with the objective to find out the concentration of polysaccharides in oyster mushroom and to determine the antioxidant activity of polysaccharides in oyster mushroom (*Pleurotus ostreatus*). **Methods:** The fresh plant of *P. ostreatus* was collected from Carrefour supermarket in Hefei, Anhui, China. After collection of plant samples, these were proceeded for extraction and fractioning. The plant was turned into powder form and then using different solvents for further process. The powder crude extract obtained from the different solvents were further used for subsequent analysis. Further investigations were done using TGA, XRD, DSC, FTIR, UV spectra, HPLC and the DPPH analysis to find out the physiochemical properties and antioxidant activities of *P. ostreatus*. **Results:** In XRD analysis, there was a weak diffraction peak for each OM with different intensity was recorded when the 2θ was nearly 47°, which was indicating the presence of both of the amorphous nature and crystalline nature of the different polysaccharides. The FTIR spectrum showed a broad peak around 3400cm⁻¹ for hydroxyl, bunches extending vibrations; the top around 2900 cm⁻¹ showed retention for C-H extending vibrations; the band around 1735cm⁻¹ showed assimilation for carboxyl gatherings extending vibrations, which can be utilized to decide the presence of uronic corrosive; a top around 1650cm⁻¹ was happened because of the related water. Biochemical analysis confirmed that purified fraction OM-1 has strong free radical scavenging activity against DPPH, hydroxyl and ABTS free radical. **Conclusion:** The results revealed the polysaccharide's important role as potential natural antioxidant agents during the investigation and application of bioactive polysaccharide. The overall study suggested that *P. ostreatus* polysaccharide could be used as a potential natural antioxidant drug resource and has value for further scientific research in food and pharmaceutical industry. Further studies are now in progress to gain more insight into its structures and identify other potential biological activities of these novel *P. ostreatus* derived polysaccharides.

KEYWORDS: *P. ostreatus*, Mushroom, Antioxidant, DPPH analysis, Polysaccharides

INTRODUCTION

As a vegetable mushroom can play an important role in meeting the nutritional needs of the worldwide population. A healthy person requires 200-250g vegetable per day.^[1] But in some countries like Bangladesh, Pakistan only 40-50g vegetable per day is available to people. To get rid of this situation, it is necessary to increase the production of vegetable which need and huge land areas.^[2] Mushroom may be used to reduce shortage of vegetable, since it required minimum land area. Mushroom fungi have low calorie they are cholesterol free and have certain medicinal properties.^[3]

There are various types of mushrooms such as oyster mushroom, milky white mushroom, shitake mushroom and button mushroom etc. which are cultivated in many countries.^[4,5] Among them, some popular species of an oyster mushroom such as; *Pleurotus ostreatus*, *P.*

djamor, *P. florida*, *P. cystidiosus* and *P. geesteranus* can be cultivated in countries like Pakistan, China, India and Bangladesh because the climate and weather of these countries is more suitable for the mushroom cultivation.^[6] Though the weather and climate of Pakistan is suitable for year-round oyster mushroom cultivation but the farmers cannot cultivate the mushroom due to lack of autoclave sterilization of substrate.^[7] Without sterilization of spawn packets contaminations occur. In the country mushroom spawn is prepared by using sawdust which is sterilized with autoclave sterilization but corn cobs, rice straw, wheat straw, pulse straw, sugarcane baggage, water hyacinth and tea leaves may be used as substrates after sterilizing them with hot water treatment, drum pasteurization or chemical treatment for spawn production.^[5,8]

Clam mushroom (*Pleurotus* species) is financially

significant on the planet mushroom market.^[9] It is generally developed and devoured in various areas of the world. Numerous individuals respect the mushroom because of its taste, flavor, high healthy benefits, and some restorative properties. *Pleurotus* are wealthy in proteins with fundamental amino acids, physiologically significant polysaccharides and fundamental unsaturated fats, dietary filaments, significant minerals, and a few nutrients.^[10] The presence of some bioactive substances, significantly polysaccharide-protein complex in the variety *Pleurotus* has been accounted for to present some pharmacological expected like antimicrobial, cancer prevention agent, anticancer, against aggravation, hostile to hypercholesterolemia, against hypertensive, against diabetic, hepato-defensive and against unfavorably susceptible exercises.^[11]

Substrate assumes a significant part in the yield and supplement substance of shellfish (Oyster) mushroom.^[12] The substrates on which mushroom bring forth (Commercial generate/Merely vegetative seed materials) is developed, influences the mushroom creation.^[13] The National Mushroom Development and Extension Centre (NAMDEC), Savar, Dhaka grows oyster mushroom using rice straw with hot water treatment and sawdust with autoclave sterilization.^[14] Farmer of some countries like Pakistan and India cannot produce mushroom spawn due to high cost autoclave sterilization.^[15] Therefore, it is necessary to identify the less expensive sterilization technique without autoclave for mushroom production. If low cost method of sterilization is introduced in place of high cost autoclave sterilization, farmers will be interested to grow mushroom in respective agriculture lands. Hot water treatment of substrate may be an effective alternative of autoclaving. With this view in mind a research project was designed with the objectives to find out the concentration of polysaccharides in oyster mushroom (*P. ostreatus*) and to determine the antioxidant activity of polysaccharides in oyster mushroom (*P. ostreatus*).

MATERIAL AND METHODS

Extraction and fractionation

P. ostreatus plant was cleaned with sterilized distilled water to eliminate unwanted contaminants and dust. It was dried at room temperature (25°C) for a span of 02 weeks. Post-drying grinding was done until fine powder was obtained. The powder was extracted using different solvents like chloroform, *n*-hexane, methanol, ethyl acetate *n*-butanol and water for three days at room temperature. After incubation, the solvent was separated from the rest of biomass and dried to obtain powder. The powder crude extract obtained from different solvents were further used for subsequent analysis.

Preparation of Extract

10-KG of raw *P. ostreatus* was washed with water to remove the traces of dirt on it. Then it was grated with grater and was spreaded on a piece of cloth in order to dry under shade for 30 days. After the *P. ostreatus* was

completely dried, it was ground into coarse powder with the help of a grinder. The total weight of *P. ostreatus* powder obtain was 400grams. 50grams of *P. ostreatus* was weighed separately and extracted by mixing in each 6 different polar solvents which were; ethanol, chloroform, ethyle acetate, *n*-hexane, *n*-butanol, and distilled water. The quantity of each of the solvent was 150ml. The mixture was shaken with hands few times daily and kept for 15 days. After 15 days each mixture was filtered via What man filter paper and filtrates were obtained in separate glass bottles. The filtrates were then evaporated by using a rotary evaporator and semi solid form of *P. ostreatus* was obtained. The weight of each crude extract left behind was 3gram to 4gram. Then extracts were dissolved in Dimethyle sulfoxide (DMSO) to make different concentrations.

Preparation of plant extraction solution for tests

In an Eppendorf tube 30% of DMSO was taken and 1gram of semi solid plant extract was dissolved into it. The Eppendorf tube was placed on vertex mixer to mix the sample efficiently. Similarly, all of the 6 plant extract solutions were prepared.

Isolation and the purification of polysaccharides

The raw polysaccharide was dissolved in D-H₂O and characterized with the Sevag method and enzyme hydrolysis, respectively; The starch was removed using α -thermostable amylase. The resulting aqueous phase was concentrated and lyophilized against water for 48 h after dialysis (cut-off 3500 da). 70 mg of polysaccharide was dissolved in 7 mL of distilled water, centrifuged and filtered with a 0.4 μ m filter, then applied to the DEAE-52 column (26.6 \times 126 mm). The polysaccharide was sequentially augmented with a solution of distilled water, 0.2 M, 0.4 M, 0.6 M and 0.8 M NaCl with a flow rate of 0.6 mL / min to 200 mL, and a refractive index detector (Learning Science) was monitored by an automatic collector equipped with. (Suzhou) Company Limited). According to the Peak Position Collect sample, fractions with NaCl solution were dialed against water for 48 h, when they were concentrated and lyophilized. Further segmentation was performed using the Cipadex G-100 column (31.2 \times 195 mm) and eluted with 0.1 M NaNO₃ / 0.05% NaN₃ with a flow rate of 0.5 mL / min, according to the extreme conditions of the collected samples. Pure polysaccharide fractions were obtained.

(TGA) of *Pleurotus ostreatus*

Thermal analysis (TGA) of the polysaccharide samples were conducted using the thermos-gravimetric analyzer. First of all, 2mg of sample was placed on the sample pan and then it is heated from 25°C and upto 600°C at 10°C per minute under a protective nitrogen atmosphere (20mL per minute). Nitrogen was used at a flow rate of 50mL per minute. Thermal analysis was used for studying the thermodynamic properties of polysaccharides. One of the important characteristic to be used for the biological applications is, the thermal stability of biomolecules.

X-ray diffraction (XRD) analysis of *Pleurotus ostreatus* polysaccharide

The stage arrangement and precious stone construction of cleansed polysaccharides were resolved utilizing X-beam powder diffractometer with 20 in the range of 5°C to 60°C at room temperature (25°C). The progression size and time/step were 0.01° and 0.1 s/step, individually. XRD spectra of polysaccharides were observed to analyze their translucent or shapeless nature. The XRD profile displayed the trademark diffraction bend of each sanitized polysaccharides. Effects was checked at normal temperature, diluted alkali, concentrated alkali and high pressure.

Differential scanning calorimetry (DSC) analysis of *Pleurotus ostreatus* polysaccharide

DSC spectra was utilized to gauge the event of endothermal changes or exothermal with an expanded temperature. The exothermal valley showed up at 304.6°C, 231.9°C, and 300.1 °C, separately for OM-1, OM-2 and OM-3 in DSC bend, while there was an endothermal top for OM-1 at 246.9°C. Thusly, these distinctions in warm dependable qualities might be brought about by the differentiations between extraction, purging techniques and atomic compliance, monosaccharide creations, constructions of polysaccharide portions.

Fourier-transform infrared spectroscopy (FTIR) analysis of polysaccharide

The FT-IR spectra was resolved utilizing a Fourier change infra-red spectro-photometer on instrument Thermo, NICOLET 6700. Test was squeezed into pellets, and afterward exposed to FT-IR spectro-photometer in the range of 4000–400 cm⁻¹. Effects was checked at temperature 25°C, diluted alkali, concentrated alkali, hot water, and high pressure.

ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)

Cytotoxicity Test

The cytotoxicity of the extract was determined using hemolytic assay using by using UV-VIS double beam Spectrophotometer (HALO DB-20 Dynamica). Titron X-100 was used as standard. The absorbance was recorded at 576 nm.

Ultraviolet (UV) spectroscopy analysis of polysaccharide

The divisions were set up into 1 mg/mL watery arrangements and Ultra violet-obvious (UV-vis) spectra were produced utilizing an UV spectrophotometer ranging from 190–400 nm. Effects was checked at temperature 25°C, diluted alkali, concentrated alkali, temperature 100°C, and high pressure.

HPLC analysis

Total phenolic contents were estimated using standard methods. 5mg extract was dissolved in 1mL methanol and filtered through 0.2µm syringe filter. The filtrate

then added into the HPLC vials screwed with cap and placed in HPLC machine. HPLC analysis was performed in HPLC LC-10A series equipped with liquid pump LS-10AS, C-18 column (CLC-ODS 25cm×4.6 mm 5µm) and a fluorescence UV-visible detector. A mobile phase containing methanol and water with the ratio 95:1 was used with the flowrate of 1mL/min. The compounds in HPLC were resolved using isocratic elution techniques.

Antioxidant assay

Free radical scavenging activity

The 2,2-Diphenyl-1 picrylhydrazyl (DPPH) method was used to measure the free radical scavenging activity. For DPPH solution, 1 mg of the reagent (DPPH) was dissolved in 50mL of solvent (methanol). 5mL DPPH solution was added into six different test tubes containing 5mg extract and 5mL methanol, separately. Samples were incubated at room temperature for 20 minutes. Blank was taken without extract. Optical density (OD) value was calculated by spectrophotometer (HALO DB-20 Dynamica) at 517nm wavelength. Antioxidant potential was measured by the following mathematical equation.

% activity =

$$\frac{(\text{Absorbance}_{(\text{blank})} - \text{absorbance}_{(\text{sample})}) \times 100}{\text{Absorbance}_{(\text{blank})}}$$

Statistical analysis

The results were taken in triplicates and $p < 0.05$ was considered as statistically significant. The resulted data was analyzed statically using IBM SPSS statistics version 21.

RESULTS

The protocol and method for the sequential extraction of polysaccharides from dried *P. ostreatus* is described in material and method section. After extraction of polysaccharides and parts of the phenolic compounds with ethanol, the *P. ostreatus* material was subsequently extracted with normal temperature, diluted alkali, concentrated alkali, hot water and high pressure. 03 raw fractions produced underwent the same purification process, resulting in 03 semi-purified fractions of polysaccharide.

Thermal gravimetric analysis (TGA) of *P. ostreatus* polysaccharide

The TGA spectra was used to study the thermodynamic properties of polysaccharides. The thermal stability of biomolecules is one of the important characteristic to be used for the biological applications. An important characteristic is the thermal stability of biomolecules to be used for the biological applications. The characteristics of thermal properties of the different extractions are showed in Figure 1. 02 different stages were very well defined during the TGA spectra. The first stage was mainly associated with the loosing of bounding water, and the thermal decomposition was responsible for second stage. The beginning

decomposition temperature of the polysaccharides could be set as at normal temperature, diluted alkali, concentrated alkali, hot water and high pressure, this result showed that, the purification method and extraction methods can affect the thermostability of different polysaccharides. The fractions which were

extracted with H₂O obtained more thermostable polysaccharides than the polysaccharides which were extracted with alkaline and acid; simultaneously, the stability of the acidic fractions might be lower than the neutral fraction.

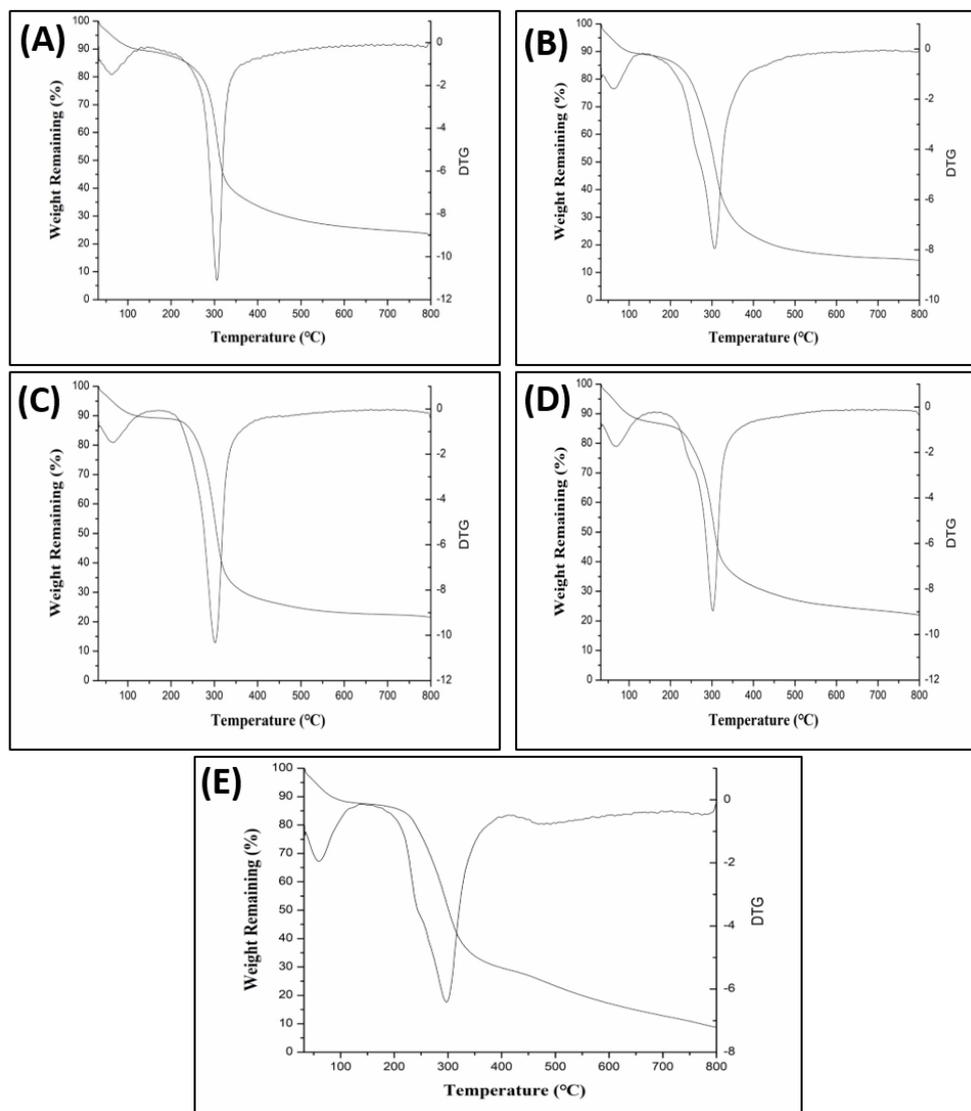


Figure 1: TGA spectra of *P. ostreatus* polysaccharide at (A) normal temperature, (B) diluted alkali, (C) concentrated alkali, (D) hot water and (E) high pressure.

X-ray diffraction (XRD) analysis of *P. ostreatus* polysaccharide

To examine crystalline or amorphous nature of the polysaccharides, the X-ray diffraction (XRD) spectra of polysaccharide was recorded. The characteristic diffraction curve of each OM purified polysaccharides was exhibited by the XRD profile. There was a weak diffraction peak for each OM with different intensity was recorded when the 2θ was nearly 47° , which was indicating the presence of both of the amorphous nature and crystalline nature of the different polysaccharides. A diffraction peak approximately at 18° was recorded for

OM-1, and the comparative results demonstrated the influence of extractions process on the physiological structure of different polysaccharides.

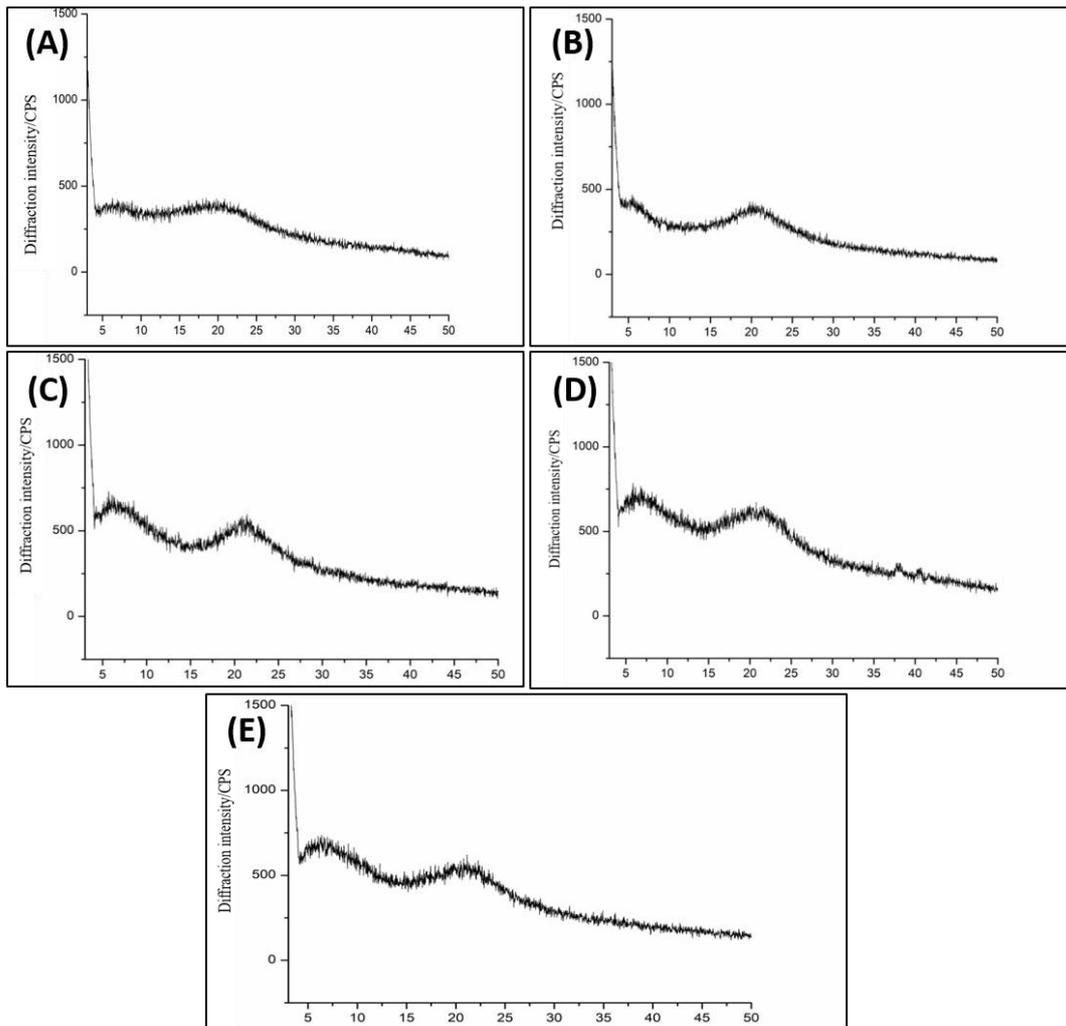


Figure 2. XRD spectra of *P. ostreatus* polysaccharide at (A) normal temperature, (B) diluted alkali, (C) concentrated alkali, (D) hot water and (E) high pressure.

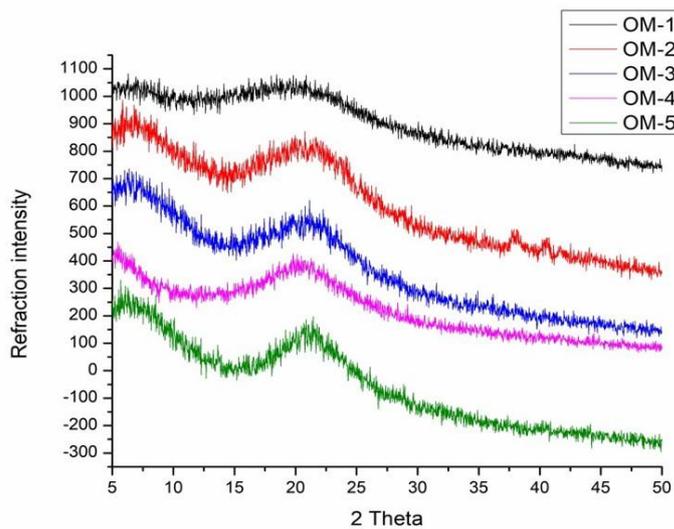


Figure 3: XRD spectra of polysaccharides.

Differential scanning DSC spectra of *P. ostreatus* polysaccharide

To measure the occurrence of exothermal or endothermal

changes, the DSC spectra was used with an increased temperature. At 231.9 °C, 304.6 °C and 300.1°C, an exothermal valley appeared, respectively for OM-1, OM-

2 and OM-3 in DSC curve. While for OM-1 there was an endothermic peak at 246.9°C. Similarly, these distinctions between extraction were may be caused by these differences in purification methods, thermal

stabilities, and molecular conformation, structures of polysaccharide fractions and monosaccharide compositions.

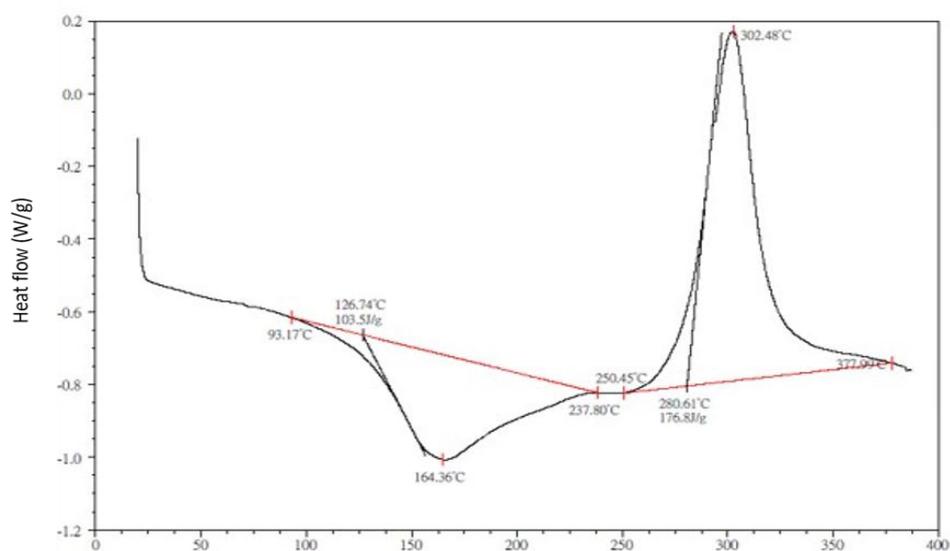


Figure 4: Temperature (°C): DSC spectra of *P. ostreatus* polysaccharide.

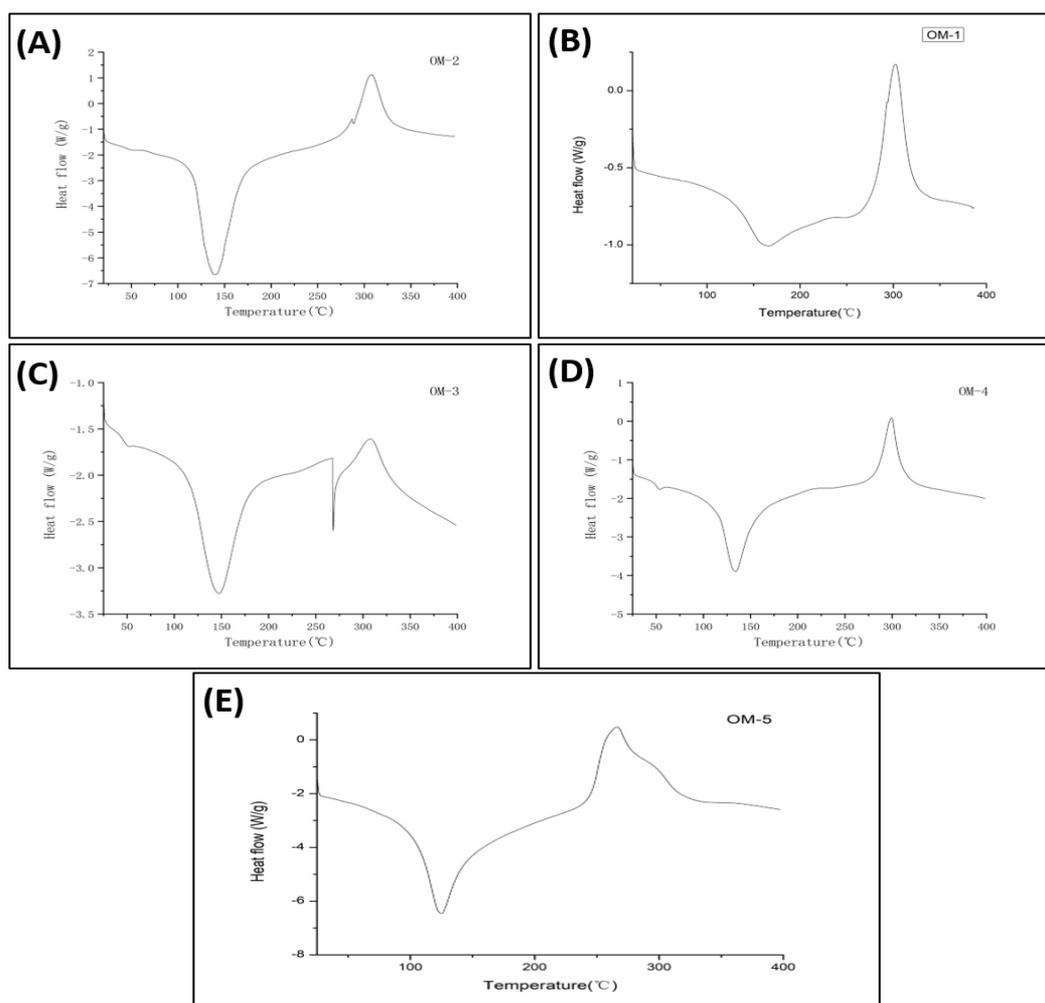


Figure 5. DSC spectra of (A) OM-1, (B) OM-2, (C) OM-3, (D) OM-4 and (E) OM-5 polysaccharide.

Fourier-transform infrared spectroscopy (FTIR) analysis of polysaccharide:

The FTIR spectrum is shown in Figure 6, a broad peak around 3400cm^{-1} for hydroxyl, bunches extending vibrations; the top around 2900cm^{-1} showed retention for C-H extending vibrations; the band around 1735cm^{-1} showed assimilation for carboxyl gatherings extending vibrations, which can be utilized to decide the presence of uronic corrosive; a top around 1650cm^{-1} was happened because of the related water. Three feeble assimilation tops somewhere in the range of 1000 and 1200cm^{-1} were relegated to C-O-C extending of glycosidic bonds, which showed the conceivable presence of pyranose ring, the noticed band around at 850cm^{-1} demonstrated the presence of α -design on the

whole of these sanitized polysaccharides; the powerless ingestion groups at 900cm^{-1} in OM-1 was credited to be normal for β -glycosidic linkages in the polysaccharide chains. The portrayal of the divisions by FTIR spectra investigation showed average ingestion pinnacles of polysaccharides. Figure 6 shows the vibration groups in the territory $3600\text{--}1200\text{cm}^{-1}$ and, in the embedded development, the zone of the recorded spectra somewhere in the range of 1200 and 900cm^{-1} . The wideband around 3220cm^{-1} was doled out to the O-H extending, because of both polysaccharide hydroxyl gatherings and test dampness. The assimilation groups somewhere in the range of 2920 and 2850cm^{-1} were, thusly, doled out to C-H (pyranoid ring and CH_2) bond extending.

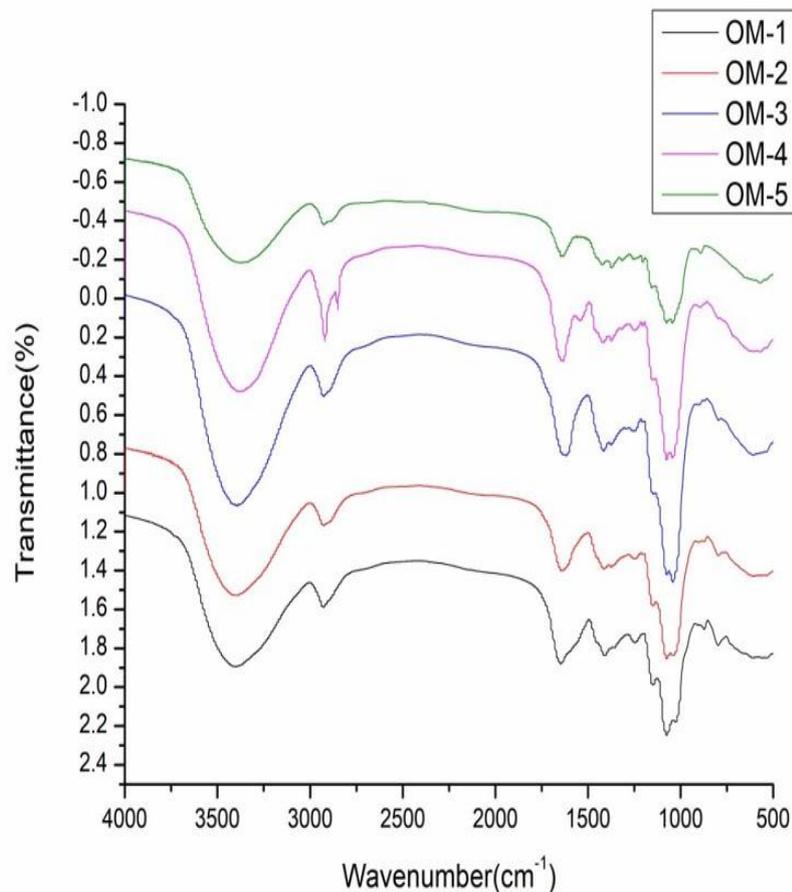


Figure 6: FT-IR spectra of *P. ostreatus* polysaccharide.

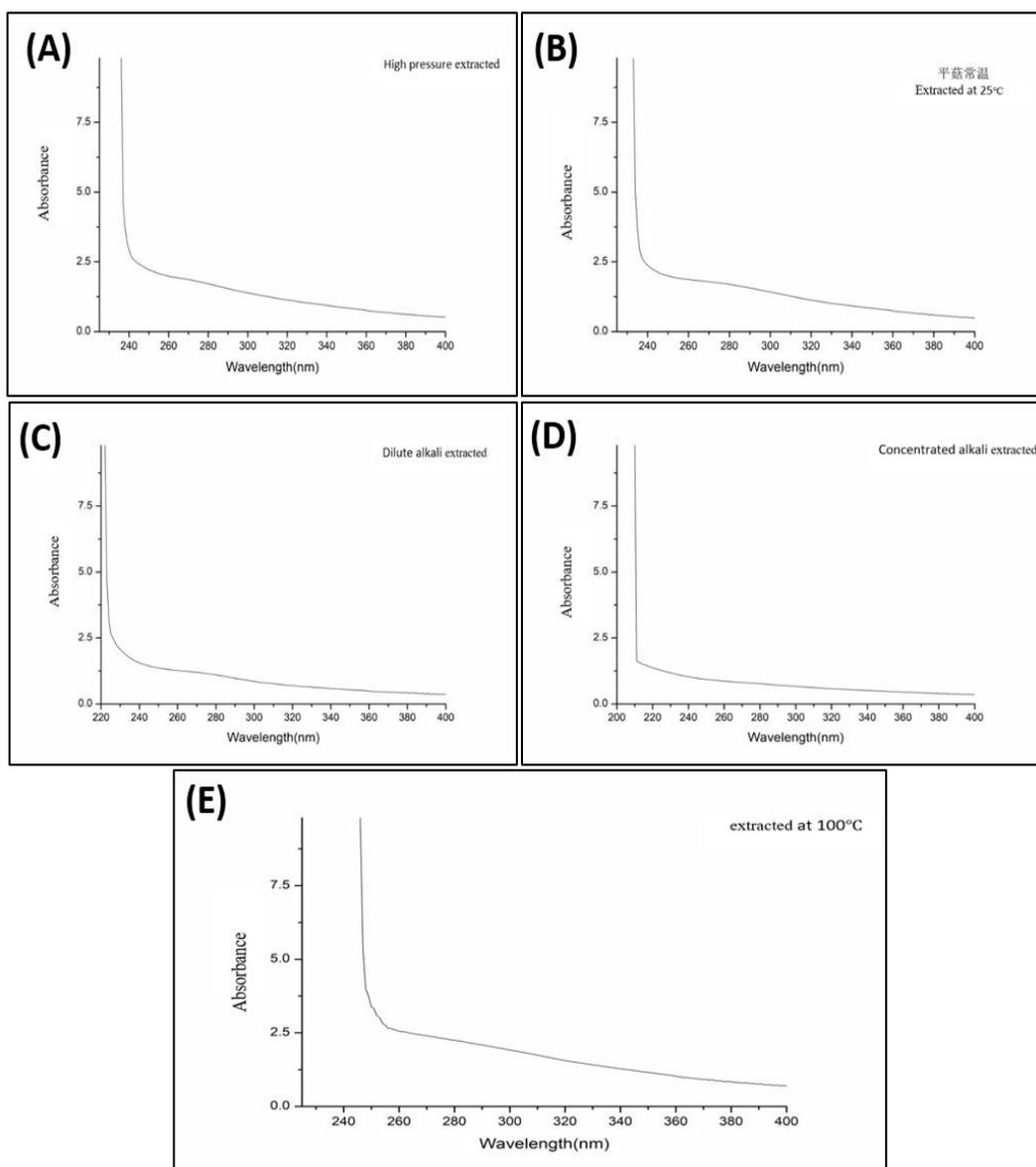


Figure 7. FTIR spectra of *P. ostreatus* polysaccharide at (A) normal temperature, (B) diluted alkali, (C) concentrated alkali, (D) hot water and (E) high pressure.

Ultraviolet spectra of polysaccharide

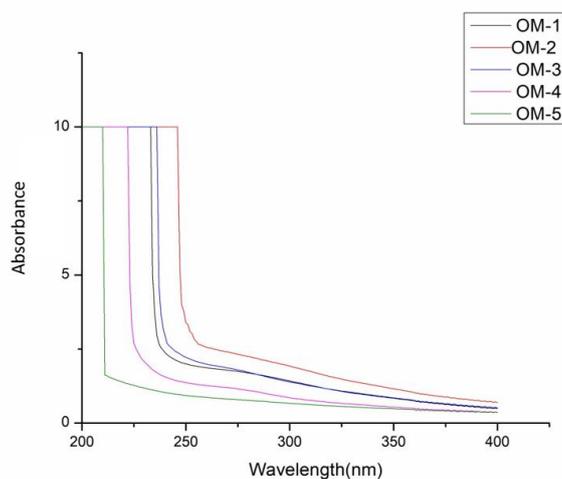


Figure 8: UV spectra of *P. ostreatus* polysaccharide.

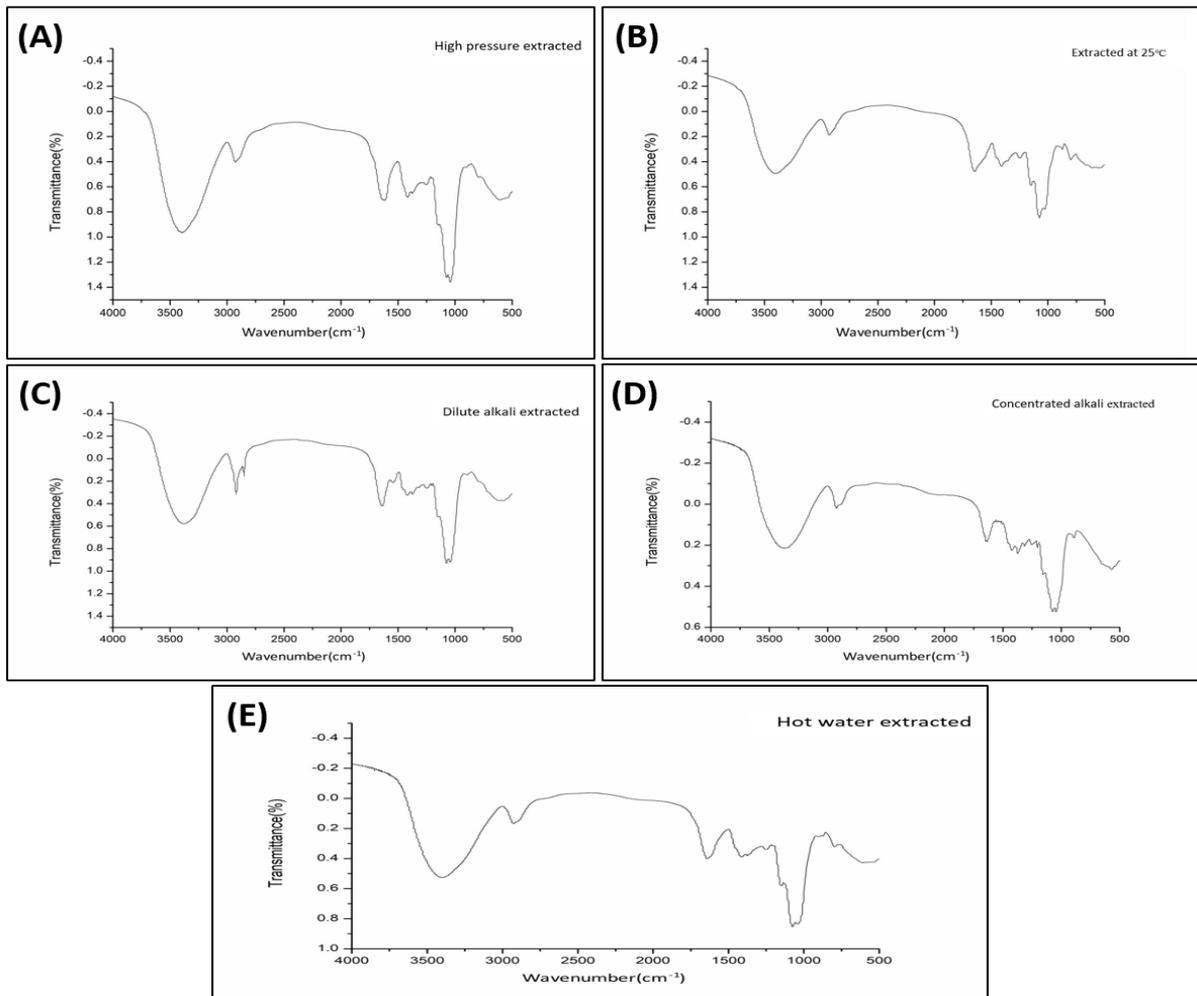


Figure 9. UV spectra of *P. ostreatus* polysaccharide at (A) normal temperature, (B) diluted alkali, (C) concentrated alkali, (D) hot water and (E) high pressure.

DPPH analysis

Antioxidant potential of the *P. ostreatus* extract was evaluated by DPPH assay. The highest antioxidant

activity was found in OM-5 and OM-4 at the concentration of 5mg/mL with the percentage scavenging activity as (50± 0.005) and (48±0.001) respectively.

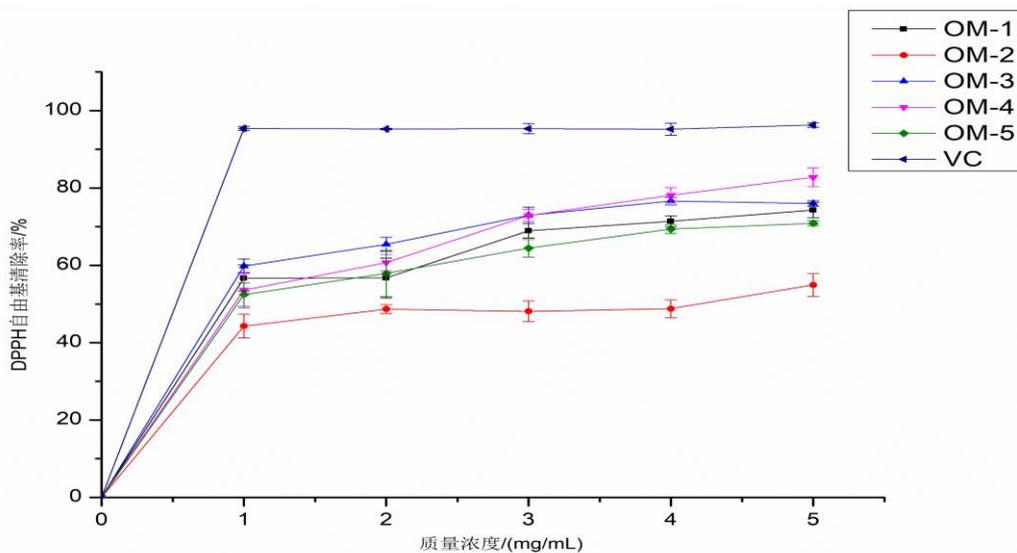


Figure 10: DPPH analysis of polysaccharides.

DISCUSSION

Results of the previous study from China showed that the physico-chemical composition of dehydrated fruit bodies of *Pleurotus* species among different species *P. eous* was rich in protein (33.89%), moderate in fat (3.10%), carbohydrate (32.60%) and ash (8%) followed by *P. florida*.^[16] However, *P. flabellatus* was rich in crude fibre, carbohydrate and ash but low in protein and fat content as compare to *P. eous* and *P. florida*.^[17] carried out an experiment to examine the effects of different levels of cow dung (0, 5, 10, 15 and 20%) on the yield and the proximate composition of *P. ostreatus*. The highest number of primordia (70.63) and fruiting body (51.92) per packet were observed in rice straw supplemented with 5% level of cow dung. The highest weight of individual fruiting body (4.71g), biological yield (234.24g), economic yield (227.72g), dry yield (22.83g) per 500 g packet, biological efficiency (140.26%) and benefit cost ratio (5.69) were observed in 10% cow dung. The highest protein content (30.90%), crude fiber (24.03%) and the lowest lipid (3.34%) was found in 10% cow dung.

Results of a previous study conducted by Cilerdzic et al. (2015) showed that the most elevated level of DPPH searching capacity was gotten by a concentrate of *P. ostreatus* mycelium developed in wheat grain enhanced medium, while control medium supported the cancer prevention agent capability of *A. cylindracea* mycelium.^[18] A similar study was also conducted by Chirinand et al. (2009) who showed that the *P. ostreatus* had more cancer prevention agent than *P. sajor-caju*. The EC₅₀ of *P. ostreatus* and *P. sajor-caju* water extricates were 11.56 and 13.38 mg/ml, individually, while those of the ethanol separates were 31.75 and 58.44 mg/ml, separately.^[19] Outcomes of the study conducted by Hong et al. (2019) showed that the subatomic load of WSP1 was 9 kDa, and it was for the most part made out of fucose and galactose in a molar proportion of 1:3.09. The cancer prevention agent test uncovered that, in the focus range tried in this analysis, WSP1 had solid searching capacity on DPPH revolutionary, recommending that WSP1 could be possibly utilized as an amazing extremist scrounger.^[20]

Amin et al. (2007) observed that minimum time (4.5 days) for primordial initiation in the MP at 20% level and the highest number of effective fruiting bodies (60.75) was obtained in WF at 50% level. The highest biological yields (247.3gram per packet) was recorded at 10% level of (WBr)^[21] While results of Zape et al. (2006) found that time required for spawn run and pinning was significantly less in *P. eous* followed by *P. florida*. However, the yield and biological efficiency did not differ significantly but was higher in *P. florida* than *P. flabellatus* and *P. eous*.^[16]

Similar another study Ayappan et al. (2000) used sugarcane trash and coir waste alone and in combination with paddy straw (3:1, 1:1 and 1:3 w/w) for sporophore

production of two species of *Pleurotus*. The highest yields of *P. florida* (1395 g) and *P. citrinopileatus* (1365 g) were recorded in a mixture of sugarcane. Similarly,^[22] cultivated the oyster mushroom on banana leaves, sugarcane, water hyacinth and beetle nut husk. They found that considerable variation in the composition of fruit bodies grown on different substrates was observed. Moisture content varied from 88.15 to 91.64%. On dry matter basis, the percentage of nitrogen and crude protein varied from 4.22 to 5.59 and 18.46 to 27.78%, respectively and carbohydrate from 40.54 to 47.68%.^[23]

A similar study was conducted by Golak et al. (2018).^[24] The bioactive substances contained in the mycelium and fruiting assortments of *Pleurotus* species show immunestimulatory, against neoplastic, hostile to diabetic, against atherosclerotic, mitigating, antibacterial and hostile to oxidative properties. While,^[25] performed a study. In the warm oxidative soundness test, utilizing fat, the request for antioxidative movement of test materials showed comparable propensities, with the exception of the concentrate of *Lentinula edodes*.

A similar study was conducted by Fakoya et al. (2020). They showed that, various zones of antibiotics were exceptionally articulated in *E. coli* having 18.0 ± 1.41 mm followed by *K. pneumoniae* of 17.0 ± 0.58 mm then *S. aureus* of 14.0 ± 1.53 mm lastly *P. aeruginosa* 13.0 ± 0.58 mm.^[26] A similar study was conducted by Et Batal et al. (2018) who showed that, CuNPs were a solid antimicrobial specialist against microorganisms caused consume skin contamination, for example, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Candida albicans* (16.0, 15.0, and 15.0 mm ZOI, individually). Moreover, CuNPs have a solid cell reinforcement with 70% searching movement against DPPH.^[27]

Results of a research study conducted by Espinosa et al. (2017), demonstrated that the organism positively affects the substrates when contrasted with the controls. The cancer prevention agent action (39.5% on kidney beans and 225% on oats comparable to the controls) and substance of all out polyphenols (kidney beans multiple times higher in regards to the controls) expanded essentially by the presence of the growth mycelium, even after mimicked assimilation. Generally, this aging treatment with *Pleurotus ostreatus* improved the wholesome nature of cereals and vegetables, making them expected elements for the elaboration as well as stronghold of food varieties for human nourishment.^[28]

Dong et al. (2019) conducted a research study. The outcomes uncovered that EnRPS at 400 mg/kg bw showed unrivaled liver defensive impacts by enhancing the AI, lowering the hepatic MPO, FFA, ADPN, TC and TG pointers, and improving the cancer prevention agent status by upgrading liver compound exercises (SOD, CAT, LEP, INS and T-AOC), disposing of the MDA content, diminishing the degrees of LDL-C, TC, TG, ALT, AST and ALP, and expanding the HDL-C in

serum. Their results proposed that EnRPS could be utilized as utilitarian food varieties of oxidative pressure and hostile to hyperlipidemic against liver injury.^[29] Deveci et al (2019) worked on a study in Brazil.^[31] FT- IR investigation showed the trademark pinnacles of the polysaccharides and elite fluid chromatography- diode exhibit recognition was utilized to decide the sub-atomic load of the polysaccharides. In β - carotene–linoleic corrosive measure FF (IC50: $2.55 \pm 0.40 \mu\text{g/ml}$) showed the most noteworthy cell reinforcement movement, while GAP demonstrated the most noteworthy cancer prevention agent action in cupric decreasing cancer prevention agent limit (A0.50: $59.90 \pm 0.53 \mu\text{g/ml}$), ABTS•+ (IC50: $16.62 \pm 0.31 \mu\text{g/ml}$), and DPPH• (IC50: $45.58 \pm 0.21 \mu\text{g/ml}$) examines.^[30]

Wani et al. (1998) reported that among the various edible fungi, oyster mushroom (*Pleurotus spp.*) has a broad adaptability due to having a wide range of suitable substrates, a simple cultivation technique and minimal cultural requirements. Various substrates on which oyster mushroom can be cultivated are mentioned.^[31]

Zhang et al. (2012) found that the protein substance of mushrooms created was 27.2% on a normal.^[32] The dry matter loss of the substrate after mushroom development changed from 30.1 to 44.3%. Yields were higher from substrates which had been ground-up to 2.5cm lengths; further size decreases brought down yields. Mushroom development is a profoundly effective technique for discarding farming deposits just as creating nutritious human food. Another study from China reported that the fruiting bodies of mushrooms contained (82.5-92.2) % moisture, (4.30-50.7) % carbohydrate, (26.6-34.1) % crude protein and (1.1-8.0) % fat.^[33]

CONCLUSION

In this study, sequential extractions of polysaccharides from *P. ostreatus* were performed with water, acid and alkaline, then further purified by anion exchange and gel permeation chromatography. The physicochemical properties of purified polysaccharides were characterized by using different analytical tools and methods. TGA spectra, XRD, DSC, FT-IR, UV spectra, HPLC and DPPH analysis provided valuable information. The structural characteristics and antioxidant activity of polysaccharides were affected by extraction methods. Four kinds of purified polysaccharide fractions have different content, molecular weight, monosaccharide composition, surface morphology, thermal stability and antioxidant activity. Biochemical analysis confirmed that purified fraction OM-1 has strong free radical scavenging activity against DPPH, hydroxyl and ABTS free radical. The overall study suggested that *P. ostreatus* polysaccharide could be used as a potential natural antioxidant drug resource and has value for further scientific research in food and pharmaceutical industry. Further studies are now in progress to gain more insight into its structures and identify other potential biological activities of these novel *P. ostreatus*-derived

polysaccharides.

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