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Research Article

Microbiology

Production of endo-polysaccharides from cultivated *Pleurotus eryngii* fruiting bodies

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KEY WORDS

Polysaccharides, *Pleurotus eryngii*, Di phenyl Pichryl hydrocarbon radical scavenging activity, Fourier Transform-Infrared spectroscopy, Nuclear Magnetic Resonance and Half Inhibition Concentration.

ABSTRACT

Pleurotus eryngii was cultivated on four different sterilized agro wastes as wheat, rice, sugarcane and wood. Wheat substrate gave the maximum yield of *P. eryngii* fruiting bodies (63.4 and 11.3 gm) for 100 gram of substrate for first flush and second flush respectively. Also, cultivation of tested mushroom on wheat substrate produced high quantity of polysaccharides (0.703 mg/ml) which was colormetrically evaluated by Phenol-Sulfuric acid method. The structure of endo-polysaccharides was studied by Fourier Transform-Infrared spectroscopy (FT-IR) was indicated the presence of O-H, C-H, C-O-C, CH2 and C-O bonds that indicated the appearance of C_1-C_5 by ^{1}H & ^{13}C NMR. Polysaccharides analysis with HPLC indicated that it is consisting of glucose, galactose, glucournic acid, ribose, rhamnose and mannose. Polysaccharides were obtained from *Pleurotus eryngii* approved high antioxidant activity by Di phenyl Pichryl hydrocarbon (DPPH) radical scavenging activity which increased by increasing the concentration of polysaccharides. The obtained polysaccharides had IC_{50} at 0.32 mg/ml.

1. Introduction

Mushrooms were the macro-fungi with fleshy spore-bearing fruiting bodies (epigeous or hypogeous) and viewed by the naked eyes and to be picked by hand (**Cheung, 2013**). It is very important in the food and biopharmaceutical industry due to their high nutritional values and for a wide range of bioactive compounds (**Taofiq *et al*., 2016**). *Pleurotus* was a genus of gilled mushrooms which involved one of the most widely eaten mushrooms, *Pleurotus ostreatus*. *Pleurotus* species were some of the most commonly cultivated edible mushrooms in the world (**Oyetayo and Ariyo, 2013**). *Pleurotus* species had been defined as mushroom with dual functions to humans; both as food and medicine (**Oloke, 2017**). They contained on good quantity of proteins, vitamins and minerals, with of low caloric value and very low sugar without starch so they were been recommended for obese persons and diabetes patients. **Wang *et al*., (2017)** studied the bioactive compounds such as polysaccharides, lectins, lactones, terpenoids, and alkaloids that were obtained from medicinal mushrooms. Polysaccharides were the main component for the bioactivities of some mushroom specie. **He *et al*., (2017)** reported that mushroom polysaccharides had many biological activities such as antitumor, immunomodulatory, anti-inflammatory, antinociceptive, antiviral, antioxidative, hypoglycemic, and hepatoprotective activities, as well as protection against chronic radiation stress. **Zhang *et al*., (2014)** demonstrated that crude polysaccharides from *Pleurotus eryngii* had the best antioxidant activity.

2. Material and methods

2.1. Cultivation of tested mushroom

The *Pleurotus eryngii* mycelium was grown on potato dextrose agar medium plate that was incubated at 25-27°C for 10 days.

2.2 Effect of different waste substrates (wheat, rice, sugarcane and wood) on yield of *P. eyngii*

2.2.1 Spawn preparation

It was prepared by boiling wheat grains in equal volume of distilled water a period of half hour as modified by **Dehariya and Vyas, (2013)**. After boiling, excess water was removed by spreading the boiled grains on a wire mesh till its moisture content reach to 50% and then PH was adjusted by adding calcium sulphate and calcium carbonate at the rate of 2% (w/w) and 0.5% (w/w), respectively on dry weight basis of grains. The grains were putted in Erlenmeyer flasks (250ml) for sterilization in autoclave (121°C, 1.5 atm for 15-20 min). The sterilized prepared grains flasks were inoculated with 5 cubes (1cm in diameter) of media containing *P. eryngii* mycelium under a septic condition and incubated at 26 ± 2°C in dark for 10-15 days until the mycelium completely enveloped the grains.

2.2.2. Substrate preparation

The collected dried agricultural wastes (rice straw, wheat straw, sugarcane and wood) were cut into small

parts (2-3 cm in length) for mushroom cultivation. The substrates were steeped in water overnight to remove impurities and then were dried in shed to remove excess water to retain 65-70% moisture content. The moisten substrates were sterilized for hour at 121°C (Pasteuralization) and kept in plastic bags. Plastic bags containing substrates were inoculated by spreading spawn (25% of substrate dry weight). Over the spawn, a layer of substrate was overlaid and the bags top was covered with used straw and pressed in tight. Three replications were made for each substrate. Holes were punctured for gas exchange. The spawn over layers substrate bags were moved into incubation rooms for 21 days at 25-27°C (**Govindaraju *et al.*, 2013**). For spawn run, relative humidity was adjusted between 65-90%. After complete mycelia run, Polythene bags were opened and the appeared substrate beds were moistened by sprinkling with distilled water three times a day until harvesting.

2.3. Extraction and purification of polysaccharides from *P. eryngii* fruiting bodies

The harvested fruiting bodies of *P. eryngii* were dried at 70ºC in oven and were ground into fine particles and then, were boiled with 20 volumes of distilled water for 3 hours and centrifuged at 8,000 rpm for 30 min and the supernatant was deproteinized by adding one volume of Sevag's reagent (chloroform : n-butyl alcohol (5:1)). Three volumes 95% cold ethanol was added to the deproteinized solution and left it at 4ºC overnight for extraction crude polysaccharide. The mixture was again centrifuged, and the obtained precipitate was polysaccharides (**Udchumpisai and Bangyeekhun, 2019**).

2.4. Polysaccharides characterization by colorimetric (Phenol-Sulfuric acid method)

The extracted polysaccharide was measured by Phenol-Sulfuric acid method (**Dubois *et al.*, 1956**). 1 ml dis water containing *P. eryngii* polysaccharides (1mg) were pipetted into a test tube and 1 ml of 5% phenol solutions were added. Then 5ml of concentrated sulfuric acids were added carefully on the wall of containing tubes and shaken well. The tube was incubated into water bath at 30ºC for 10-20 minutes and then left to be cool and the change of color was measured at 490 nm spectrophotometerically where the characteristic yellow-orange color was appeared for hexose monosaccharide and 480 nm for pentose monosaccharide and uronic acid. Blank was prepared by adding all content without polysaccharides.

2.5. Characterization of polysaccharides from *P. eryngii* fruiting bodies

2.5.1 High Performance Liquid Chromatography (HPLC) of polysaccharides obtained from *P. eryngii* fruiting bodies

10 mg of sample was hydrolyzed according to modified method of **Seedevi *et al.*, 2019** with 2 ml of 3M TFA at 95ºC for 8 hours in a10 ml sealed sample filled with nitrogen gas, and then cooled down and centrifuged in 1500 rpm for 5 min. the supernatant was transferred to a 5-

ml micro-round flask and was dried under reduced pressure before dissolving with 1 ml ultrapure water. Finally, the aqueous layer was passed through a 0.45 mm membrane for HPLC analysis (Shimadzu Class-VPV5.03 (Kyoto, JAPAN) equipped with refractive index RID-10A.

2.5.2 Fourier Transform-Infrared Spectroscopy (FT-IR) of polysaccharides obtained from *P. eryngii* fruiting bodies

Fourier Transform-Infrared (FT-IR) spectroscopy (FT/IR-4100, Japan) was employed to analyze the obtained polysaccharides to detect of functional groups using the potassium bromide (KBr). The sample pellets were prepared according to the method of **Xiong *et al.*, (2020**). It was carried out at IR unit at Chemistry division, Faculty of Science, Tanta University.

2.5.3 Nuclear magnetic resonance (NMR) analysis

The 50 mg of polysaccharides sample were dissolved in 0.5ml of 99% deuterium (D_2o) and 30µl acetone-d6 was added (**Xiong *et al.*, 2020)**. The ^{1}H and ^{13}C NMR spectra of polysaccharides were performed at 30°C with a MH JOEOL-Japazn at 500 MHz.

2.6. Antioxidant activity of polysaccharides obtained from *P. eryngii* fruiting bodies by Di phenyl Pichryl hydrocarbon (DPPH) radical scavenging activity

DPPH radical scavenging activity of *P. eryngii* polysaccharides was assayed according to a modified method of **Uddin Pk *et al.*, (2019)**. Tubes containing 50 µl of each polysaccharides concentrations (0.625, 0.3125, 0.208, 0.156, 0.125 mg/ml) was mixed with 5 ml (0.004% w/v in ethanol) solution of DPPH and then was vortexed and covered with Aluminum foil and incubated for 30 min in dark at room temperature. The blank control was 80% ethanol. After end of incubation, changing of color was noticed which was measured spectrophotometery at 515 nm. Antioxidant activity was calculated by the following equation:

DPPH Scavenging % = $(A_O - A_S / A_O) \times 100$

Where A_o: is the absorbance of the blank

A_s: is the absorbance of sample at 515 nm

3. Results and Discussion

3.1. Effect of different wastes substrates (wheat, rice, sugarcane and wood) on yield of *P. eyngii*

By comparing weight of fruiting bodies of *P. eyrngii* on different four tested agriculture wastes, revealed that wheat substrate is the best substrate for maximum yield where it recorded 63.4 and 11.3 gm for 100 gm of substrate for first flush and second flush respectively as shown in Fig. (1), followed by rice, sugarcane and wood substrate. This result was constituent with result of **Jamil *et al.*, (2019)** who stated that wheat straw and newspaper mixture was the favourable substrate for maximum yield of *P. sajor-caju*, *P. sapidus* and *P. erynjii*. Our results disagree with studying of **Ashraf *et al.*, (2013)** where their results stated maximum total yield of oyster mushroom was on cotton substrate.

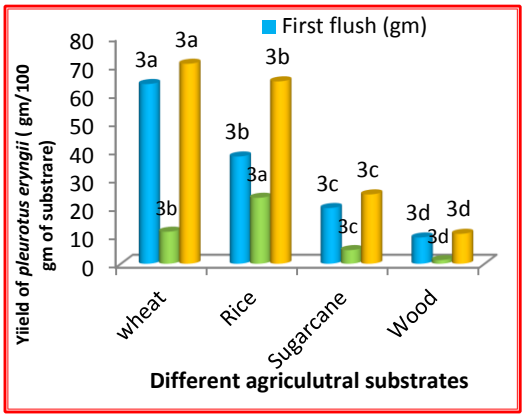


Fig. (1): Effect of different substrates on yield performance of *P. eyngii*.

3.2. Polysaccharides characterization by colorimetric (Phenol-Sulfuric acid method)

It was found that cultivation of *P. eryngii* on wheat substrate produced maximum amount of polysaccharides (about 0.703mg/ml) which was followed by rice giving 0.686 mg/ml of polysaccharides, followed by sugarcane and finally by wood Fig. (2).

Chen *et al.*, 2021 demonstrated that addition of proportions of maize straw to all the substrate (wheat bran and maize straw) produced high amount of polysaccharides of *Auricularia cornea.*

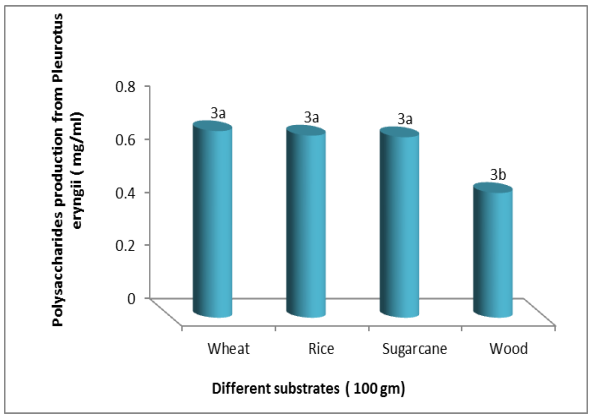


Fig. (2): Effect of different substrates on polysaccharides production from *P. eryngii.*

3.3. Characterization of Polysaccharides from *P. eryngi* fruiting bodies

3.3.1. High Performance Liquid Chromatography (HPLC) of Polysaccharides obtained from *P. eryngii* fruiting bodies

In our study, polysaccharides were extracted from fruiting bodies of *P. eryngii* were analyzed by HPLC spectrum by refractive index for monosaccharides components identification. It was found that the polysaccharides consisting of glucose, galactose, glucournic acid, ribose, rhamnose and mannose with its concentrations as 75.23, 4.96, 1.38, 0.94, 2.35 and 3.87(%) respectively as shown in Fig. (3). Irradiated polysaccharides structure not change by gamma irradiation (**Xiong *et al.*, 2020**).

Seedevi *et al.*, 2019 reported that HPLC analysis of *P. sajor-caju* polysaccharide showed that it is monomer structure is glucose (80.7%) and galactose (16.3%) confirming that the mushroom polysaccharides are rich in glucans.

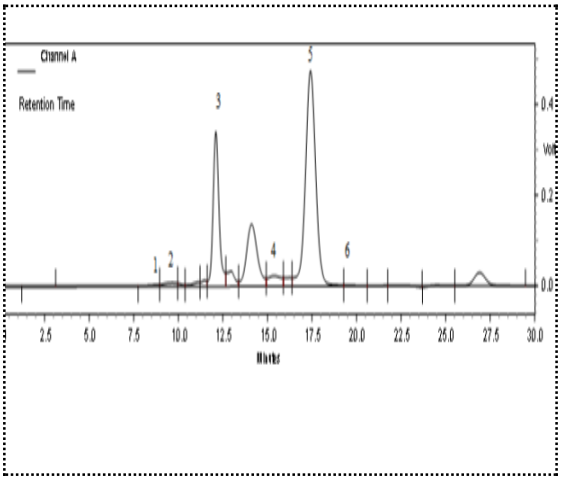


Fig. (3): HPLC analysis of monomer of polysaccharides obtained from *P. eryngii:* peaks No. 1, 2, 3, 4, 5 and 6 refers to mannose, ribose, rhammnose, glucouronic, glucose and galactose sugars.

3.3.2. Fourier Transform-Infrared Spectroscopy (FT-IR) of Polysaccharides obtained from *P. eryngii* fruiting bodies

The FT-IR spectrum of *P. eryngii* fruiting bodies polysaccharides displayed the typical signal pattern expected for carbohydrate moiety, with serval bands in the anomeric region (Fig. 4). O-H stretching peak was seen at 3340.39cm^{-1}, while band at 2928.38cm^{-1} was referred to C-H stretching. The water associated band was shown around 1637 cm^{-1} while S-H stretching was at 2364 cm^{-1} and CH_3 stretching Band was at 1388 cm^{-1}. The absorption at 1000-1200 was assigned to be stretching vibration C-O-C. Our results indicated presence of polysaccharides with sugar ring structures. **Udchumpisai** and **Bangyeekhun, (2019**) studied structure of polysaccharides from *Lentinus velutinus* by FT-IR spectroscopy and found that O-H, C-H, CH_2 , C-O and C-O-C stretching was seen at 3200–3400 cm^{-1}, 2800–3000 cm^{-1}, 1460 cm$^{-1,}$ 1200 cm^{-1} and between 1000-1200 cm^{-1} respectively.

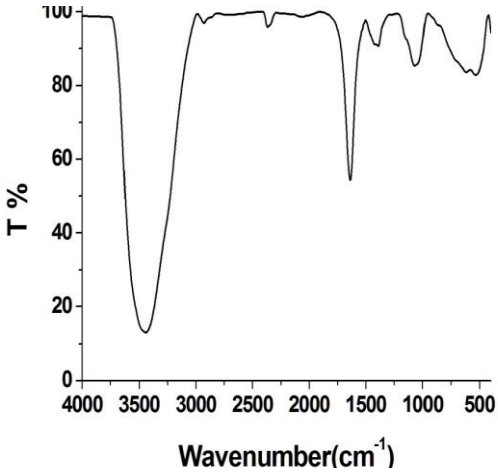
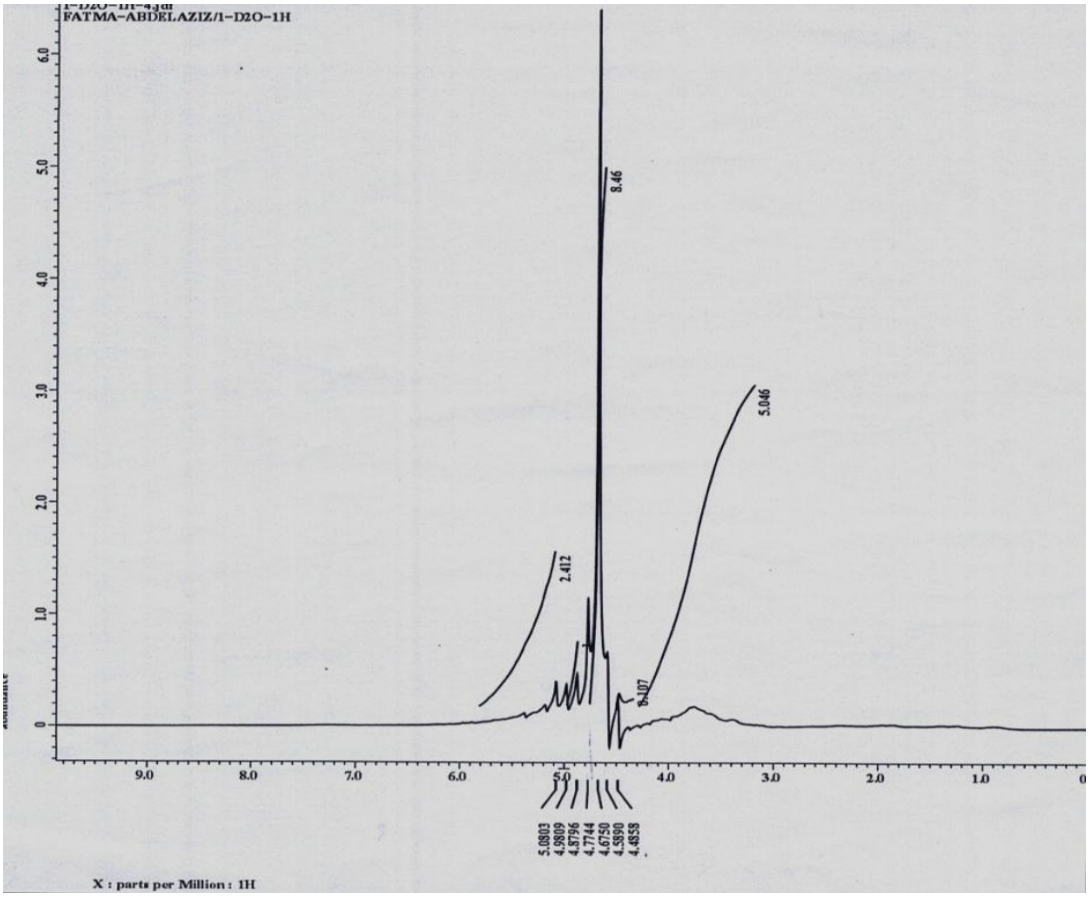


Fig. (4): FT-IR spectrum of Polysaccharides obtained from *P. eryngii* fruiting bodies

3.3.3. Nuclear magnetic resonance (^{1}H &^{13}CNMR) analysis of polysaccharides *P. eryngii*

The ^{1}HNMR spectrum of polysaccharides obtained from *P. eryngii* fruiting bodies was shown in Fig. (5). All the signals were in the range δ3.5-5 ppm which indicated that polysaccharide had α configuration. The main signals were at δ 4.4-4.8 ppm indicating the main chain had α configuration. The ^{13}C NMR spectrum was shown in Fig. (5), the peaks at 93.26, 71.08, 73.10, 69.29 and 69.73 referred to presence of C_1, C_2, C_3, C_4 and C_5 respectively. **Xiong *et al*., (2020**) reported that the *Morchella sextelata* fruiting bodies polysaccharides had signal at 5.28– 4.41 ppm which indicated to the terminal hydrogen signal of its polysaccharides in the ^{13}CNMR spectrum.

Nakahara *et al*., (2020) characterized *P. eyrngii* polysaccharides by ^{13}C-Nuclear magnetic resonance and found typical signals of polysaccharides was appeared in a region ranging from 60 to 106 ppm and other carbon signals at 60–80 ppm were assigned to C_2–C_6 of the sugar residues.



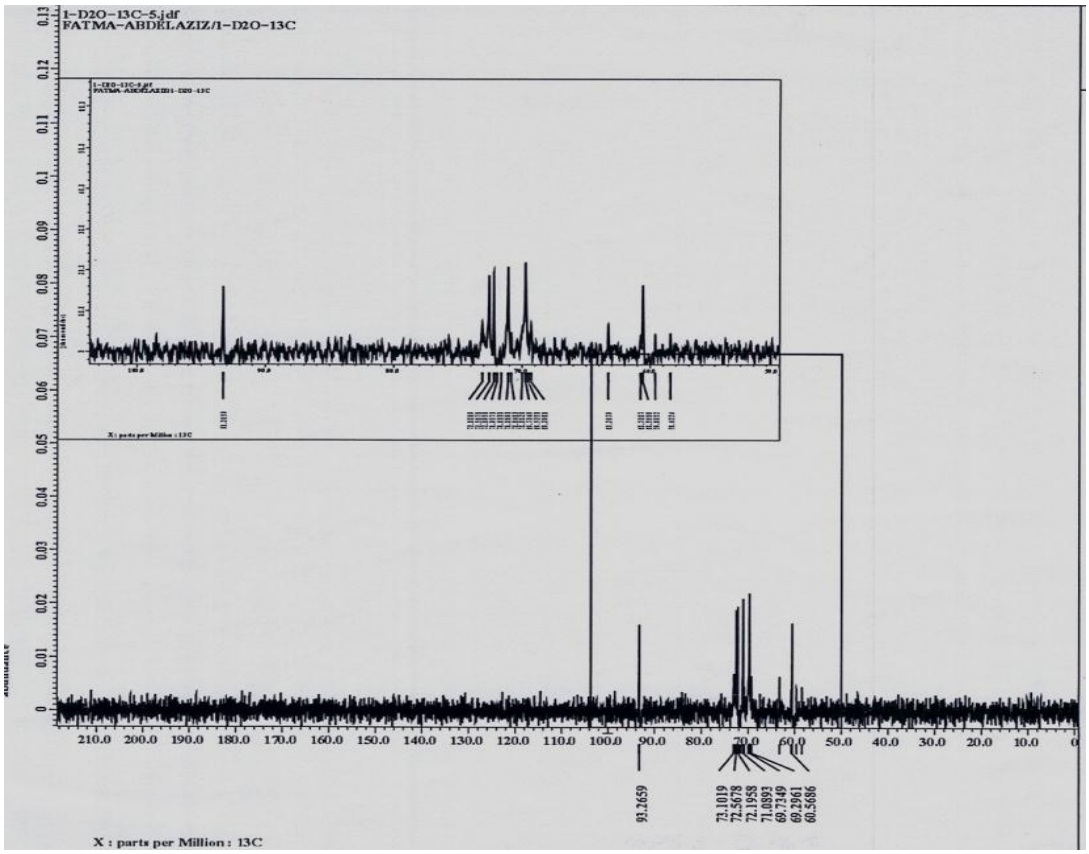


Fig. (5): Nuclear magnetic resonance (^{1}H &$^{13}CNMR$) analysis of polysaccharides *P. eryngii* fruiting bodies

3.4. Di phenyl pichryl hydrocarbon (DPPH) radical scavenging activity

DPPH radical scavenging ability is responsible for hydrogen donating efficiency of antioxidants. As shown in Fig. (6), the DPPH radical scavenging activity of extracted polysaccharides increased gradually with increasing of its concentrations. Polysaccharides had IC_{50} at 0.32 mg/ml. **Zhang *et al.*, 2020** demonstrated that *P. eryngii* polysaccharide had a good antioxidant activity. **Zhang *et al.*, 2014** stated that the concentration of three crude polysaccharides types from *P. eryngii* fruiting body is positively correlated with DPPH free radical scavenging rate.

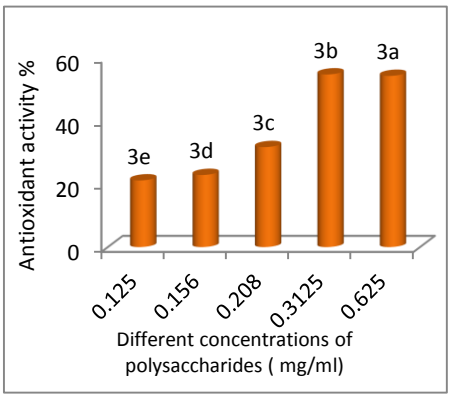


Fig. (6): Antioxidant activity of polysaccharides from *P. eryngii* fruiting bodies by DPPH

4. Conclusion

Maximum yield of *Pleurotus eryngii* fruiting bodies was achieved by grown on wheat straw, rice straw, sugarcane bagases and wood respectively. The obtained polysaccharides was characterized by FTIR spectrum analysis and it was found it's containing OH, CH, CH_3, CH_2, SH, C=O, C-C and C-S functional groups. The ^{1}H and ^{13}C NMR spectrum showed that polysaccharides were with α and β configuration and the signals of C-1, C-2, C-3, C-4 and C-5 were appeared. The obtained polysaccharides had antioxidant activity.

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إنتاج عديدات التسكر الداخلية من الاجسام الثمرية المزروعة لفطر البلورتس اراينجي

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أوضحت الدراسة أن زراعة فطر البلورتوس اراينجي علي قش القمح كمخلف زراعي أعطي انتاجية عالية من الاجسام الثمرية للفطر، كما اعطي أيضا إنتاجيه عاليه من السكريات العديدات التسكرالداخلية المستخلصة من الاجسام الثمرية للفطر. ايضا تحليل ^{1}H & ^{13}C NMR أظهر وجود الرابطة الفا الجلوكوزيدية. أوضح تحليل الاشعة فوق الحمراء للسكريات عديات التسكر وجود المجموعات الفعالة وهى الكربوكسيل و الكيتون و الهيدروكسيل والالكيل ومجموعات اخري. أثبتت السكريات عديدات التسكر المستخلصة كفاءتها كمضادات للاكسدة من خلال مركب ثنائى فينل هيدردو كربون. وبدراسة تركيب السكريات عديدات التسكر باستخدام جهاز HPLC وجد أنها تتكون من الجلوكوز والجالكتوز والاربينوز والمانوز والراميوز وحمض الجلوكويورنيك اسيد.

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