



# Purification, chemical, structural characterization and biological activity of polysaccharide from blue oyster mushroom *Hypsizygus ulmarius*

Alamelu Thimmaraju<sup>1</sup> · Palaniappan Seedeve<sup>2</sup>

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## Abstract

The polysaccharide (HUP-1) was fractionated and purified from crude polysaccharide of *Hypsizygus ulmarius* by using ion-exchange and gel-permeation chromatography. The chemical composition analysis of the HUP-1 noted 60.07% of carbohydrate, 2.22% of protein, 11.08% of uronic acid, and 4.39% of sulfate content respectively. The molecular weight of HUP-1 was 27,887 Da, and its monosaccharide composition disclosed 44.4% of mannose, 36.4% of glucose, and 19.18% of fucose. Further, the HUP-1 was examined for the structural characterization through the FTIR, <sup>1</sup>H-NMR, <sup>13</sup>C NMR, XRD, and SEM analysis. The HUP-1 showed the DPPH and ABTS scavenging activity were 16.38–61.60% and 21.33–85.08% at 1–5mg/ml concentration, with the EC<sub>50</sub> values which were 4.160 and 2.245mg/ml and the significant value which was  $p < 0.05$ . The anticancer activity of HUP-1 reported 70% at 100µg/ml concentration against human lung carcinoma A549 cells. Hence, the above results of the HUP-1 showed good antioxidant and anticancer activity; it was well correlated for their molecular weight and monosaccharide composition such as mannose, glucose, and fucose. So, the good antioxidant and anticancer activity of the HUP-1 suggested that may be an effective chemotherapeutic treatment for lung cancer in the future pharmacological industry.

**Keywords** *H. ulmarius* · Polysaccharides · FT-IR · NMR · Antioxidant · Anticancer

## 1 Introduction

Mushrooms are broadly expended for their high nutritive esteem and therapeutic properties [1]. They contain an assortment of auxiliary metabolites such as phenolic compounds, terpenoids, alkaloids, and polysaccharides [2]. In previous studies of elm oyster mushrooms, it has been reported earlier that water and methanol extracts from elm oyster mushrooms have many bio application [3, 4], such as antioxidant activity [5], life prolonging, and immunomodulatory activity [6]. Still, few information about the bio application or structure of polysaccharides has been

published. Because of their anticancer, antioxidant, immunomodulating, and other properties, polysaccharides have been described as biological response modifiers [7, 8].

Cancer is the greatest important human malignant [9], and the second largest cause of cancer-related deaths in men in the world [10], with its predominance expanding in developing countries [11]. To lower the mortality rate, numerous helpful choices have been used, including surgery [12], radiation [13], and adjuvant chemo or hormonotherapy [14]. Unfortunately, lung cancer is especially troublesome to treat since it is very resistant to radiation and predictable chemotherapeutic drugs [15, 16], and this resistance is connected to a poor prognosis [17], especially in hormone receptor lung cancer [18]. Approximately 30–40% of men with this cancer will metastasize and eventually die of the disease [19]. In this manner, novel recuperating venders are to address the developing predominance of human lung cancer.

Currently, the purified polysaccharides of *Lentinus velutinus* demonstrated the anticancer activity against HeLa (human epithelial cervix carcinoma) and HepG2 (human hepatocellular liver carcinoma) cells and conformed the

✉ Palaniappan Seedeve  
seedevisalem@gmail.com

<sup>1</sup> Department of Biochemistry, School of Biosciences, Periyar University, Salem, Tamil Nadu 636011, India

<sup>2</sup> Department of Product Development, Institute of Biotechnology, Saveetha School of Engineering, Saveetha Institute of Medical and Technical Sciences (SIMATS), Chennai, Tamil Nadu 602105, India

structural characterization [7]. In addition, the mushroom polysaccharides from *Pleurotus sajor-caju* and *Lactuca Sativa* had anticancer activity against colon (HCT 116), liver (HEPG2), cervical (HELA), and breast (MCF7) carcinoma cell lines [20]. Seedeivi et al. [21] investigated the chemical structure and biological properties of a polysaccharide derived from *Pleurotus sajor-caju* and discovered that it has antioxidant and anticancer activity. Hence, the biological activities of the mushroom polysaccharide, in the present study, were to evaluate the biological activity of polysaccharide from blue oyster mushroom *Hypsizygus ulmarius*, and their chemical and structural properties were analyzed

## 2 Materials and methods

### 2.1 Collection of mushroom

The fruit bodies of the *H. ulmarius* (fresh) were collected by VG Mushroom Culture, Salem, Tamil Nadu, India. The *H. ulmarius* was shade-dried, at that point broiler dried at  $40\pm 2^\circ\text{C}$  for 48 h, and processed into powder through 0.5-mm strainer employing a pound process (IKAWERKEMF10; IKA-Works, Staufenim Breisgau, Germany), and stored in sealed shut plastic packs to further investigations.

### 2.2 Hot water extraction

Fifty grams of powder was added to 250 ml of petroleum ether by stirring with 2 days after filtered in no. 1 Whatman filter paper for evacuating the fat substance and dried to get pre-extract. Then the sample was mixed with 750 ml of  $\text{dH}_2\text{O}$  were boiling at  $90^\circ\text{C}$  for 3 h and filtered at the same process three times after that centrifuged at (300rpm) 1000 g for 15 min. The supernatant was diluted with 95% of ethanol and kept at  $4^\circ\text{C}$  overnight. It was then filtered and oven-dried, after which it was deproteinized using the savage strategy chloroform with n-butanol (4:1 v/v) at 6 times [22]. Thus, it was dialysed against double-distilled water for 72 h in a cold room. The dialysed solution was dried in the oven, and 200 mg of sample was dissolved in 10 ml of double-distilled water. Then the polysaccharides were precipitated using ethanol and centrifuged at 1000 g for 15 min (3000 rpm); supernatant was collected and further treated for purification.

### 2.3 Purification of polysaccharide

The polysaccharides were purified through DEAE-52 ion exchange chromatography [22]. The column was eluted with distilled water and aqueous NaCl solutions (0.1, 0.3, 0.5 mol/l) were significantly increased gradually at a flow rate of 5.0 ml/min. The different fractions were collected

(HUP-1, HUP-2, HUP-3), and the presence of carbohydrate was assessed by phenol-sulfuric acid method [23]. The higher yield of the fraction was purified with Sepharose 6B fast flow, and the purified fraction was lyophilized and encoded as HUP-1 have been obtained. HUP-1 was collected for further investigation due to its highest antioxidant activity in our preliminary experiment.

### 2.4 Molecular weight determination

High-performance liquid gel permeation chromatography (HPGPC) was used to determine the molecular weight distribution of the polysaccharide from *H. ulmarius* using an Agilent 1260 system, a TSK-Gel G4000 SWXL (7.8 mm 300 mm) gel chromatography column, and a refractive index detector. Each dextran standard was dissolved and turned into a standard solution containing 4.0 mg/ml. This sample was run through a 0.22-m aqueous phase filtration membrane and subjected to high-performance liquid chromatography for analysis. In order to solve the regression equation, the horizontal axis is the retention period ( $x$ , min) of each standard and the vertical axis is the logarithm of the dextran standard molecule ( $y$ , log MW). The precise detection conditions were as follows: an ultrapure water mobile phase, a 15  $\mu\text{l}$  injection volume, a column temperature of  $30^\circ\text{C}$ , and a flow rate of 0.7 ml/min.

### 2.5 Monosaccharide composition

The monosaccharide composition of HUP-1 was determined by pre-column derivatization with phenyl-3-methyl-5-pyrazolinone (PMP). The 10-ml polysaccharide sample was hydrolyzed for 4 h at  $120^\circ\text{C}$  with 5.0 ml of 2 mol/l trifluoroacetic acid (TFA). Methanol was added after acidolysis, and TFA was dried with a nitrogen blowing apparatus. Then, at  $70^\circ\text{C}$  for 1 h, 250  $\mu\text{l}$  of sample solution was accurately absorbed into a 5-ml Eppendorf tube, followed by 250  $\mu\text{l}$  of 0.6 mol/L NaOH and 500  $\mu\text{l}$  of 0.4 mol/L PMP-methanol. After 10 min of cooling in cold water, 500  $\mu\text{l}$  of 0.3 mol/l HCl was added for neutralization, followed by 1 ml of chloroform added to whirlpool for 1 min and centrifuged at 3000 r/min for 10 min. The supernatant was carefully collected and extracted three times before the aqueous phase was analyzed using HPLC. The HPLC system was an LC-20AD system outfitted with an Agilent ZORBAX Eclipse Xtimate C18 column (5 m, 4.6 mm 200 mm) at  $30^\circ\text{C}$ . The mobile phase was 0.05 M phosphate buffer solution (pH 6.7) and acetonitrile (83:17, v/v) at a flow rate of  $1\text{ ml min}^{-1}$  and sample injection volume of 20  $\mu\text{l}$ . To acquire chromatograms, the DAD detector was set to 250 nm. The standards were xylose, arabinose, ribose, rhamnose, fucose, mannose, galactose, glucose, glucuronic acid, galacturonic acid, N-acetyl glucosamine, N-acetyl galactosamine, and fucose.

## 2.6 Characterization of polysaccharides (HUP-1)

The HUP-1 was measured using UV-Spectrophotometer 1800 (Shimazu Corporation, Japan), using 200–400 nm [24]. The polysaccharide region was resolved with a Fourier shift infrared (FTIR) spectrophotometer. The sample was ground with spectroscopic potassium bromide (KBr) powder and then ground into 1-mm granules for FTIR confirmation in the repeating range of 4000–400  $\text{cm}^{-1}$ . XRD energy-dispersive X-ray spectroscopy (EDS, Oxford Energy Max 80) was used for elemental analysis Cu K radiation, 40 kV and 200 mA, and scan rate 0.5°/min in a nitrogen atmosphere. Twenty milligrams of HUP-1 is dissolved in 0.5 ml of double-distilled water and set into a 4-mm NMR tube.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR were performed on a BRUKER 600M spectrometer (Rheinstetten, Germany) at 400 and 100 MHz, separately. Scanning electron micrographs were procured with a biological analyzing electron amplifying instrument (ESEM, Philips XL-30). The polysaccharide tests of HUP-1 were set on a specimen holder with the assistance of two-fold sided adhesive tapes and covered with gold powder. The test was intensified at an animating capability of 20-kV micrography.

## 2.7 Antioxidant activity

### 2.7.1 DPPH radical scavenging activity

The DPPH assay was performed as different concentrations of polysaccharide extracts (1–5 mg/ml) were mixed with 1 ml of methanolic solution containing DPPH, which resulted in the final concentration of DPPH being fraudulent of 0 and 2 mM [25]. The mixture was stirred enthusiastically, and rinsed for 30 min at room temperature, and the absorbance at 517 nm was measured. Ascorbic acid was used as positive controls [26]. The radical excavation movement DPPH was calculated as follows:

$$\text{Scavenging activity(\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A0 represented the absorbance without the extract and A1 represented the absorbance with the extract.

The  $\text{EC}_{50}$  value (mg/ml) was obtained by interpolation from linear regression analysis and was the effective concentration at which DPPH radicals were scavenged by 50%.

### 2.7.2 ABTS radical scavenging activity

The reactions were started by expanding of 1.0 ml ABTS+ diluted to 50  $\mu\text{l}$  of various concentrations of various polysaccharide extracts (1–5 mg/ml) or 10  $\mu\text{l}$  of methanol as a control

[27]. The absorbance at 734 nm was read after 6 min and the percent inhibition was calculated. Ascorbic acid was used as positive controls [24]. Inhibition was calculated giving to the formula:

$$\text{Scavenging activity(\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

where A0 represented the absorbance without the extract and A1 represented the absorbance with the extract.

The  $\text{EC}_{50}$  value (mg/ml) was obtained by interpolation from linear regression analysis and was the effective concentration at which ABTS radicals were scavenged by 50%.

## 2.8 Anticancer activity

### 2.8.1 Cell culture

The National Center for Cell Sciences, Pune, India, provided the lung adenocarcinoma human alveolar basal epithelial cells (A549). Cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine and adjusted salt solution containing 1.5 g/l  $\text{Na}_2\text{CO}_3$ , 0.1 mM immaterial amino acids, 1 mM sodium pyruvate, 2 mM L-glutamine, 1.5 g/l glucose, and 10 mM (4-(2-hydroxyethyl)-1-piperazine (GIBCO, USA). Penicillin and streptomycin (100 IU/100 g) were reduced to 1ml/l upon comprehension. In a humidified  $\text{CO}_2$  incubator, cells were maintained at 37°C with 5%  $\text{CO}_2$  [28, 29].

### 2.8.2 Cytotoxicity assay

The MTT assay was used to assess the efficacy of polysaccharide (HUP-1) against the A549 human lung cancer cell line [30]. A549 cells were seeded in 96-well plates (1×10<sup>4</sup>/well) with 100 ml of DMEM growth media containing 10% FBS mixture, and then incubated at 37°C in a 5%  $\text{CO}_2$  environment. After 24 h of monolayer cell growth, the medium was discarded, and the relevant wells were filled with 100 ml of HUP-1 at various concentrations (10–100  $\mu\text{g/ml}$ ) in DMEM medium with 2% FBS. After 24 h of incubation with 20 ml of MTT (5 mg/ml) in PBS solution/well, the crystal formation was identified after 4 h of incubation under the prescribed conditions. The medium in each well was replaced with 100 ml of DMSO solution, and the optical density (OD) of each well was measured at 570 nm using an Elisa reader. Dox was used as a positive control and untreated A549 cells as a negative control. Following the formula, the OD value was used to calculate the percentage of viability.

$$\% \text{ of viability} = \frac{\text{OD value of experimental sample}}{\text{OD value of experimental control}} \times 100$$

### 2.8.3 Confocal microscopic analysis

To study the intracellular localization, 300  $\mu$ l of A549 cell suspension was seeded in a 6-well plate on a sterile glass cover slip and allowed to adhere for 24 h.  $IC_{50}$  concentration of HUP-1 incubated with the cells for 12 h. After that, cell medium was discarded every incubation time, PBS buffer was used to wash cells 3 times, and cells were fixed with paraformaldehyde 4%. FITC and DAPI stained with cells were further investigated. Microscopy slide was applied, cells mounted on images were obtained under the FV3000 confocal laser scanning microscope.

### 2.9 Statistical analysis

All data was subjected to one-way analysis of variance was performed and Dunnett's multiple comparison test (Graph-Pad Software, USA) was also employed to find differences in means at the level of  $p < 0.05$ .

## 3 Results and discussion

### 3.1 Isolation and purification

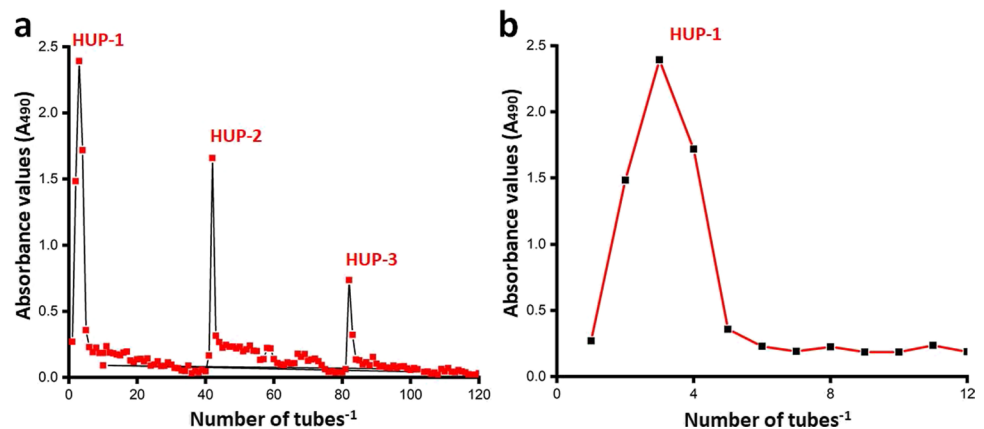
In present study, the HUP of polysaccharide from *H. ulmarius* by extraction of hot water, centrifugation, ethanol precipitation, and fat emissions dialyzed against double-distilled water. The extracted polysaccharide HUP was eluted in DEAE 52 cellulose anion exchange chromatography and gradually eluted with water, 0.1–0.3 M sodium chloride. The elution selection pecks of three as HUP-1, HUP-2, and HUP-3 were obtained in Fig. 1a. Among, the fractions, the HUP-1 was significantly higher percentage of carbohydrate, than those of HUP-2 and HUP-3. Because of their comparable

properties, we chose HUP-1 with huge substance and purified in advanced pre-clean per column of Sepharose. The Fig. 1b appeared in HUP-1 consisting of all the individual components after purification and the yield rate was 11.54%.

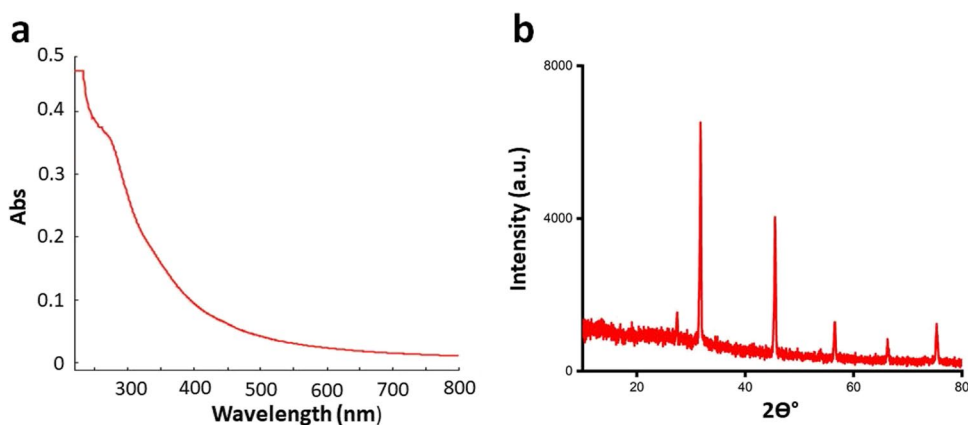
### 3.2 UV spectrometer, and biochemical and XRD analysis

After purification, HUP-1 was checked by a UV spectrometer at a wavelength of 200 to 800 nm. In Fig. 2a, as shown, weak assimilation peak of purified HUP-1 at 260 nm indicated that the polysaccharides purified from *H. ulmarius* contained exceptionally few proteins. But HUP-1 did not have an absorption peak at 260 nm [31], suggesting that all proteins were displaced during the purification system. Were there no absorption peaks discovered at 280 nm region after expansion, indicating they did not contain nucleic acid [32] so as not to appear in purified HUP-1 polysaccharides. The biochemical composition of the HUP-1 recorded 60.07% of carbohydrate, 2.22% of protein, 11.08% of uronic acid, and 4.39% of sulfate content respectively. Subsequently, the other mushroom *Pleurotus sajor-caju* polysaccharide was reported to have 90.16% of total carbohydrate and 0% of protein content [21]. HUP-1 crystalline nature was confirmed using an XRD analysis. The HUP-1 result is represented in Fig. 2b, which was obtained using an XRD spectrum with a range of 10–80°. There are 5 main crystal reflection peaks recorded at  $2\theta = 31.76^\circ, 45.5^\circ, 56.58^\circ, 66.26^\circ,$  and  $75.32^\circ$ . These peaks confirm the semi-crystalline nature of the polymer framework [33]. Several studies with various types of polysaccharides found a similar profile [34]. The molecule crystallinity is also related to solubility. For example, higher crystallinity in some drugs implies lower water solubility.

**Fig. 1** Purification of HUP. **a** Stepwise elution curve of crude HUP by DEAE-52 column **b** Elution curve of HUP-1 by Sepharose 6B column



**Fig. 2** **a** UV spectrum of HUP-1, **b** X-ray diffraction analysis of HUP-1



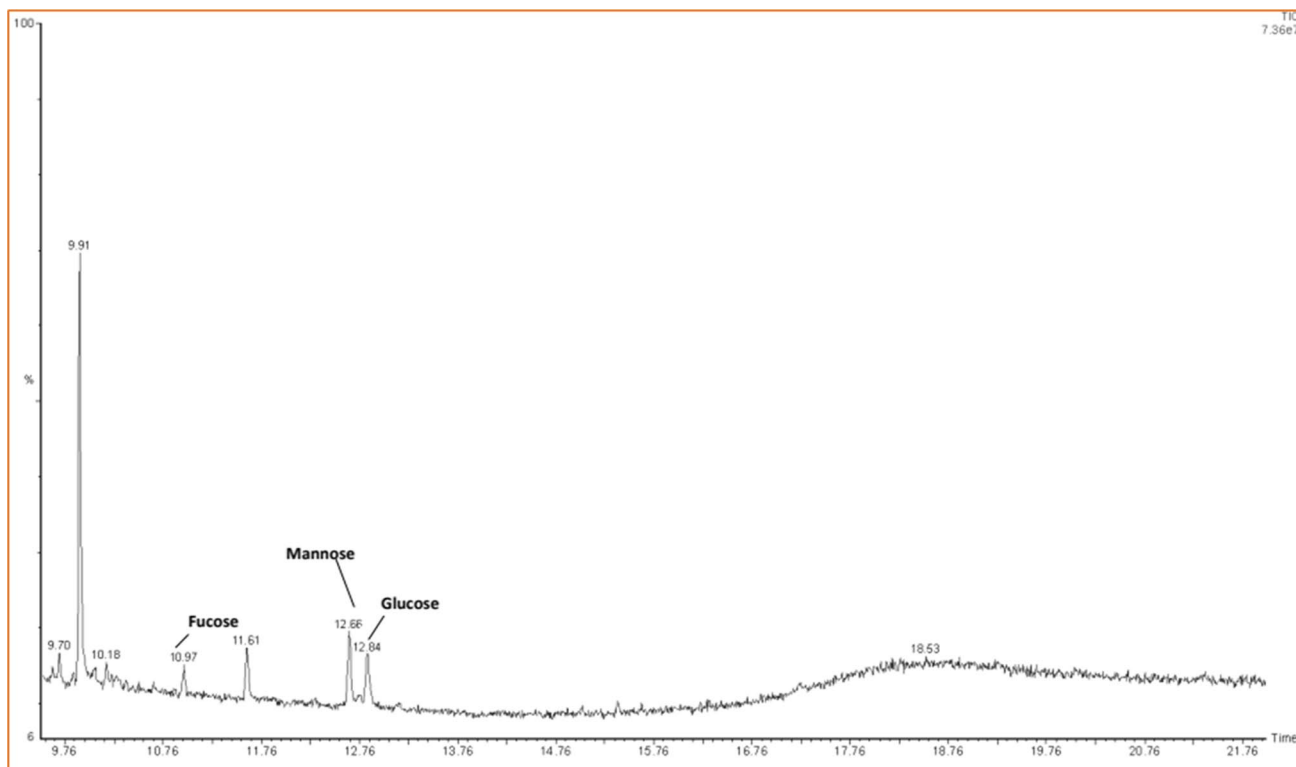
### 3.3 Monosaccharide composition

The monosaccharide content of polysaccharides was determined by HPLC. The HPLC chromatogram on various conventional monosaccharide assays. Various monosaccharide measurements were used to compare maintenance time was xylose (tR = 10.09), arabinose (tR = 10.2), ribose (tR = 10.27), rhamnose (tR = 10.69), fucose (tR = 10.9), mannose (tR = 12.7), galactose (tR = 12.8), glucose (tR = 12.88), glucuronic acid (tR = 13.26) galacturonic acid (13.8), N-acetyl glucosamine (14.87), and N-acetyl

galactosamine (tR = 15/02). The monosaccharide composition of HUP-1 is shown in Fig. 3. HUP-1 was basically composed of fucose (tR = 10.90), mannose (tR = 12.69), and glucose (tR = 12.88), and its monosaccharide composition disclosed 44.4% of mannose, 36.4% of glucose, and 19.18% of fucose.

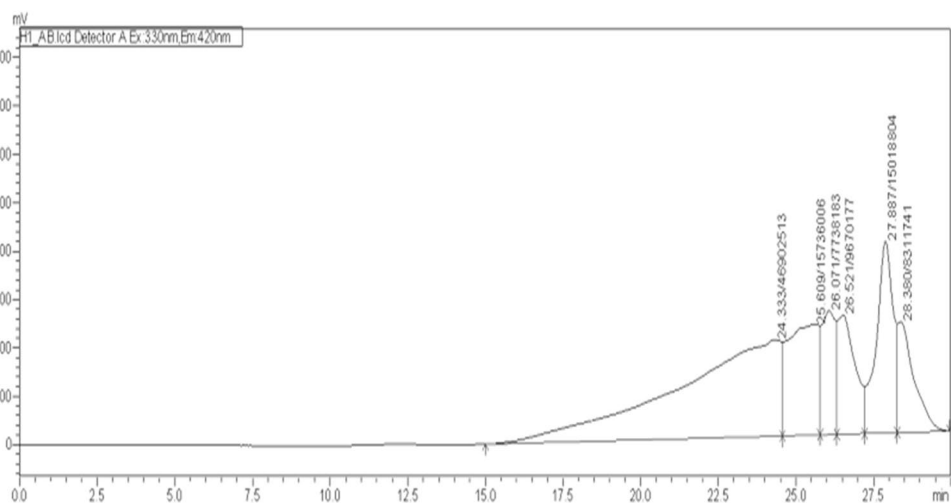
### 3.4 Molecular weight analysis

The molecular weight was determined by HPGPC. As shown in Fig. 4, the normal molecular weight of HUP-1



**Fig. 3** HUP-1 monosaccharide composition

**Fig. 4** Molecular weight of HUP-1

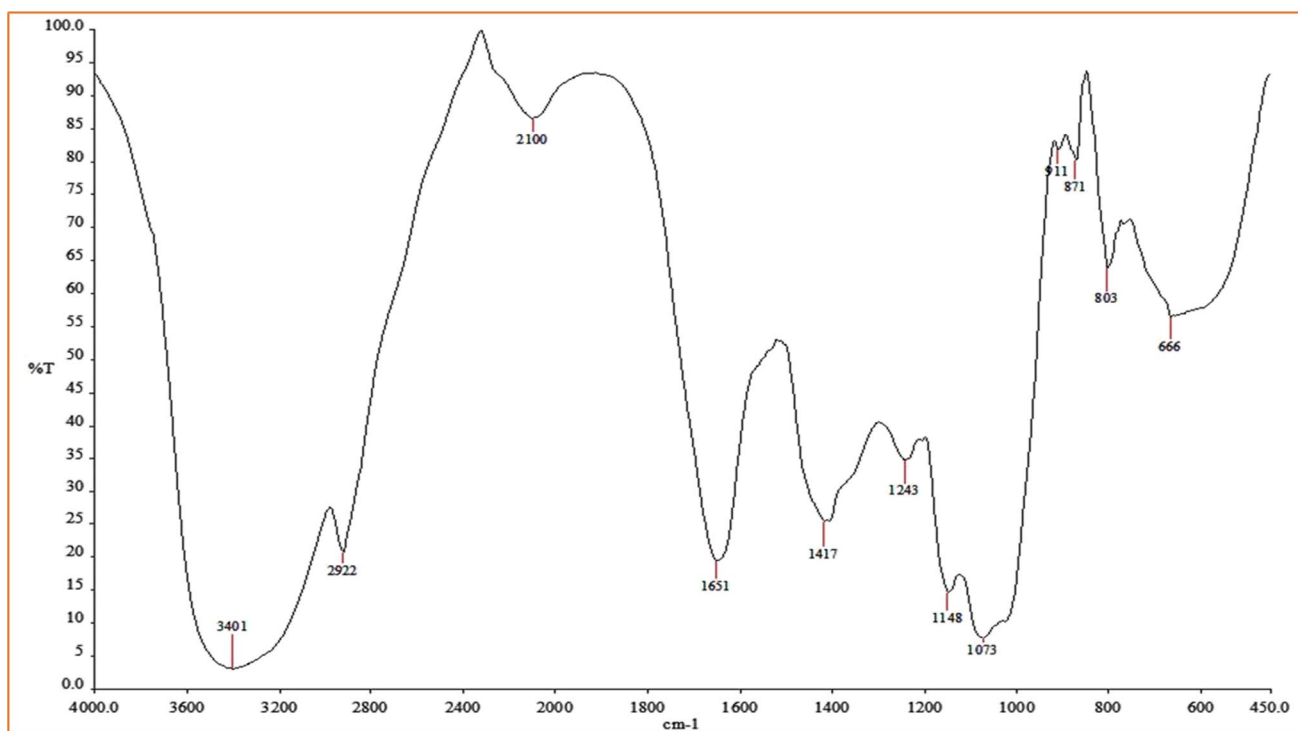


was 27,887 Da. The percentages of the peak absorption of the component were close to 100%, demonstrating that the virtue of HUP-1 was huge. In comparison with *Lepista nuda* polysaccharide with an ordinary molecular weight was 11,703 Da [35]. In the present study, the molecular weight of HUP-1 polysaccharide was lower, while that of *Porphyra haitanensis* polysaccharide was the largest, with  $6.3 \times 10^5$  Da [36]. Moreover, the polysaccharide from the mushroom *Pleurotus sajor-caju*

has a molecular weight of 79 kDa [21]. The variation in molecular weight is caused by the solvent system's extraction method.

### 3.5 FT-IR analysis

The HUP-1-FTIR results are shown in Fig. 5. The functional substances such as methylene and hydroxyl groups were accepted from the peaks at  $3401 \text{ cm}^{-1}$  and



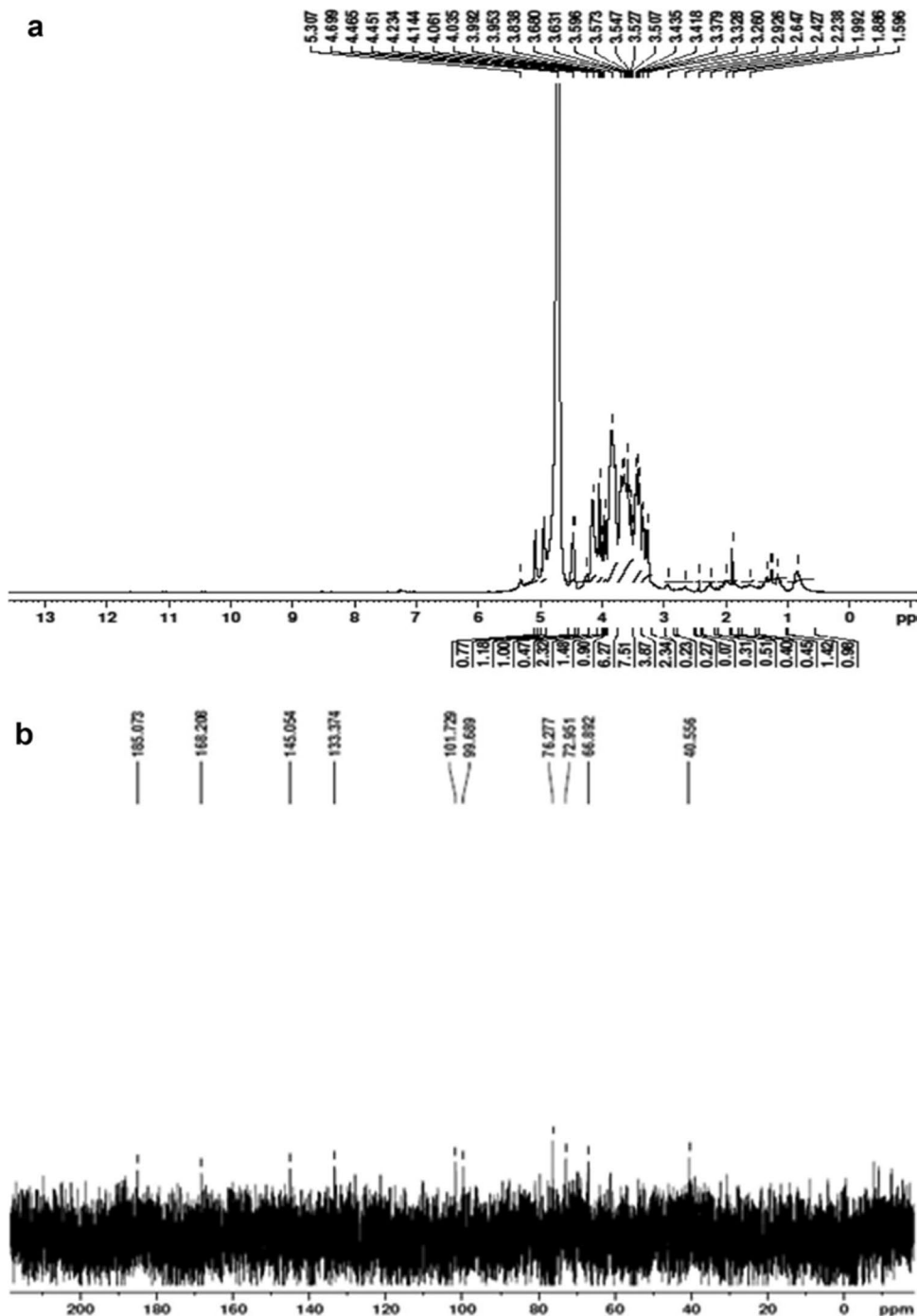
**Fig. 5** FTIR analysis of HUP-1 isolated from *H. ulmarius*

2922  $\text{cm}^{-1}$  [37]. The spectrum observed at 1243  $\text{cm}^{-1}$  and 871  $\text{cm}^{-1}$  was distributed between the expansion vibration (S=O) and the expansion vibration COS of the sulfate at the vibration point C-4 [38]. These outcomes confirm the existence of sulfate groups in the backbone of HUP-1. The peak at 1073  $\text{cm}^{-1}$  demonstrates the occurrence of C-6 accumulations of fucose, galactose, and the CO expansion vibration linked with a C–O–SO<sub>3</sub> group [39].

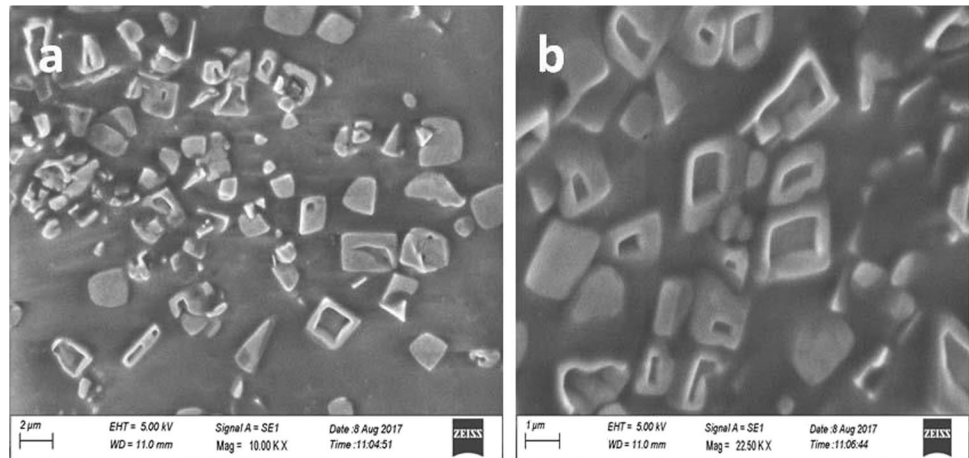
### 3.6 <sup>1</sup>H NMR and <sup>13</sup>C NMR analysis

The <sup>1</sup>H NMR and <sup>13</sup>C NMR investigation of HUP-1 illustrates the auxiliary data (Fig. 6a, b). In the <sup>1</sup>H NMR studies, peaks within the range among 3 and 5 ppm showed the typical polysaccharide signal [40]. Furthermore, the <sup>1</sup>H NMR signals of the anomeric protons in the down-field (5–6 ppm) showed the proximity 6-OH group in the polysaccharide [41]. The existence of contained mannose,

**Fig. 6** NMR Spectrum of HUP-1 **a** <sup>1</sup>H NMR and **b** <sup>13</sup>C NMR



**Fig. 7** SEM image of HUP-1. **a** 2000× magnification, **b** 1000× magnification



glucose, and fucose the position was confirmed by the ratio peaks at (3.7, 5.0, and 1.2 ppm). Furthermore, the ratio of peaks at 99.68/4.3 (C–H) signified the confirmation of N-acetyl galactosamine debris. The peak at 66.892 ppm showed in presence of glucuronic acid.

### 3.7 SEM analysis

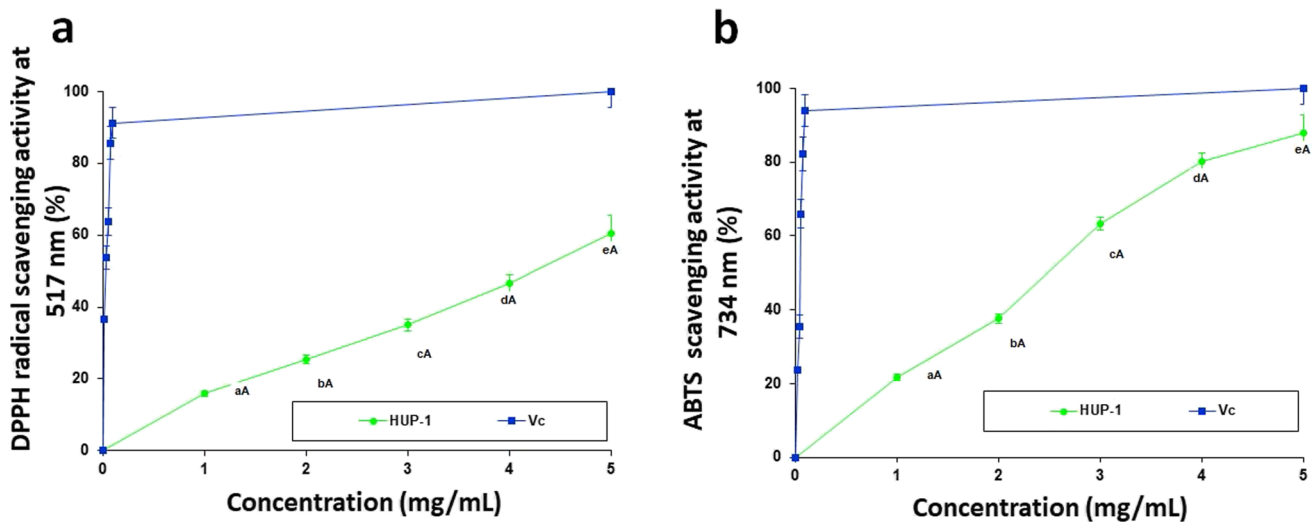
In SEM analysis, the morphological properties of HUP-1 are presented in Fig. 7; the HUP-1 had a smooth surface and an uneven structure with gaps between the crystals and was colorless. Figure 7 a and b show different amplifying controls of 2000× and 1000×. SEM has been described as a qualitative tool for analyzing the surface morphology of polysaccharides [42]. The molecular weight of the polysaccharides was used to determine the differences in

morphological properties, which could also explain the differences in activities [43].

### 3.8 Antioxidant activity

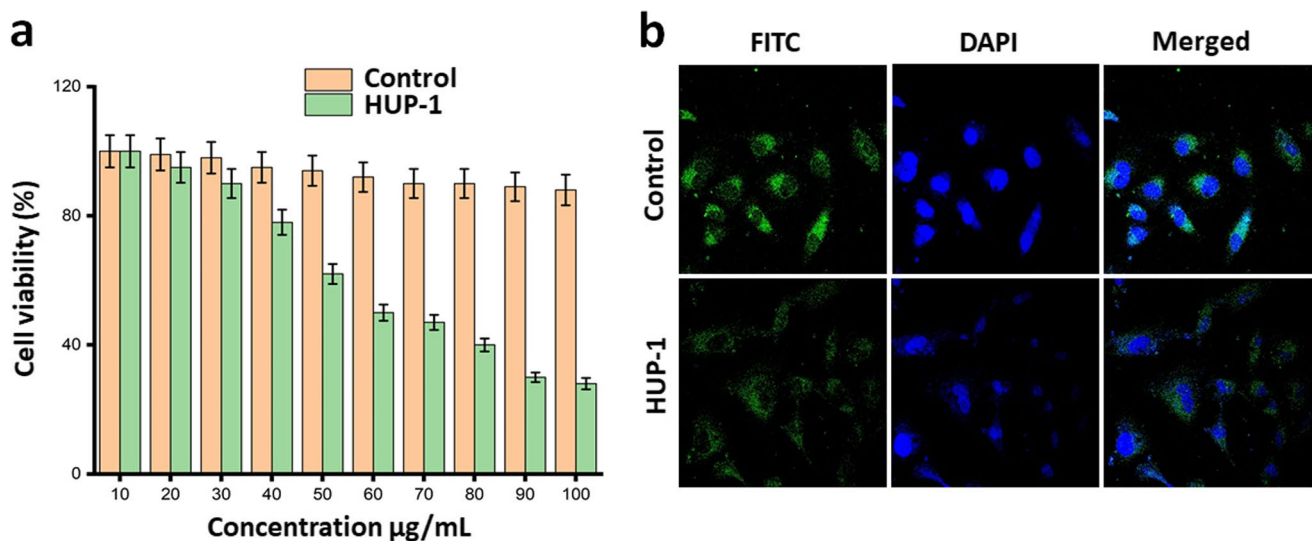
#### 3.8.1 DPPH radical scavenging activity

DPPH can be a stable free radical compound, showing an absorption maximum at 517 nm that can be instantly trapped by an antioxidant. It has been widely recognized as a device for evaluating the radical scavenging activities of characteristic compounds [26]. DPPH radical scavenging activity of HUP-1 noted 16.38–61.60% at 1–5 mg/ml concentration, compared to the standard Vc which showed 34–93.38% above the same concentration (Fig. 8a). The DPPH radical polysaccharide inhibition



**Fig. 8** In vitro antioxidant activities of HUP-1, **a** DPPH radicals scavenging activity and **b** ABTS radical scavenging activity





**Fig. 9** **a** Anticancer activity of HUP-1 against A549 cell, **b** fluorescence image

capture activity was related to the concentration of the samples. The scavenging activities of Vc and HUP-1 were significantly different from the experimental concentration. The HUP-1 polysaccharide showed the strongest removal capacity, which increased significantly as a function of concentration. At a concentration of 5.0 mg/ml,  $EC_{50}$  was 4.160 mg/ml. Similarly, the DPPH radical scavenging activity of crude polysaccharide and  $\alpha$ -L-rhamnose from *Grateloupia lithophila* recorded as 22.5–69.91% and 29.89–83.31% at 10–160 µg/ml concentration [44].

### 3.8.2 ABTS radical scavenging activity

The ABTS test was widely used to evaluate the overall antioxidant control of compounds. A special absorption at 734 nm can be used as log in both organic and aqueous solvents, reflecting the antioxidant movement of the extracted polysaccharides [45]. The ABTS radical scavenging activity of the HUP-1 was 21.33–85.08%, and the standard Vc showed 23.61–93.87% above the same concentration (Fig. 8b). The free radical scavenging activity of ABTS was increased in a concentration-dependent manner. The crude polysaccharide and  $\alpha$ -L-rhamnose from *Grateloupia lithophila* showed the ABTS radical scavenging activity were 25.04–74.46% and 33.01–87.32% at 25–125 µg/ml concentration reported by Seedeivi et al. [44]. Furthermore, CCPP-1 showed the radical scavenging activity of 99.4% at 500 µg/ml concentration [46]. Comparatively, the results showed that HUP-1 has strong ABTS radical scavenging power and could be explored as a potential new antioxidant.

### 3.9 Anticancer activity

The cellular toxicity and viability of HUP-1 treatment on A549 cells, with various concentration of HUP-1, range from 10 to 100 µg/ml. The HUP-1 did not show apparent cytotoxicity, and the concentration was less than 40 µg/ml. However, at high concentrations of HUP-1, cytotoxicity was observed with a slight increase in all treatments. Therefore, compared to the control, HUP-1 showed a high inhibitory effect on cellular development in A549 cells. Higher cytotoxicity of HUP-1 was apparent in all the concentrations at different time treatment. As it were, 60.5 µg/ml of HUP-1 caused almost 53% of cell death compared to a single major cell death of 25% caused by the control. The cytotoxicity was also noticeable at a concentration of HUP-1, since 70% of cell death was recognized at 100 µg/ml and the differences in means at the level of  $p < 0.05$  (Fig. 9a). Its clear indication of HUP-1 was a high level of cellular internalization of A549 cells. Crude glycerin and glycerin polysaccharide extract from *Chaetomium globosum* verified the viability of A549 cells in only 49% [47]. The observed HUP-1-treated A549 cells suggest that cell internalization with optical imaging can be efficiently achieved by confocal laser scanning microscopy which no other imaging probe was used. We calculated the cellular internalization of HUP-1 in A549 cell line with incubation times of 12 h. Additionally, as shown in our data, the MTT assay findings with varied concentrations of the positive control drug doxorubicin (10–100 µg/ml) demonstrated that the control treatment had a significant impact on A549 cells. The confocal study shows in Fig. 9b that DAPI and FITC were incubated with HUP-1 in A549 cells, suggesting cellular internalization. This highlights HUP-1 as a productive anti-cancer property against A549 cellular internalization.

## 4 Conclusion

In the present study, the HUP-1 purified polysaccharide from *H. ulmarius* has good antioxidant (DPPH and ABTS scavenging activity) and anticancer active potential against human lung carcinoma A549 cells. The antioxidant activity of HUP-1 exposed the inhibition rate of 61.60% and 85.08% on DPPH and ABTS scavenging activity and 70% inhibition rate against human lung carcinoma A549 cells. The bioactive potential of the HUP-1 was well correlated for their molecular weight and monosaccharide composition such as mannose, glucose, and fucose. So, the good antioxidant and anticancer activity of the HUP-1 suggested that may be an effective chemotherapeutic treatment for lung cancer in the future pharmacological industry.

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**Author contributions** Alamelu Thimmaraju: extraction, and biological activity evaluation in whole manuscript; Palaniappan Seedeve: conceptualization, writing—review, editing, and validation.

**Data availability** The data used to support the finding of this study are included within the manuscript.

## Declarations

**Ethical approval** Not Applicable.

**Competing interests** The authors declare no competing interests.

## References

- Valverde ME, Hernández-Pérez T, Paredes-López O (2015) Edible mushrooms: improving human health and promoting quality life. *Int J Microbiol* 2015:376387–376387
- Cheung PCK (2010) The nutritional and health benefits of mushrooms. *Nutr Bull* 35(4):292–299
- Venturella G, Ferraro V, Cirlincione F, Gargano ML (2021) Medicinal mushrooms: bioactive compounds, use, and clinical trials. *Int J Mol Sci* 22(2):634
- Fogarasi M, Socaci SA, Dulf FV, Diaconeasa ZM, Fărcaș AC, Tofană M, Semeniuc CA (2018) Bioactive compounds and volatile profiles of five transylvanian wild edible mushrooms. *Molecules* 23(12):3272
- Sánchez C (2017) Reactive oxygen species and antioxidant properties from mushrooms. *Synth Syst Biotechnol* 2(1):13–22
- Pan H, Han Y, Huang J, Yu X, Jiao C, Yang X, Dhaliwal P, Xie Y, Yang BB (2015) Purification and identification of a polysaccharide from medicinal mushroom *Amauroderma rude* with immunomodulatory activity and inhibitory effect on tumor growth. *Oncotarget* 6(19):17777–17791
- Chen H, Li S, (2015) Polysaccharides from Medicinal Mushrooms and Their Antitumor Activities. In: Ramawat, K., Mérillon, JM. (eds) *Polysaccharides*. Springer, Cham. [https://doi.org/10.1007/978-3-319-16298-0\\_3](https://doi.org/10.1007/978-3-319-16298-0_3)
- Udchumpisai W, Bangyeekhun E (2019) Purification, structural characterization, and biological activity of polysaccharides from *Lentinus velutinus*. *Mycobiology* 48(1):51–57
- Fares J, Fares MY, Khachfe HH, Salhab HA, Fares Y (2020) Molecular principles of metastasis: a hallmark of cancer revisited. *Signal Transduct Target Ther* 5(1):1–17
- Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F (2021) Global Cancer Statistics 2020: GLOBOCAN Estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J Clin* 71(3):209–249
- Siegel RL, Miller KD, Jemal A (2020) (2020) Cancer statistics. *Cancer J Clin* 70(1):7–30
- Hanna TP, Kangolle ACT (2010) Cancer control in developing countries: using health data and health services research to measure and improve access, quality and efficiency. *BMC Int Health Hum Rights* 10:24–24
- Tohme S, Simmons RL, Tsung A (2017) Surgery for cancer: a trigger for metastases. *Cancer Res* 77(7):1548–1552
- Strobel O, Hank T, Hinz U, Bergmann F, Schneider L, Springfield C, Jäger D, Schirmacher P, Hackert T, Büchler MW (2017) Pancreatic cancer surgery. *Ann Surg* 265(3):565–573
- Luo D, Johnson A, Wang X, Li H, Erokwu BO, Springer S, Lou J, Ramamurthy G, Flask CA, Burda C, Meade TJ, Basilion JP (2020) Targeted radiosensitizers for MR-guided radiation therapy of prostate cancer. *Nano Lett* 20(10):7159–7167
- Torres Royo L, Antelo Redondo G, Áquez Pianetta M, Arenas Prat M (2020) Low-dose radiation therapy for benign pathologies. *Rep Pract Oncol Radiother* 25(2):250–254
- Student S, Hejmo T, Poterała-Hejmo A, Leśniak A, Bułdak R (2020) Anti-androgen hormonal therapy for cancer and other diseases. *Eur J Pharmacol* 866:172783
- Serzan MT, Farid S, Liu SV (2020) Drugs in development for small cell lung cancer. *J Thorac Dis* 12(10):6298–6307
- Horvath L, Thienpont B, Zhao L, Wolf D, Pircher A (2020) Overcoming immunotherapy resistance in non-small cell lung cancer (NSCLC) - novel approaches and future outlook. *Mol Cancer* 19(1):1–15
- Moharib SA, Abd El Maksoud N, Ragab HM, Shehata M (2014) Anticancer activities of mushroom polysaccharides on chemically-induced colorectal cancer in rats. *J Appl Pharm Sci* 4(7):54–63
- Seedeve P, Ganesan AR, Mohan K, Raguraman V, Sivakumar M, Sivasankar P, Loganathan S, Rajamalar P, Vairamani S, Shanmugam A (2019) Chemical structure and biological properties of a polysaccharide isolated from *Pleurotus sajor-caju*. *RSC Adv* 9(35):20472–20482
- Seedeve P, Moovendhan M, Sudharsan S, Sivasankar P, Sivakumar L, Vairamani S, Shanmugam A (2018) Isolation and chemical characteristics of rhamnase enriched polysaccharide from *Grateloupia lithophila*. *Carbohydr Polym* 195:486–494
- Dubois M, Gilles KA, Hamilton JK (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28(3):350–356
- Rial-Hermida MI, Rey-Rico A, Blanco-Fernandez B, Carballo-Pedrares N, Byrne EM, Mano JF (2021) Recent progress on polysaccharide-based hydrogels for controlled delivery of therapeutic biomolecules. *ACS Biomater Sci Eng* 7(9):4102–4127
- Nicklisch SCT, Waite JH (2014) Optimized DPPH assay in a detergent-based buffer system for measuring antioxidant activity of proteins. *MethodsX* 1:233–238
- Bermúdez-Oria A, Bouchal Y, Fernández-Prior Á, Vioque B, Fernández-Bolaños J (2020) Strawberry puree functionalized with natural hydroxytyrosol: Effects on vitamin C and antioxidant activity. *Molecules* 25(24):5829

27. Lin S, Li HY, Wang ZY, Liu X, Yang Y, Cao ZW, Du G, Zhao L, Zhang Q, Wu W, Qin DT (2019) Analysis of methanolic extracts and crude polysaccharides from the leaves of *Chuanminshen violaceum* and their antioxidant activities. *Antioxidants* 8(8):266
28. Rejeeth C, Vivek R, Kannan S (2015) A novel magnetic drug delivery nanocomplex with a cisplatin-conjugated Fe<sub>3</sub>O<sub>4</sub> core and a PEG-functionalized mesoporous silica shell for enhancing cancer drug delivery efficiency. *RSC Adv* 5(115):94534–94538
29. Rejeeth C, Vivek R, Nipunbabu V, Sharma A, Ding X, Qian K (2017) Cancer nanomedicine: from PDGF targeted drug delivery. *MedChemComm* 8(11):2055–2059
30. Seedeivi P, Moovendhan M, Vairamani S, Shanmugam A (2016) Structural characterization and biomedical properties of sulfated polysaccharide from the gladius of *Septoteuthis lessoniana* (Lesson, 1831). *Int J Biol Macromol* 85:117–125
31. Jiang Y, Zi W, Pei Z, Liu S (2018) Characterization of polysaccharides and their antioxidant properties from *Plumula nelumbinis*. *Saudi Pharm J* 26(5):656–664
32. Olson ND, Morrow JB (2012) DNA extract characterization process for microbial detection methods development and validation. *BMC Res Notes* 5:668–668
33. Tanaka H, Kanahashi K, Takekoshi N, Mada H, Ito H, Shimoi Y, Ohta H, Takenobu T (2020) Thermoelectric properties of a semicrystalline polymer doped beyond the insulator-to-metal transition by electrolyte gating. *Sci Adv* 6(7):1–8
34. López-Legarda X, Rostro-Alanis M, Parra-Saldivar R, Villa-Pulgarín JA, Segura-Sánchez F (2021) Submerged cultivation, characterization and in vitro antitumor activity of polysaccharides from *Schizophyllum radiatum*. *Int J Biol Macromol* 186:919–932
35. Shu X, Zhang Y, Jia J, Ren X, Wang Y (2019) Extraction, purification and properties of water-soluble polysaccharides from mushroom *Lepista nuda*. *Int J Biol Macromol* 128:858–869
36. Dong M, Jiang Y, Wang C, Yang Q, Jiang X, Zhu C (2020) Determination of the extraction, physicochemical characterization, and digestibility of sulfated polysaccharides in Seaweed-*Porphyra haitanensis*. *Mar Drugs* 18(11):539
37. Musto P, La Manna P, Moon JD, Galizia M, Freeman BD (2018) Infrared spectroscopy of polybenzimidazole in the dry and hydrate forms: a combined experimental and computational study. *ACS Omega* 3(9):11592–11607
38. Zhuang J, Li M, Pu Y, Ragauskas AJ, Yoo CG (2020) Observation of potential contaminants in processed biomass using fourier transform infrared spectroscopy. *Appl Sci* 10(12):4345
39. Fernando IP, Sanjeeva KK, Samarakoon W, Lee WW, Kim HS, Kim EA, Gunasekara UK, Abeytunga DT, Nanayakkara CM, De Silva ED, Lee HS (2017) FTIR characterization and antioxidant activity of water soluble crude polysaccharides of Sri Lankan marine algae. *Algae* 32(1):75–86
40. Korva H, Kärkkäinen J, Lappalainen K, Lajunen M (2016) Spectroscopic study of natural and synthetic polysaccharide sulfate structures. *Starch-Stärke* 68(9-10):854–863
41. Brown GD, Bauer J, Osborn HMI, Kuemmerle R (2018) A solution NMR approach to determine the chemical structures of carbohydrates using the hydroxyl groups as starting points. *ACS Omega* 3(12):17957–17975
42. Liu F, Zhu ZY, Sun X, Gao H, Zhang YM (2017) The preparation of three selenium-containing *Cordyceps militaris* polysaccharides: characterization and anti-tumor activities. *Int J Biol Macromol* 99:196–204
43. Zhu ZY, Dong F, Liu X, Lv Q, Liu F, Chen L, Wang T, Wang Z, Zhang Y (2016) Effects of extraction methods on the yield, chemical structure and anti-tumor activity of polysaccharides from *Cordyceps gunnii* mycelia. *Carbohydr Polym* 140:461–471
44. Seedeivi P (2022) Antioxidant and anticoagulant activity of crude polysaccharide and  $\alpha$ -L-rhamnose from *Grateloupia lithophila*. *Biomass Convers Biorefin* 29:1–9
45. Chaves JO, De Souza MC, Da Silva LC, Lachos-Perez D, Torres-Mayanga PC, Machado AP, Forster-Carneiro T, Vázquez-Espinosa M, González-de-Peredo AV, Barbero GF, Rostagno MA (2020) Extraction of flavonoids from natural sources using modern techniques. *Front Chem* 8:507887
46. Aryal S, Baniya MK, Danekhu K, Kunwar P, Gurung R, Koirala N (2019) Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from Western Nepal. *Plants* 8(4):96
47. Wang Z, Chen P, Tao N, Zhang H, Li R, Zhan X, Wang F, Shen Y (2018) Anticancer activity of polysaccharides produced from glycerol and crude glycerol by an endophytic fungus *Chaetomium globosum* CGMCC 6882 on human lung cancer A549 cells. *Biomolecules* 8(4):171

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