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■■■ ABSTRACT

Introduction: Cancer is the second worldwide cause of death. Polysaccharides may act as new safe alternative remedies for cancer treatment. **Aim:** The present work aimed to survey the ability of different fungi from local habitat to produce polysaccharides; and to evaluate their antitumor activity against different types of tumor cells. **Materials and methods:** Fifteen different locations in the Gharbiya governorate were surveyed for higher and filamentous fungi. Collected isolates were tested for polysaccharide production, then polysaccharide producers were identified. The produced polysaccharides were characterized by high-performance liquid chromatography (HPLC) and for antitumor activity. **Results:** The highest polysaccharide yield was recorded for *Beauveria bassiana* ethanolic extract (8.68% w/w), followed by *Aureobasidium pullulans* ethanolic extract (7.42% w/w), then *Trametes odorata* cold water extract (3.17% w/w), *Inonotus hispidus* hot water extract (2.84% w/w), while the least value was recorded for *Bjerkandera adusta* ethanolic extract (0.79% w/w). Arabinose was the most common sugar for *Aureobasidium pullulans* (24.01%) and *Trametes odorata* (17.86%). *Trametes odorata* extract showed normal appearance and a high count of healthy cells, and high mortality of HepG-2. While both HepG-2 and MCF-7 were distorted and their count was observably reduced under the effect of *Aureobasidium pullulans* extract, with no effect on liver or breast normal cells. **Conclusion:** Polysaccharides produced by *Aureobasidium pullulans* were promising safe antitumor agents, that should be more investigated for accurate drug delivery and safety tests.

Keywords: Fungi, mushrooms, polysaccharides, antitumor, cancer, *Aureobasidium*, *Trametes*

INTRODUCTION

Among death causes, cancer is considered the second most common around the world and has become a global health concern due to its high incidence in the 21st century; about 1.5 million patients die yearly and the number increases every day by the formation of malignant cells (WHO, 2020). Surgery, radiation therapy and chemotherapy alone or in combination are the most conventional methods for the treatment of cancer (Subramaniam et al., 2019). A variety of chemotherapeutic anti-cancer drugs still cause serious side effects on host organs, including lungs, liver and kidneys; the risk of cancer development progression increases with the emergence of multidrug resistance (Bhadresha et al., 2022).

Polysaccharides, proteins, fats, ergosterol, folate, phenolics, alkaloids, enzymes, and organic acids are common fungal bioactive compounds; especially polysaccharide, sulfated polysaccharide, lentinan, cordycepin, antroquinonol, hispolon, lectin and krestin, which have recorded antitumor activity (Ayeka, 2018). Polysaccharides share other proteins and nucleotides in many biological activities such as molecular recognition, adhesion and cell–cell communication in the immune system (Yang and Zhang, 2009). They are the most abundant macromolecules, obtained from algae, plants, fungi and bacteria (Darge et al., 2019).

β-glucan is an active polymer of glucose units linked by β-(1→3)-glycosidic bonds, with side-chains of glucose units bound by β-(1→6) linkages (Chen and Seviour, 2007). β-Glucan

activates phagocytes and leukocyte formation as an external non-self-stimulant for the immune system (Ayeka, 2018). Mushroom polysaccharides have potent antitumor and immunomodulatory properties under clinical trials (Subramaniam et al., 2019).

Antitumor activity is reported for basidiomycetic polysaccharides that can prevent breast cancer after menopause; the first isolated anti-tumour compound was obtained from *Boletus edulis* mushroom in 1957. Many mushrooms are used as an immunosuppressive agent to treat AIDS and cancer in traditional medicine (Meng et al., 2016).

Fungi produce a wide range of bioactive monosaccharides and polysaccharides; depending on their location in the fungal growth, they may be intracellular or extracellular, (Zhang et al., 2019). Different extraction methods can help in obtaining higher anti-tumour activity of polysaccharides by changing the native polysaccharides' viscosity and spatial configuration (Li et al., 2020).

Recently, Usage of these natural polysaccharides has grown by 17% (during 2017–2021), presenting 10 billion USD; that is expected to reach more than 22 billion USD by (2030). In this sense, there is a need to improve yields or purity of polysaccharide extracts by alternative extraction methods; and to extract new polysaccharides from natural sources like fungi (de Souza et al., 2022).

The present work aimed to survey the ability of different fungi from local habitat to produce polysaccharides; and to evaluate their antitumor activity against different types of tumor cells.

MATERIALS AND METHODS
A. Survey for local higher and filamentous fungal samples: A survey was conducted during 2021/2022 to search for higher fungi and other filamentous fungi within the area of Gharbiya governorate. Special attention was directed to damp farms, canal edges and tree trunks in different 15 localities. The Bracket fungi were carefully picked from their growing point on the ground or on the trees by using a sterilized sharp knife. The collected samples were ensured to be free of insect attack or infection, then wrapped with tissue paper and stored inside a sterilized polyethylene bag. (Drábková and Izmailova, 2014). In the workplace, the surface of collected fruiting bodies was sterilized by 1% sodium hypo chloride solution and washed by dist. Water successively; then cut into small slices, air-dried and ground into powder. Each powdered fruiting body was preserved in a tightly closed glass jar away from humidity to be ready for further work.

From the same localities, soil samples were collected from damp soils with decaying roots of dead trees, diluted with sterile dist. Water, cultured on sterile PDA agar plates. All plates were incubated till the appearance of filamentous fungal growth, then subcultured for separation of individual pure isolates. Pure isolates were preserved on sterile agar PDA slants in the fridge to be ready for further work.

The most potent polysaccharide producers among the collected fungi were identified as follows: Samples of higher fungi were morphologically examined directly on the same day of collection for a detailed description of fresh body characters and identified according to Lincoff (1981) and Phillips (1981). Other filamentous fungi were identified morphologically with the assistance of Assuit University Moubasher Mycological Center, according to Moubasher (1993), Klich (2002) and Samson et al. (2010).

B. Extraction of polysaccharides from the collected fungi: Both stored powdered bracket fungi and preserved filamentous fungi were tested for polysaccharides as follows: Five grams of each powder (for higher fungi) or 30 ml of culture filtrate (for filamentous fungi) were mixed with about 60 ml of each one of different 7 solvents (cold water, hot water (60°C), ethanol, methanol, acetone, diethyl ether, petroleum ether and chloroform) separately; they were soaked overnight at room temperature, then each extract was centrifuged for 10 min. at 4500 rpm. Each supernatant was evaporated, and the precipitated poly-saccharides were washed with dist. Water and dried 3 times, and then each powder was stored at 4°C for further work.

C. Quantitative assay of the extracted polysaccharides from the tested fungi: The total polysaccharide content of the tested fungi was estimated according to (Molish and Montash, 1963) by mixing 0.1 ml of each tested fungal extract with 1 ml of 5% phenol and 5 ml of concentrated sulfuric acid at room temperature for 20 min. The light absorbance was recorded for each separate mixture at 490 nm, and then the amount of the present polysaccharide was calculated by the following formula:

Conc. of polysaccharide (mg/ml) = {[(As-Ab) × 1/0.2089 × total volume of reaction mixture × original solvent volume] / dry weight} × 0.95

Where: As= reading of the absorbance of the sample.

Ab= reading of absorbance of blank.

D. Characterization of different extracted fungal polysaccharides by High-Performance Liquid Chromatography (HPLC): Polysaccharides were characterized and quantified according to Smith and Dube (2005). Each stored polysaccharide powder was redissolved in dist. water separately, then each solution sample was cooled, dried and lyophilized by a rotary evaporator to get a fine powder. Pure 100 mg of each sample was transferred into a 100 ml volumetric flask, dissolved in 60 ml of methanol, left for 10 minutes, then diluted to 100 ml with mobile phase, shaken well, and finally filtered through a 0.22 µ Millex filter (PVDF membrane). The number of polysaccharides was determined by comparing it with a standard mixture in the automated apparatus.

For estimation of the monosaccharide content of the extracted fungal polysaccharides, ten millilitres were hydrolyzed in a closed glass tube (13 ×100 mm) with 2 mol/L tri-fluoro-acetic acid (200 ml), filled with N2 gas at 121°C for 2 h. The hydrolyzed solution was dried at 45°C, and tri-fluoro-acetic acid was completely removed by adding 2-propanol (500 ml). The hydrolysate was pre-fractionated with 1-phenyl-3-methyl-5-pyra-zolone (PMP), as a chemical marker. Forty millilitres of NaOH (0.3 mol/L) and 40 ml of PMP solution (0.5 mol/L in methanol) were added to each tube. Tubes were incubated under agitation at 120°C for 2 h. The mixture was neutralized with 40 ml HCl (0.3 mol/L)., monosaccharide derivatives were extracted by adding 0.5 ml of ethyl tertiary butyl ether with agitation for 5 seconds. The layers were centrifuged (at 5000 rpm, 4°C, 5 min.), discarding the upper organic phase. The residue was dissolved in water (1.0 ml) after repeating the extraction steps 5 times, then filtered through 0.45 mm filter paper (Waters Millipore Bedford, MA, USA). Concentrations were read under standard conditions in mg/ml, comparing with standard sugars; Rhamnose 0.05, Galacturonic acid 0.05, Xylose 0.05, Galactose 0.05, Arabinose 0.05 and Glucose 0.05 (Smith and Dube, 2005).

HPLC Isocratic System Conditions (Agilent 1200, USA): for the chromatographic separation of sugars, they were analyzed on a polysaccharide column (250 × 4.6 mm I.D., 5 µm) equipped with a guard column (12.5 × 4.6 mm I.D.) at 30°C by a thermo-regulator. The mobile phase (acetonitrile and water: 75:25 v/v) flowed at the rate of 1.0 ml/min. Ten µL were injected by auto-sampler with a vacuum degasser. The solution was filtered and digested for 3 minutes in a sonicator (Smith and Dube, 2005).

E. Survey for biological activity of the selected fungal polysaccharides:
i. Total antioxidant capacity (TAC): Stored polysaccharides were redissolved in water; then three millilitres of the mixture (28 mM Na3PO4, 0.6 M H2SO4 and 4 mM Amm. molybdate) was added to 0.3 ml of each tested solution, separately. Each mixture was cooled in the dark for 90 minutes. Light absorbance was detected at 695 nm against blank. TAC was calculated as (mg ascorbic acid equivalents / g crude extract) according to Sun et al. (2011).

ii. Cytotoxicity assay (MTT assay) of the tested fungal polysaccharides against human tumour cell lines: The protocol of cell treatment was approved by the Research Ethics Committee, Faculty of Science, Tanta University, with the approval code of IACUC-SCI-TU-0178. Each one of the stored polysaccharide powders was redissolved in dist. water and was tested for its cytotoxicity effect against both liver and breast cancer cells, in comparison with their normal cells. The percentage of cell viability and

mortality were calculated; morphological effects were illustrated microscopically. This protocol was conducted by (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) (MTT) assay according to (van de Loosdrecht et al., 1994) as follows: Each selected human cell was inoculated with a concentration of 1 × 105 cells/ml in a 96 well tissue culture plate (100 μL/well), incubated at 37°C for 24 h. to form a complete regular monolayer sheet, separately. Then the excess medium was discarded and the cell monolayer was washed twice.

Each tested polysaccharide extract was two-fold diluted in a maintenance medium with 2% serum, separately. 0.1 ml of each dilution was applied in 3 different wells. After incubation of the plate at 37°C, cells were checked for any physical signs of toxicity. Twenty μl MTT solution (Bio Basic Inc., Canada) was added to each well, shaken for 5 minutes at 150 rpm, and incubated at 37°C under 5% CO_2 for 4 hours to metabolize MTT. The formed formazan (MTT metabolic product) was re-suspended in 200 μl DMSO, and shaken at 150 rpm for 5 min. Optical density was detected at 560 nm, which had a direct proportion with the cell quantity.

RESULTS

Many fungal samples were collected during the present survey through once-monthly regular visits to different locations in the Gharbiya governorate. The type of sample and its location were recorded in Table 1 for both higher and filamentous fungi. Whereas 12 locations possessed filamentous isolates and only 3 locations had higher fungal bodies.

All the collected fungi were screened for polysaccharide production, estimating the highest polysaccharide concentration (64.6 mg/g dry wt.) for the filamentous fungus isolated from location no.8 (Morus leaves, Bashbeesh village, El-Mahalla El-Kobra, Gharbiya); followed by another filamentous fungus (33.1 mg/g dry wt.) isolated from location no.12 (Grape leaves, Basiyon town, Basiyon, Gharbiya); then the higher fungus (30.6 mg/g dry wt.) from location no.3 (Casuarina tree trunk, El-Delebshat village, Kafr El-Zayat, Gharbiya); followed by another higher fungus (24.8 mg/g dry wt.) from location no.10 (Morus

tree trunk, Kom El-Naggar village, Basiyon, Gharbiya) and the bracket fungus (1.92 mg/g dry wt.) from location no.13 (Salix tree trunk, Mahallet El-Laban village, Basiyon, Gharbiya); as represented in Table 2.

The most efficient polysaccharide producers among the collected fungi were identified morphologically as bracket fungi, namely: *Bjerkandera adusta* Karsten, 1880; *Inonotus hispidus* Karsten, 1880; *Trametes odorata* Fries, 1836; filamentous fungi, namely: *Aureobasidium pullulans* Arnaud, 1918 and *Beauveria bassiana* Vuillemin, 1912 (Table 3).

Different solvents were used to obtain the highest available amount of total polysaccharide from each selected fungus, so *Trametes odorata* cold water extract produced the highest polysaccharide concentration (31.7 mg/g dry wt.); followed by (28.41 mg/g dry wt.) for *Inonotus hispidus* hot water extract; then (7.92 mg/g dry wt.) for the ethanolic extract of *Bjerkandera adusta*. Whereas higher amounts were recorded for filamentous fungi, *Beauveria bassiana* and *Aureobasidium pullulans* (86.82 and 74.23 mg/g dry wt.) as illustrated in Figure 1. HPLC analysis of the bracket fungus *Trametes odorata* hot water extract showed that arabinose is the most abundant monosaccharide part (17.86 %) followed by N-Acetyl muramic acid (16.3 %) then galacturonic acid (14.87 %); while xylose had the lowest persistence (10.97 %) among the components of the analyzed polysaccharide (Figure 2).

These monosaccharide ratios differed for the filamentous fungus *Aureobasidium pullulans* ethanolic extract; as the most common part was arabinose (24.01 %), followed by galactose (18.54 %) then N-Acetyl muramic acid (16.92 %); and the lowest part was mannose (11.23 %), as illustrated in Figure 3. Many other components of the tested crude fungal extracts might share in their biological activity. Total antioxidant capacity was measured for the five selected fungi and represented in Table 4, referring to the highest TAC value for *Aureobasidium pullulans* (11.82 mg/g dry wt.), followed by *Trametes odorata* (10.37 mg/g dry wt.). while the lowest TAC value was recorded for extract (5.19 mg/g dry wt.).

Table 1. Survey for local higher and filamentous fungal samples

Location no.	Location name	Macro-fungi	Filamentous fungi
1	Beans soil, Dalgmon, kafr El-Zayat, Gharbiya	-ve	+ve
2	Corn soil, Abo El-Ezz village, kafr El-Zayat, Gharbiya	-ve	+ve
3	Casuarina tree trunk, El-Delebshat village, Kafr El-Zayat, Gharbiya	+ve	-ve
4	Legume root, El-Korashiya, El-Santa, Gharbiya	-ve	+ve
5	Orange tree roots, El-Shenawy village El-Santa, Gharbiya	-ve	+ve
6	Pond water, Mahallet Roh, Tanta, Gharbiya	-ve	+ve
7	Grape leaves, Shobra melles village, Zefta, Gharbiya	-ve	+ve
8	Morus leaves, Bashbeesh village, El-Mahalla El-Kobra, Gharbiya	-ve	+ve
9	Pond water, El-Moatmdiya village, El-Mahalla El-Kobra, Gharbiya	-ve	+ve
10	Morus tree trunk, Kom El-Naggar village, Basiyon, Gharbiya	+ve	-ve
11	Banana soil, Kom El-Naggar village, Basiyon, Gharbiya	-ve	+ve
12	Grape leaves, Basiyon twon, Basiyon, Gharbiya	-ve	+ve
13	Salix tree trunk, Mahallet El-Laban village, Basiyon, Gharbiya	+ve	-ve
14	Non-cultured soil, Damat, Kotour, Gharbiya	-ve	+ve
15	Raddish leaves, Meet Assas village, Samannoud, Gharbiya	-ve	+ve

-ve: no samples were collected from this location. +ve: a sample was collected from this location.

Table 2. Quantitative screening for polysaccharide production by the collected fungi

Location no.	Produced polysaccharides (mg/g dry wt.)	
	Macro-fungi	Filamentous fungi
1	-ve	0.76
2	-ve	0.0
3	30.6	-ve
4	-ve	0.68
5	-ve	0.0
6	-ve	1.54
7	-ve	0.0
8	-ve	64.6
9	-ve	1.32
10	24.8	0.0
11	-ve	0.43
12	-ve	33.1
13	1.92	-ve
14	-ve	0.0
15	-ve	1.02

-ve = no samples tested in this location.
0.0 = the tested sample doesn't produce polysaccharides.

Table 3. Identification of the most polysaccharide-producing fungi

Location no.	Location name	Identified isolates
3	Casuarina tree trunk, El-Delebshat village, kafr El-Zayat, Gharbiya	*Trametes odorata* (Fries, 1836)
8	Morus leaves, Bashbeesh village, El-Mahalla El-Kobra, Gharbiya	*Beauveria bassiana* (Vuillemin, 1912)
10	Morus tree trunk, Kom El-Naggar village, Basiyon, Gharbiya	*Bjerkandera adusta* (Karsten, 1880)
12	Grape leaves, Basiyon twon, Basiyon, Gharbiya	*Aureobasidium pullulans* (Arnaud, 1918)
13	Salix tree trunk, Mahallet El-Laban village, Basiyon, Gharbiya	*Inonotus hispidus* (Karsten, 1880)

The obtained data in Figure 4 showed that the water-redissolved *Trametes odorata* extract had no toxic effect on the growth of the human liver normal cell line (THLE-2) even at the highest concentrations, which at 1000 µg/ml the percentage of viability was 90.47%. In contrast, *Trametes odorata* extract had a low ratio of mortality in normal liver cell line and this ratio increased gradually with increasing the concentration of the extract till reaching 9.53% at 1000 µg/ml; indicating that Half Maximal Inhibitory Concentration (IC50) of this extract on normal liver cells was more than 1000 µg/ml.
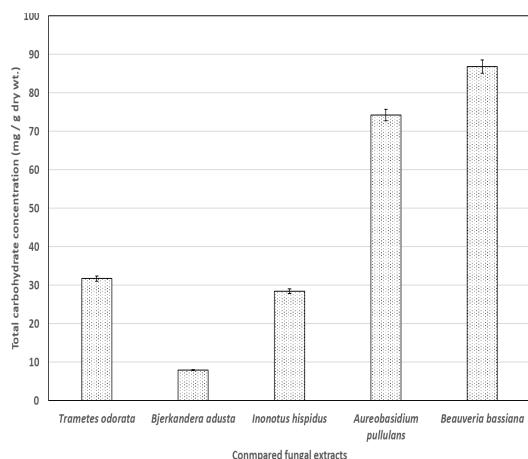
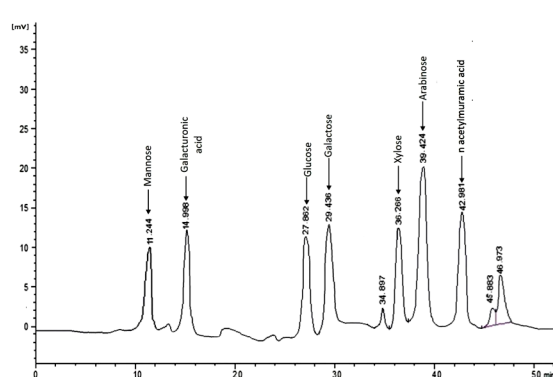**Figure 1.** Comparison among total carbohydrate yield for different extracts of the collected fungi

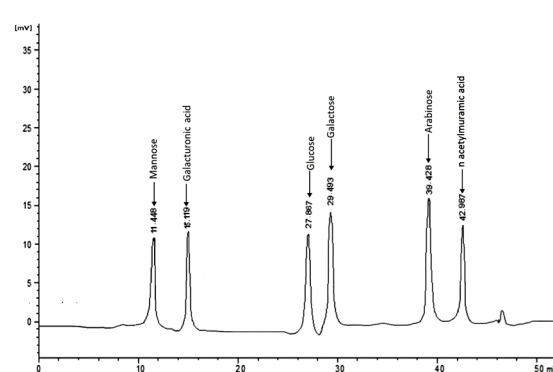**Figure 2.** HPLC analysis profile of *Trametes odorata* hot water extract

Figure 3. HPLC analysis profile of Aureobasidium *pullulans* ethanolic extract

Table 4. Quantitative estimation of total antioxidant capacity for different extracts of the collected fungi

Fungus	Total antioxidant capacity (mg/g dry wt.)
Trametes odorata	10.37
Bjerkandera adusta	8.56
Inonotus hispidus	5.19
Aureobasidium pullulans	11.82
Beauveria bassiana	7.64
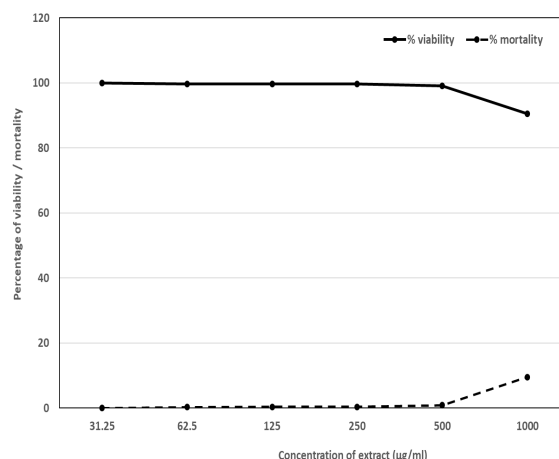
Figure 4. Effect of different concentrations of *Trametes odorata* hot water extract on the growth of human liver normal cell line (THLE-2)

The second type of the extract tested on THLE-2 for its toxicity was *Aureobasidium pullulans* extract as illustrated in Figure 5; first, the percentage of viability was high at low concentrations, then slowly decreased till 35.14% of viability at maximum concentration (1000 µg/ml). The percentage of mortality of the extract was Zero at 31.25 µg/ml of extract concentration, then increased regularly till 64.86 at (1000 µg/ml) with IC50 of 365 µg/ml.

The different polysaccharide extracts were tested for their cytotoxicity effect against both liver and breast cancer cells; *Trametes odorata* extract recorded a decreasing percentage of viability of human liver hepatoma cell line (HepG-2) with an arising concentration of the tested extract till 23.99% at 1000 µg/ml, as illustrated in Figure 6.

In contrast, the percentage of mortality of the liver cancer cells increased with rising concentrations of the *Trametes odorata* extract till reaching 76.01% at 1000 µg/ml; with consideration that IC50 revealed at the concentration of 405 µg/ml.

Secondly, the cytotoxicity effect of *Aureobasidium pullulans* extract was illustrated in Figure 7; the percentage of viability of HepG-2 cells decreased gradually till reached about 15.55% at 1000 µg/ml.
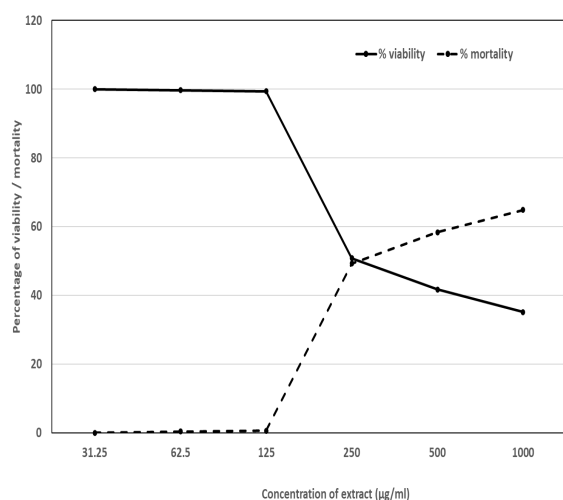
Figure 5. Effect of different concentrations of *Aureobasidium pullulans* ethanolic extract on the growth of human liver normal cell line (THLE-2)
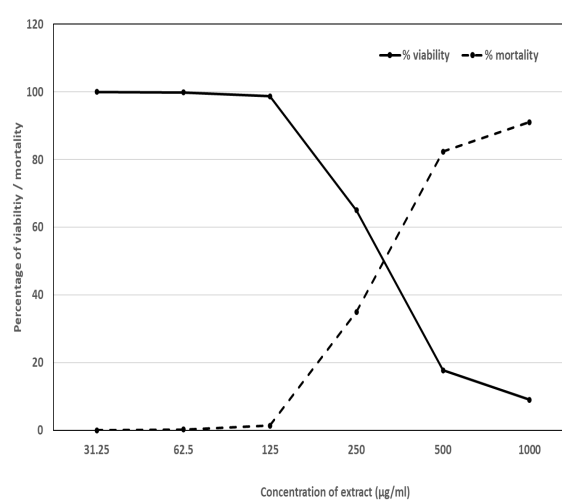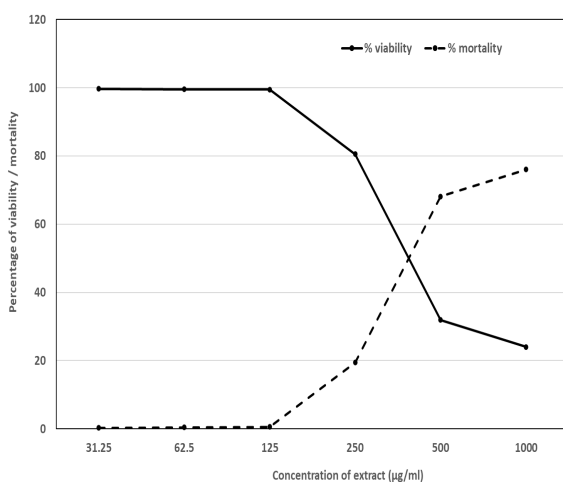

Figure 6. Effect of different concentrations of *Trametes odorata* hot water extract on the growth of human liver hepatoma cell line (HepG-2)
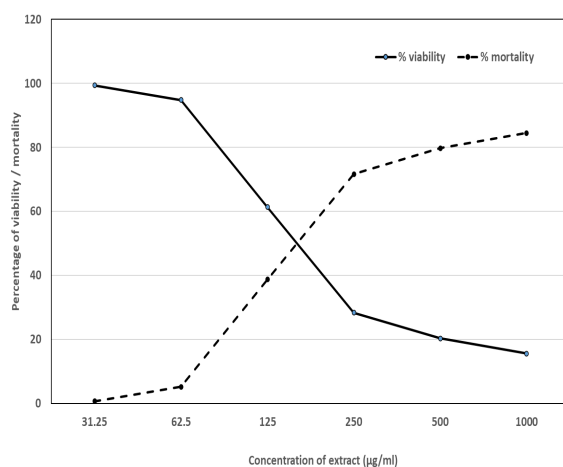

Figure 7. Effect of different concentrations of *Aureobasidium pullulans* ethanolic extract on the growth of human liver hepatoma cell line (HepG-2)

Figure 8. Effect of different concentrations of *Aureobasidium pullulans* ethanolic extract on the growth of human breast cancer cell line (MCF-7)

The percentage of mortality increased regularly with increasing concentration until extended to its highest value (84.45%) at 1000 µg/ml; recording IC50 at 180 µg/ml. Due to the promising results of the filamentous *Aureobasidium pullulans* extract against HepG-2; its antitumor activity was also tested against the human breast cancer cell line (MCF-7) as revealed in Figure 8; showing a decrease in viability of MCF-7 cells with arising the polysaccharide extract concentration gradually till reaching 8.97% at 1000 µg/ml. On the other hand, the percentage of mortality of MCF-7 cells was zero at the lowest concentration (31.25 µg/ml); then increased till reaching a maximum value of 91.03% at 1000 µg/ml; considering that IC50 was 340 µg/ml.

Overall, the lower IC50 value indicated that the tested extract had a strong effect on cancer cell lines; revealing that *Aureobasidium pullulans* extract may be suitable for the pharmaceutical drug as treatment for cancer without affecting normal human cells. As a confirmation of antitumor activity against cancer cell lines; and safe effects on healthy human cells; Photo 1 illustrated the count and morphological changes of target cells under the effect of both *Trametes odorata and Aureobasidium pullulans* extracts. They showed normal appearance and a high count of healthy cells, while HepG-2 and MCF-7 cells were distorted and their count was observably reduced under the effect of both extracts.
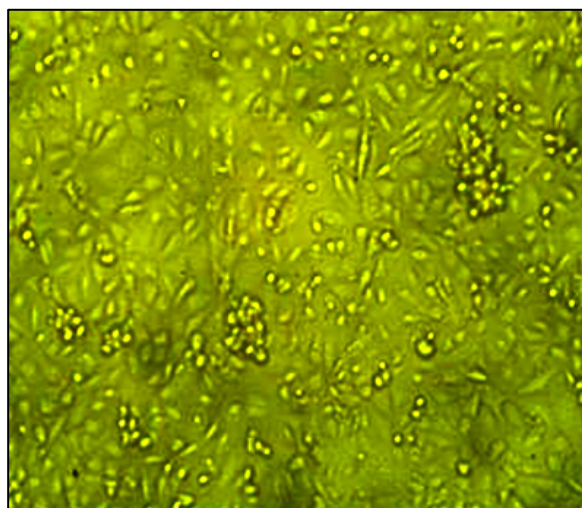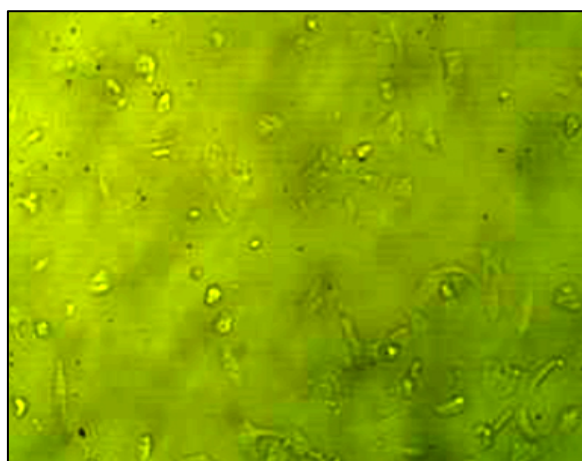
Healthy human liver cells (THLE-2), treated with 500 µg/ml *Trametes odorata* hot water extract
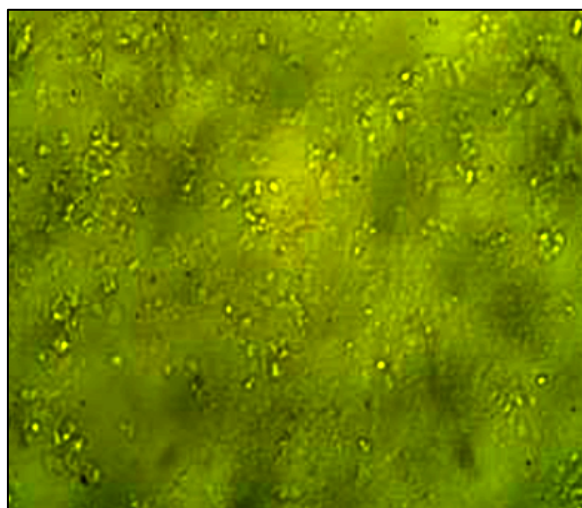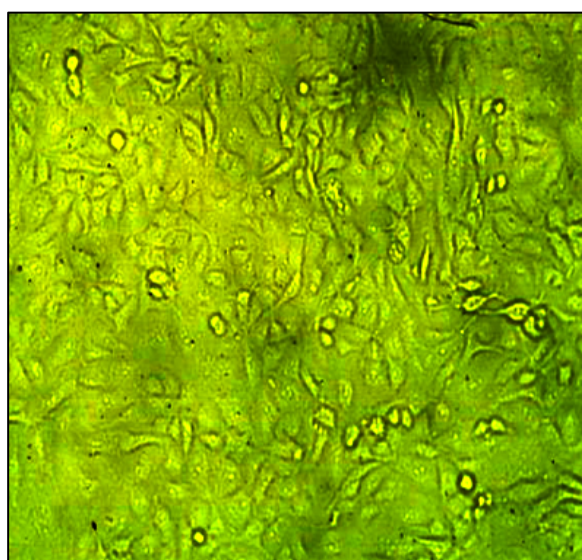
Healthy human liver cells (THLE-2), treated with 500 µg/ml *Aureobasidium pullulans* ethanolic extract
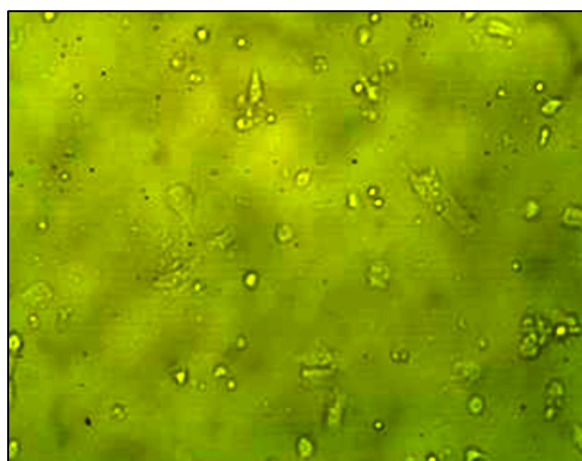
Human hepatoma cells (HepG-2), treated with 500 µg/ml *Trametes odorata* hot water extract

Human hepatoma cells (HepG-2), treated with 500 µg/ml *Aureobasidium pullulans* ethanolic extract

Non-treated human breast cancer cell line (MCF-7)
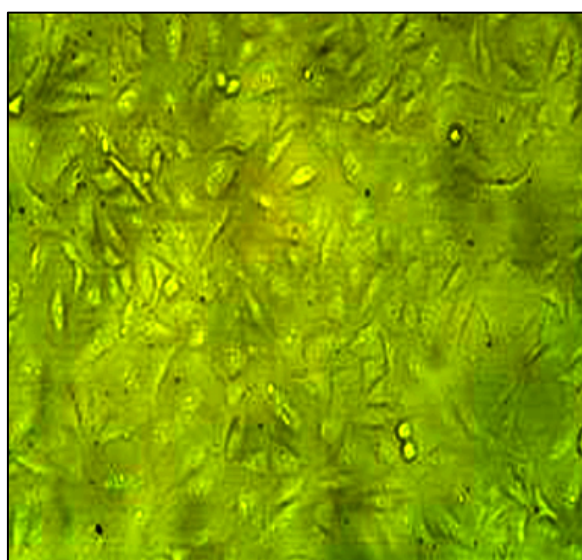
Human breast cancer cell line (MCF-7), treated with 31.25 µg/ml *Aureobasidium pullulans* ethanolic extract

Photo 1. Cytotoxic effects of the most potent fungal extracts on the healthy and tumor human liver and breast cells

DISCUSSION

Polysaccharide drugs are considered as novel antitumor agents, exhibiting a pharmacological role with many mechanisms and multi-targets, mainly immune regulation pathways (Huang et al., 2010). Fungal polysaccharides can stimulate the immune system by connecting some receptors of immune cells, such as TLRs, Dectin, CD4, CR3 and Mannose can cause differentiation of the dendritic cells and macrophages. So, they can activate various immune pathways such as respiratory burst and phagocytosis (Thompson et al., 2010). For example, activation of NK cell generation was a way of the antitumor function of lentinan (Wu et al., 2020).

This study was conducted on fifteen different isolates within the area of Gharbiya governorate, identified morphologically then both stored bracket fungi and preserved filamentous fungi were tested for polysaccharides after extraction methods by solvents were done to produce polysaccharides; estimating the production of exopolysaccharides were arranged as follow; the highest polysaccharide concentration (64.6 mg/g dry wt.) for the filamentous fungus *Beauveria bassiana*; then the higher fungus *Trametes odorata* (30.6 mg/g dry wt.). In a study by Xiang et al. (2012), *Inonotus obliquus* was a good example of the vast array of fungi that recorded high exopolysaccharide production. Another study by Wu et al. (2019) reported that *Trametes versicolor* produced protein and peptide bound β-(1→3;1→6)-glucan.

Several factors could account for the variation in yield; this includes the microbial strain, the composition and impurities in the medium (such as inhibitors or promoters), and the type and conditions of the fermentation system. Seviour et al. (1992) reported maximum levels for *Aureobasidium pullulans* polysaccharides of 6 and 3 g/L, when grown in shirred-tank fermenters synthetic medium and in airlift vessels. Qiao et al. (2009) recorded high levels of *A. pullulans* polysaccharide at 12 to 14 g/L, grown in peat hydrolysate in shake flask culture. Also, Chu et al. (2019) found that a culture of *Kluyveromyces fragilis* and *A. pullulans* produced 15.5 g/L exo-polysaccharides when cultured on Jerusalem artichoke extract. Similarly, Tang et al. (2023) recorded high rates of polysaccharide production (16.3 g/L) by *Lentinula edodes*.

HPLC analysis of *Trametes odorata* hot water extract and *Aureobasidium pullulans* ethanolic extract showed that arabinose is the most abundant monosaccharide part followed by N-Acetyl muramic acid, then galacturonic acid, while xylose had the lowest persistence, among the components of the analyzed polysaccharide. The study of Refaie et al. (2009) revealed that *Pleurotus ostreatus* M2191 and PBS281009 had EPS monomer composition of galactose, glucose, arabinose and mannose. A study by Guo et al. (2020) separated 12 labelled monosaccharides from *Polyporus umbellatus*, namely: mannose, rhamnose, glucosamine, ribose, erythrose, lyxose, galacturonic acid, glucuronic acid, galactose, glucose, xylose, and fructose.

Many other components of the used crude fungal extracts in the present study might share in their biological activity; total antioxidant capacity was measured, referring the highest value for *Aureobasidium pullulans* (11.82 mg/g dry wt.), followed by *Trametes odorata* (10.37 mg/g dry wt.). Muller et al. (2011) had the first record of the antioxidant activity of polysaccharides from the entophytic fungus *Fusarium oxysporum* Dzf17. Sodium hydroxide-extracted mycelial polysaccharide (SPS) was the most active antioxidant component among the three tested polysaccharides. Free radicals are extremely unstable and have high oxidative activity. They attack membranes of mitochondria, react with unsaturated fatty acids and enhance membrane lipid peroxidation. Strong antioxidant natural polysaccharides can remove excess free radicals and help in neglecting the effect of free radicals and raise the immune response against tumors and infections (Kim et al., 2020).

Both *Trametes odorata* hot water extract and *Aureobasidium pullulans* ethanolic extract had no toxic effect on the growth of the human liver normal cell line (THLE-2) in the present survey even at the highest concentrations, which at 1000 µg/ml the percentage of viability was more than 90%.

They had a low ratio of mortality on normal liver cell lines and this ratio increased gradually with increasing the concentration of the extract till reached maximum at 1000 µg/ml. Another study recorded Half Maximal Inhibitory Concentration (IC50), the lowest concentration of the extract reduces 50% of normal liver cells, revealed at more than 1000 µg/ml, as the fungal polysaccharide promotes the phagocytosis of tumour cells by neutrophils (Rubin-Bejerano et al., 2007).

Zhao et al. (2010) demonstrated the activity of *T. robiniophila* polysaccharides against human osteosarcoma, as a mitochondria-mediated apoptotic pathway depended on the polysaccharide-induced apoptosis. However, all the articles referred the antitumor potential to the polysaccharides and other bioactive ingredients of mushrooms like *Trametes*; this potential could be attributed to the depolarization of the mitochondrial membrane, the cell cycle arrest, the immunomodulation and the nitric oxide pathway (Khan et al., 2019).

Different fungal extracts displayed cytotoxic potential in various types of cancer. *T. versicolor* ethanol extract blocked the proliferation of human hepatocellular carcinoma (HepG-2) and human breast adenocarcinoma (MCF-7) *in vitro* (Janjušević et al., 2018). Similarly, *T. hirsuta*, *T. gibbosa*, and *T. versicolor* ethanol extracts possessed cytotoxic activity against human colon carcinoma (LS174), human lung adenocarcinoma (A549) and human cervix adenocarcinoma (HeLa) cell lines *in vitro* (Knežević et al., 2018). Finally, the antitumor effects of fungal polysaccharides can be referred to as a combined action of induction of tumour cell apoptosis, inhibition of tumour cell proliferation, suppression of tumour angiogenesis and indirect immunomodulation (Huang et al., 2023).

CONCLUSION

Aureobasidium pullulans and *Trametes odorata* were isolated from the local habitat and possessed a high ability to produce polysaccharides among the different obtained isolates. Their polysaccharides recorded observable anti-tumor effects against both liver and breast cancer cell lines with IC50 of 180 µg/ml for *Aureobasidium pullulans*; and 405 µg/ml for *Trametes odorata* with negligible cytotoxic effects on normal human cell lines.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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