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Research Article

Production of Childinan SF-2 as Bioactive Compound from *Daldinia childiae*: A Survey on Antioxidant, Antibacterial and Antitumor Properties

Tayebeh Fooladi[1], Mohammad Reza Soudi[1,*], Seyed Mahmoud Hashemi[2]. Peyman Abdeshahian[3]

[1]Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran
[2]Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Science, Tehran, Iran
[3]Department of Biotechnology, Engineering School of Lorena, University of São Paulo, Lorena, São Paulo, Brazil

[*]*Corresponding author*: Mohammad Reza Soudi, Department of Microbiology, Faculty of Biological Sciences, Alzahra University, Tehran, Iran. Tel: +98- 2188058912, Fax: +98- 2188058912, E-mail: msoudi@alzahra.ac.ir

Background: Fungal extracts have received increased attention due to their great medicinal applications including antitumor, immune-modulating, antioxidant, radical scavenging, antiviral, antibacterial, antifungal and detoxificating properties.

Objectives: This study is the first report on a novel bioactive compound, namely Childinan SF-2 which was isolated from soil ascomycete fungus. The significant antibacterial, antioxidant and cytotoxic properties of the extract may lead to development of novel, safe and useful substances.

Materials and Methods: The isolate was identified on the basis of molecular approach. Spore suspension was inoculated in the culture medium and the bioactive compound was isolated from the viscous fermented broth via ethanol precipitation of the extracellular compound. The basic chemical composition of the extract including protein, carbohydrate, sulfate radical and uronic acid content were measured. FTIR (Fourier-transform infrared spectroscopy) and GC–MS (Gas chromatography–mass spectrometry) analysis were used for further structural characterization. The extract was utilized for treatment of AGS and MDA cell lines to assess the cell cycle and apoptosis. The antioxidant activity was examined using DPPH, hydroxyl radicals scavenging, β-carotene bleaching inhibition and ferric reducing power assay methods. The extract was tested for evaluation of antibacterial activity using different Gram-positive and Gram-negative bacterial strains

Results: The fungal isolate was identified as the new strain *Daldinia childiae* SF-2. Initial biochemical characterization of the extract showed that the fungal biopolymer was composed of total sugars, protein, uronic acids and sulfated groups with values of 91.6%, 2.15%, 2.25% and 1.05% (w/w), respectively. FTIR and GC–MS analysis revealed that Childinan SF-2 might be mainly constructed from D-glucose, D-mannitol and D-galactofuranose. The *in vitro* experiments revealed that Childinan SF-2 enhanced the percentage of necrosis and apoptosis of cancer cells and blocked the cell cycle progression as shown by flowcytometry. Childinan SF-2 represented a considerable antioxidant and antibacterial activity.

Conclusions: These results indicated that Childinan SF-2 can serve as a potential source in medicinal applications.

Keywords: Exopolysaccharides, Fungal Biopolymers, Mycelial Secretions, Natural Pharmaceuticals, Xylariaceae

1. Background

Different biopolymers, especially exopolysaccharides have been found in nature. The majority of these biocompounds have potential biological functions (1). The isolation of bioactive biopolymers has received substantial attention due to the fact that most of them are eco-friendly and nontoxic with minimum side effects (2, 3). In recent decades, a large group

of bioactive biopolymers have been isolated from a variety of endophytic fungal species with showing some activities including anti-oxidant, anti-tumor, anti-viral, anti-bacterial and immune modulating properties (4-7). Chitin, Schizophyllan and β-glucan are the known fungal biopolymers with the unique applications such as anti-cancer and anti-tumor properties (8-10). *Daldinia* is an ascomycetal genus belonging to the family *Xylariaceae* (11). The researches on the genus *Daldinia* have attracted a lot of interest since it is known as a potential source of polyketides with a strong biological activity (12). Meanwhile, the fungus *D. childiae* identified by Rogers *et al.* (13) has rarely been investigated as a biopolymer producer. Much less work has been carried out regarding the chemical constituents of bioactive substances extracted from the *D. childiae* with therapeutic effects (13).

2. Objectives

The current study aimed to isolate and identify a fungal strain, namely *Daldinia childiae* SF-2 which could produce an extracellular polymer (Childinan SF-2) with considerable bioactive properties. The cytotoxicity and apoptosis effect against different cancer cell lines along with anti-oxidant and anti-bacterial efficiency was evaluated. Furthermore, the biochemical structure of the fungal biopolymer was evaluated. The current work is the first investigation on extracellular biopolymer extracted from *D. childiae* SF-2 in terms of antioxidant, antibacterial and antitumor effects.

3. Materials and Methods

3.1. Fungal Identification

The fungus was isolated from the soils of forest area in the North of Iran, [Golestan province (E 50°50′59.6″ N 36°44′13.3″)]. The isolation of the samples was carried out using Potato Dextrose Agar (PDA) at 28 °C during 7 days. Samples were preserved at 4 °C and subcultured monthly. The genomic DNA extraction was carried out from the fresh mycelia using cetyltrimethyl ammonium bromide (CTAB) buffer and glass beads (14). Polymerase chain reaction (PCR) using the ITS1 and ITS4 primers was utilized to amplify this region in the 5.8S rDNA of the genomic DNA. The PCR product was sequenced (Bioneer Corporation, South Korea), assembled and deposited in GenBank. The phylogenetic tree was originated using the neighbor-joining algorithm of MEGA software version 7.0.21 (15). Confidence levels of the clades were evaluated from bootstrap analysis according to 1000 replications.

3.2. Fungal Biopolymer Preparation

Fungal extract was produced by fermentation according to our previous reported methods (16). The ethanolic precipitate of the extract was called as Childinan SF-2 and used for further experiments.

3.3. Partial Characterization of the Biopolymer

3.3.1. Biochemical Characterization

Biochemical characteristics of the biopolymer were evaluated in terms of protein content according to the Bradford method, reducing sugars content using DNS (3, 5–dinitro salicylic acid) method and total carbohydrate content according to the phenol-sulfuric methods (16). Furthermore, the amount of uronic acid and sulfate radicals were evaluated according to methods defined by Blumenkrantz and Hansen (17) and Dodgson and Price (18), respectively.

3.3.2. Fourier Transform Infrared (FTIR) Spectroscopy

In order to find out the functional groups within biopolymer structure of the fungal extract obtained, Fourier transform infrared (FTIR) was performed by a Bruker Optics spectrometer (OPUS 3.1) in the frequency range of 4000–400 cm^{-1} (2).

3.3.3. Monosaccharide Analysis

The components of the crude extract were analyzed for monosaccharide composition using a GC-MS by the reported method of Jamshidian *et al*. (19) with some modifications. The extract was used for alditol acetates preparation. Briefly, the crude extract (10 mg in 2 M trifluoroacetic acid) was hydrolyzed at 121 °C for 3 h, reduced by $NaBH_4$ at room temperature and acetylated with acetic anhydride and pyridine. The obtained alditol acetates were analyzed by gas chromatography coupled with mass spectrometer (Agilent Technologies) which was attached with HP-5 capillary column (0.25 mm × 30 m × 0.25 µm). Peak assignments were made based on retention times and mass spectra by the library and confirmed with the standard reference sugars.

3.4. Cells and Cultures

MDA-MB-231 cell line (human breast cancer) and

AGS gastric carcinoma cell line (human stomach cancer) were a gift from Dr. R. Ramezani (Women Research Center, Alzahra University, Iran). Cell lines were cultured in DMEM supplemented with 10% (v/v) FBS at 37 ºC in 5% CO_2. Cells were digested by 1 mL trypsin and treated with different concentration of extracts.

3.5. Flow cytometry Analysis

3.5.1. Cell Apoptosis Assessment Using Annexin-V/ Propidium Iodide

The MDA and AGS cells were cultured in the presence or absence of the fungal biopolymer SF-2 (5 $mg.mL^{-1}$). The cells apoptosis was evaluated using commercially available FITC Annexin V Apoptosis Detection Kit (BD Bioscience; Franklin Lake, NJ) according to the manufacturer's instructions and subsequently analyzed by a FACS/Calibur flow cytometer (Becton Dickin-son, NJ, USA).

3.5.2. Cell Cycle Arrest Analysis

AGS and MDA cells (5×10^5 cells/ well in 6-well plates) were exposed to biopolymer SF-2 (5 $mg.mL^{-1}$) using DNA staining solution (Cell Cycle Staining Kit (MultiScience Biotech Co., Ltd). After treatment, the cells were collected with trypsin and re-suspend in PBS and added to pre-cooled ethanol (75%, v/v) for 4 h. Subsequently, cells were incubated in dark for 30 min before being analyzed by flowcytometry. The cell population was defined by the control (cells were not exposed to biopolymer).

3.6. Determination of Antioxidant Capacity

3.6.1. DPPH Radical Scavenging Assay

The DPPH assay, as previously reported by Shimada *et al*. (20) was applied to assess the radical scavenging ability of the biopolymer from strain SF-2. The absorbance was measured at 517nm and the scavenging ability was calculated according to the equation used by Fooladi *et al*. (16).

3.6.2. Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of the fungal biopolymer was measured according to the method described by Zhang *et al*. (21). The biopolymer was dissolved in the distilled water to form the final concentrations of 2-10 $mg.mL^{-1}$. The hydroxyl radicals were detected by monitoring absorbance at 510 nm. Ascorbic acid was used as the positive control. The hydroxyl radical scavenging activity was calculated according to the mathematical equation used by Fooladi *et al*. (22).

3.6.3. βeta-Carotene Bleaching Assay

In order to assess lipid peroxidation capacity, βeta-carotene bleaching assay was carried out according to the method of Mayouf *et al*. (23) with some modifications. Vitamin C and ethanol were used as positive and negative controls, respectively. The absorbance of the solution was measured at 460 nm and the percentage of the βeta-carotene bleaching inhibition was calculated in accordance with the mathematical equation used by Fooladi *et al*. (22).

3.6.4. Ferric Reducing Antioxidant Power (FRAP) Assay

The antioxidant capacity of the biopolymer from the fungal isolate was evaluated to reduce Iron (III). The FRAP assay was performed according to the method of Sobeh *et al*. (24). Ascorbic acid was used as a positive control and FRAP values were expressed as optical density at 700nm.

3.7. Antibacterial Activity Assay

Antimicrobial activity of the biopolymer was assayed by the minimum inhibitory concentration (MIC) method. Four Gram-negative (*Salmonella typhimurium* ATCC 14023, *Pseudomonas aeruginosa* ATCC 27853, *Proteus mirabilis* ATCC 43071 and *Escherichia coli* ATCC 25922) and five Gram-positive (*Enterococcus faecalis* ATCC 33186, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Streptococcus agalactiae* ATCC 12386 and *Listeria monocytogenes* ATCC 7644) were utilized for antibacterial activity evaluation. Each well of 96-well micro-titer plates contained Mueller–Hinton Broth (100 µL), bacterial suspension (100 µL, approximately 10^6 $CFU.mL^{-1}$) and fungal biopolymer (100 µL, 1% w/v) with concentration from 0.4 $mg.mL^{-1}$ up to 6.6 $mg.mL^{-1}$ (25). After incubation at 37 °C for 24 h, MIC values were measured by visual detection of the turbidity and absorbance measurement at 620 nm. Antimicrobial activity tests were carried out according to the Clinical Laboratory Standard Institute (CSLI) guideline 2012 (26).

3.9. Statistical Analysis

All data were performed in triplicate and presented as means ± SD. The data were subjected to an analysis of variance (ANOVA) and SPSS (Version 17.0, USA). Statistical significance was determined at $P < 0.05$.

4. Results

4.1. Fungal Identification

The fungal strain was identified according to the molecular characteristics by sequence analysis of ITS1 and ITS4 regions in the 5.8S rDNA of the genomic DNA. A BLAST exploration of the database and the ITS sequences length (480 bp) indicated a close genetic relation with other isolates of *Daldinia* spp. (27). According to the pair wise sequence alignments, the strain revealed a high sequence similarity value (99.8%) to *Daldinia* species. Phylogenetic analysis obtained base on the ITS dataset showed that the fungal isolate SF-2 was closely related to *Daldinia childiae* using Neighbor-Joining method (**Fig. 1**).

4.2. Production of Childinan SF-2

The results showed that maximum biopolymer (1.12 ± 0.11 $g.L^{-1}$) and biomass (10.12 ± 1.52 $g.L^{-1}$) productions were obtained during 122 h and 168 h of the fermentation, respectively. The separated and purified biopolymer, namely, Childinan SF-2, was obtained from the fungal isolate that identified as *Daldinia childiae* SF-2.

4.3. Chemical Characteristics

The biochemical composition of the Childinan SF-2 demonstrated a total protein content of 2.15% (w/w) and a high content of total sugars 91.6% (w/w). Moreover, the results obtained from the DNS evaluation showed that 22.5% (w/w) of the total sugars of the fungal extract contained reducing sugars. In addition, the extract showed a high amount of uronic acids (2.25%) and sulfated groups (1.05%).

The IR spectrum of Childinan SF-2 (**Fig. 2A**) revealed the intense peaks at 3424, 2929, 1730, 1647 and 1050 cm^{-1}. The broad IR band at 3424 cm^{-1} was assigned to hydroxyl groups in carbohydrates and the band at 2929 cm^{-1} was considered as C-H groups. The band in region of 1647 cm^{-1} was attributed to the stretching vibration of C=O. The bands at 1200-1000 cm^{-1} were considered the stretch vibration of C-O-C and C-O-H in polysaccharides (2). Moreover, the presence of absorption peak at around 1730 cm^{-1} may revealed the presence of uronic acids (16).

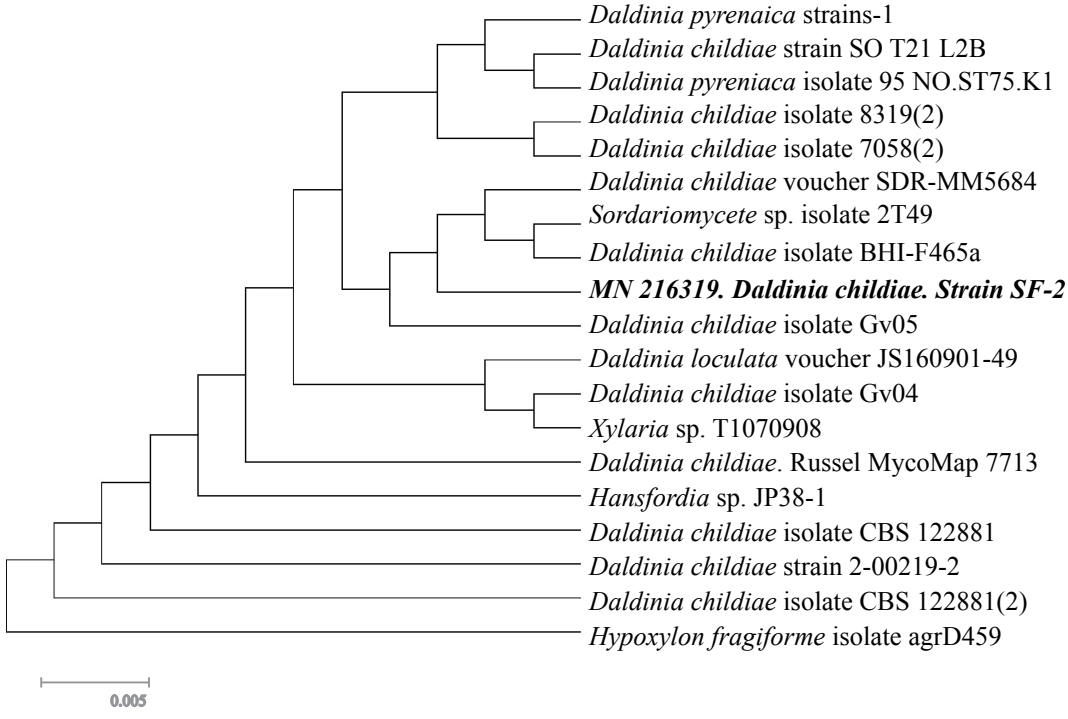

Figure 1. Phylogenetic evolutionary relationship of *Daldinia Childiae* strain SF-2 based on ITS 1-5.8S-ITS2 regions (MEGA Version 7). The isolated strain showed as *MN216319. Daldinia Childiae strain SF-2.*

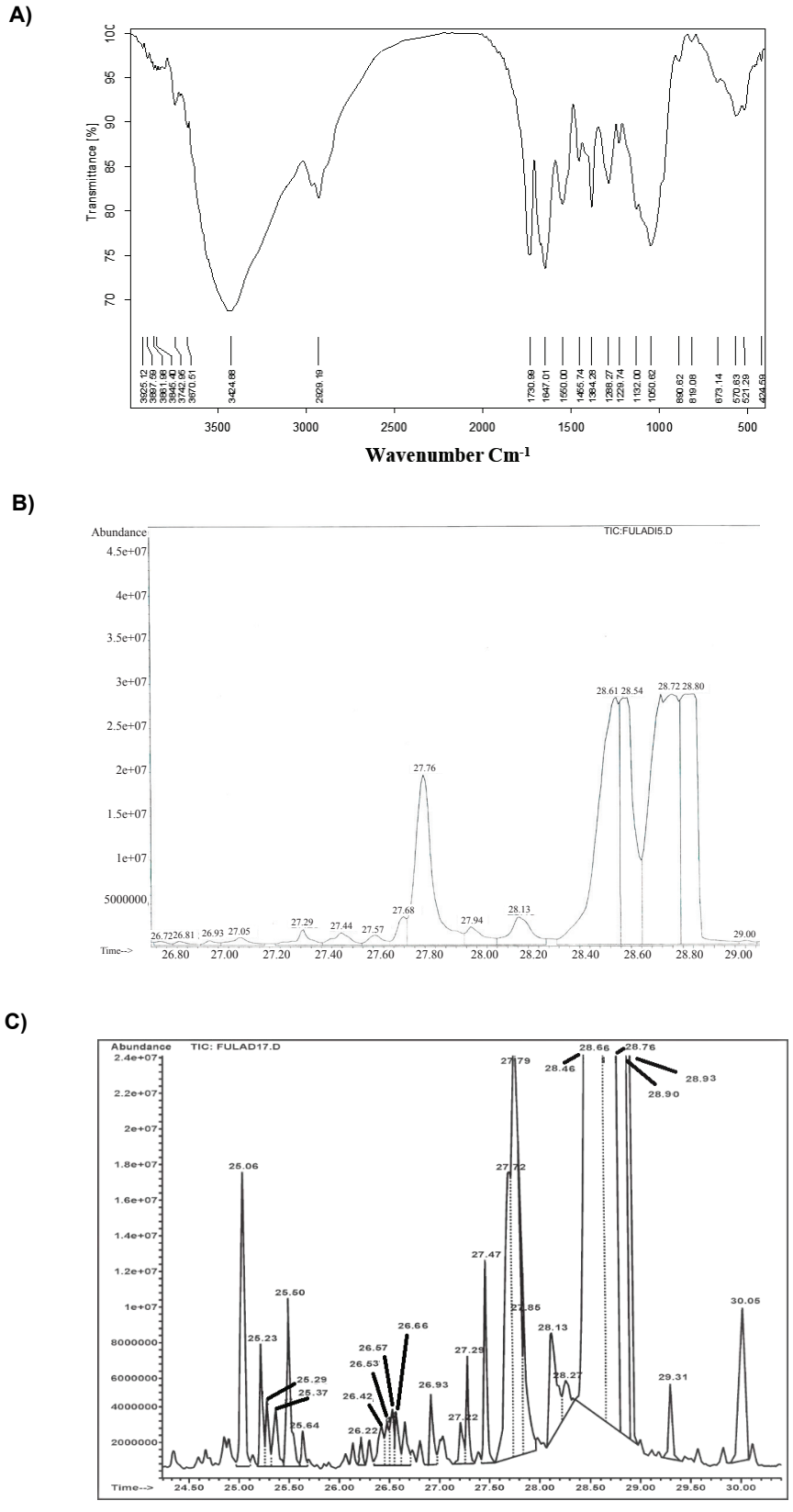

Figure 2. A) The Infrared spectra of the Childinan SF-2 , **B)** GC/MS spectrum of the derivative compound analyzed for Childinan SF-2, **C)** The mass spectra of the peaks were characterized by comparison of the spectra of the standard sugars treated by the same reaction.

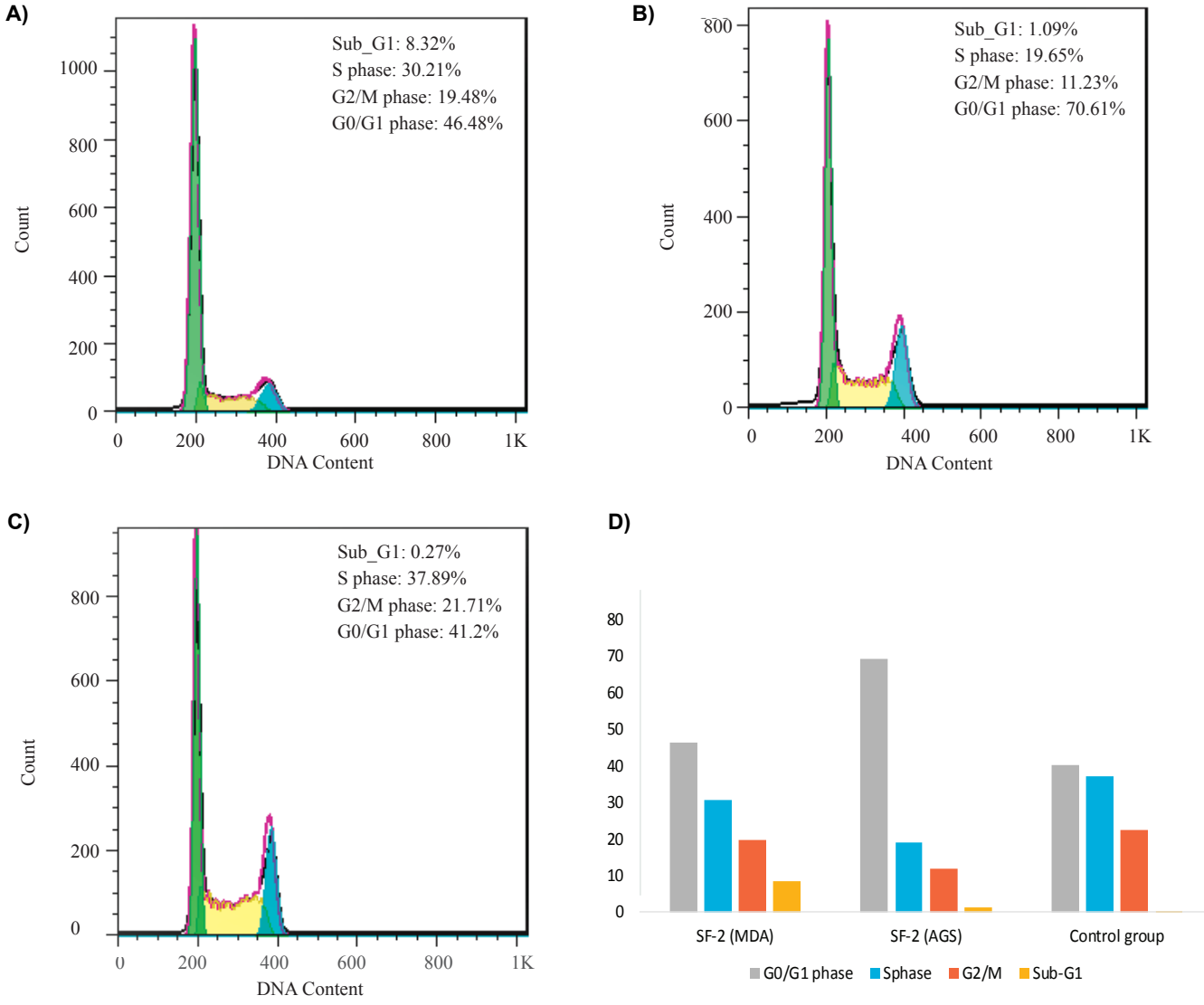

Figure 3. The blocking effect of Childinan SF-2 (5 mg.mL^{-1}) on the cell cycle of **A**) MDA, **B**) AGS, and **C**) control group **C**). **D**) Bar graph characterizes the percentage of different cell cycle phases. The data are represented as mean ± SD of three independent assays (One-way ANOVA), indicating significant differences ($p < 0.05$).

GC-MS analysis revealed that D-glucose, D-mannitol and D-galactofuranose were the monosaccharides figured out in the molecular structure of the Childinan SF-2 with the mass-to charge-ratio of m/z 429, 441 and 456 based on GC–MS library (Wiley 2007) (**Fig. 2B**) and comparison of the peaks obtained from the fungal biopolymer with the standard sugars treated with the same reactions (**Fig. 2C**).

4.4. Effect on Cell Cycle

In cell cycle distribution, the accumulation of the cells in each phase in both treated cells and control sample were depicted in **Figure 3**. In the case of Childinan SF-2 treatment, the accumulation of cells in G2 was 19.48% **(Fig. 3A)** and 11.23% **(Fig. 3B)** for MDA and AGS, respectively, compared to 21.71% of the control groups **(Fig. 3C)**. Moreover, a significant ($P<0.05$) enhancement in the population of cells was observed in the sub-G1 group for both MDA and AGS after treatment with the biopolymer. Simultaneously, in S-phase, the population of cells was reduced to 30.21% for MDA and 19.65% for AGS cells, compared to 37.89% for the

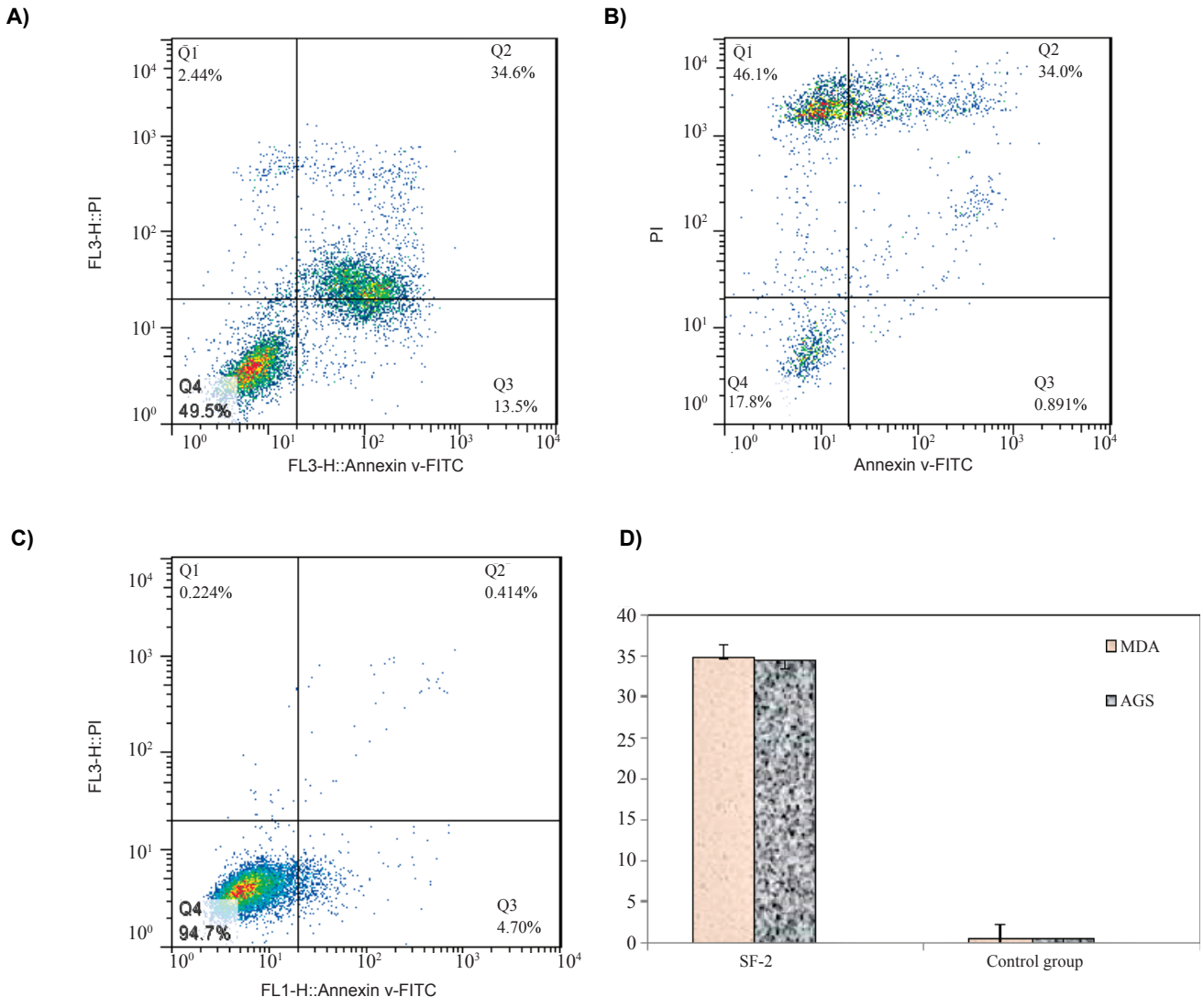

Figure 4. Effect of Childinan SF-2 on the apoptosis of **A**) MDA, **B**)AGS, and **C**) Control group. **D**) Bar graph characterizing the percentage of apoptotic cells. The data are represented as mean ± SD of three independent assays (One-way ANOVA), indicating significant differences ($p < 0.05$) .

control, inferring that cells were arrested at S and G2 phases of the cell cycle. As shown in **Figure 3D**, the data represented as mean ± SD of three independent assay which indicated significantly different.

4.5. Effect on Cell Apoptosis

For apoptosis assay, when cells were treated by the biopolymer, the percentage of the cells was reduced to 49.5% and 17.8% for MDA and AGS cells, respectively. In contrast, in the control group, the most of the cells remained healthy (94.7%) in the Q4 area, which indicated that the treated AGS cells tended to undergo apoptosis ($P<0.05$) more than MDA (**Fig. 4**). The biopolymer has induced the late apoptosis in both cell lines similarly with the value of 34.0% in Q2 area compared to the 0.41% in the control cells. Consequently, the percentage of the MDA cells significantly ($P<0.05$) increased (13.5%) in Q3 area, indicating that Childinan SF-2 can enhance early apoptosis of MDA cell line compared to that obtained by control (4.7%) and AGS cells (0.89%) (**Fig. 4A**). Interestingly, as shown in **Figure 4B,** Childinan SF-2 could induce a considerable rate of necrosis in the AGS cells in Q1 area with the percentage of 46.1% compared to 2.4% in MDA **(Fig.**

4C) and 0.22% in the control group **(Fig. 4D).**

4.6. Antioxidant Capacity

The antioxidant capacity experiments for Childinan SF-2 showed that DPPH radical scavenging activity increased dramatically in a dose-dependent manner, as the maximum scavenging ability of 77.05% was observed at the highest tested concentration (10 $mg.mL^{-1}$) of the biopolymer (**Fig. 5A**). The results showed the remarkable antioxidant potential of the biopolymer compared to that of ascorbic acid (95.25%) used as a positive control. As shown in **Figure 5B**, the potential antioxidant activity of the Childinan SF-2 for hydroxyl radical scavenging was examined at different concentrations (2-10 $mg.mL^{-1}$). At the highest concentration, the inhibition rate of the biopolymer on hydroxyl radical was recorded 56.45% compared to the Vitamin C (98.05%) as positive control. Childinan SF-2 had a considerable protection activity for β-carotene bleaching reduction about 59.5% at the highest tested concentration (5 $mg.mL^{-1}$).

As shown in **Figure 5C,** instantly after starting the experiment, ascorbic acid inhibited the oxidative deterioration of lipids and fatty acids and remained approximately stable, whereas the activity for Childinan SF-2 was observed after 30 min and gradually increased. The effect of Childinan SF-2 was studied corresponding to the ferric reducing power, as depicted in **Figure 5D**. The Childinan SF-2 showed concentration-dependent antioxidant activity, as the ferric reducing power (wavelength at 700 nm) was elevated by an increment concentration from 0.01 to 2 $mg.mL^{-1}$. Although the reducing power of the Childinan SF-2 was gently increased, its activity was significantly less than that of the positive control (ascorbic acid).

4.7. Antimicrobial Activity

The results from antibacterial activity, revealed that the biopolymer had antimicrobial activity against both Gram-negative and Gram-positive strains. In the case of Gram-positive bacteria, a higher antibacterial activity was observed against *Staphylococcus aureus* and *Listeria monocytogenes* with the MIC value of 6.6 $mg.mL^{-1}$ and 3.3 $mg.mL^{-1}$, respectively.

5. Discussion

Daldinia, a genus of fungi, belongs to the family of *Xylariaceae* with a large number of species easily grown in varying environmental conditions and substrates (11). In total, 47 taxa in *Daldinia* were recognized based on morphological and chemotaxonomic evidence. Their biogeography, chorology, ecology, as well as molecular phylogeny based on 5.8S/ITS rDNA and the importance of their secondary metabolites, all provided a basis for more comprehensive identification of these species (28). The fungus was determined as *Daldinia childiae* using morphological and molecular methods. The rDNA gene sequence data of the new fungal isolate were deposited in GenBank under accession numbers of MN 216319 as *Daldinia chiliae* SF-2.

It is widely recognized that the chemical structure and monosaccharide composition are the most important factors defining the bioactivity of polymeric carbohydrate compounds. Herein, Childinan SF-2 was successfully extracted from *D. childiae*, biochemically characterized and its initial compositional analysis revealed the presence of total sugars, protein, uronic acids and sulfated groups. It is supposed that radical scavenging ability of the Childinan SF-2 may be due to the presence of sulfated groups together with uronic acids (29).

From the author's knowledge, there is no evidence for the vast studies on characterization of the extract from *D. childiae*. According to GC-MS analysis, the m/z values of the alditol acetate derivatives from the fungal biopolymer Childinan SF-2 and the electron ionization (EI) mass spectra were compared to that obtained from the standard sugars based on GC–MS library (Wiley 2007). Although the similarity in the m/z values of the components may lead to the similarity of the fragmentation patterns of the isomers of alditol acetate derivatives, the characterization of each single sugar alone with the similarity of the mass spectra is not adequate to fulfill the structural characterization. Identification of the components was confirmed by comparison of the chromatographic characteristics such as retention time of the individual compounds with standard sugars (30). Therefore, presence of D-glucose, D-mannitol and D-galactofuranose was figured out in the molecular structure of the Childinan SF-2 according to the library. Each of these components is very common in fungal extracts (31) but the distribution of the monosaccharaides is different and variable, suggesting that Childinan SF-2 extracted from the fungal isolate was a novel biopolymer that isolated from *D. childiae* and identified for the first time for their monosaccharide

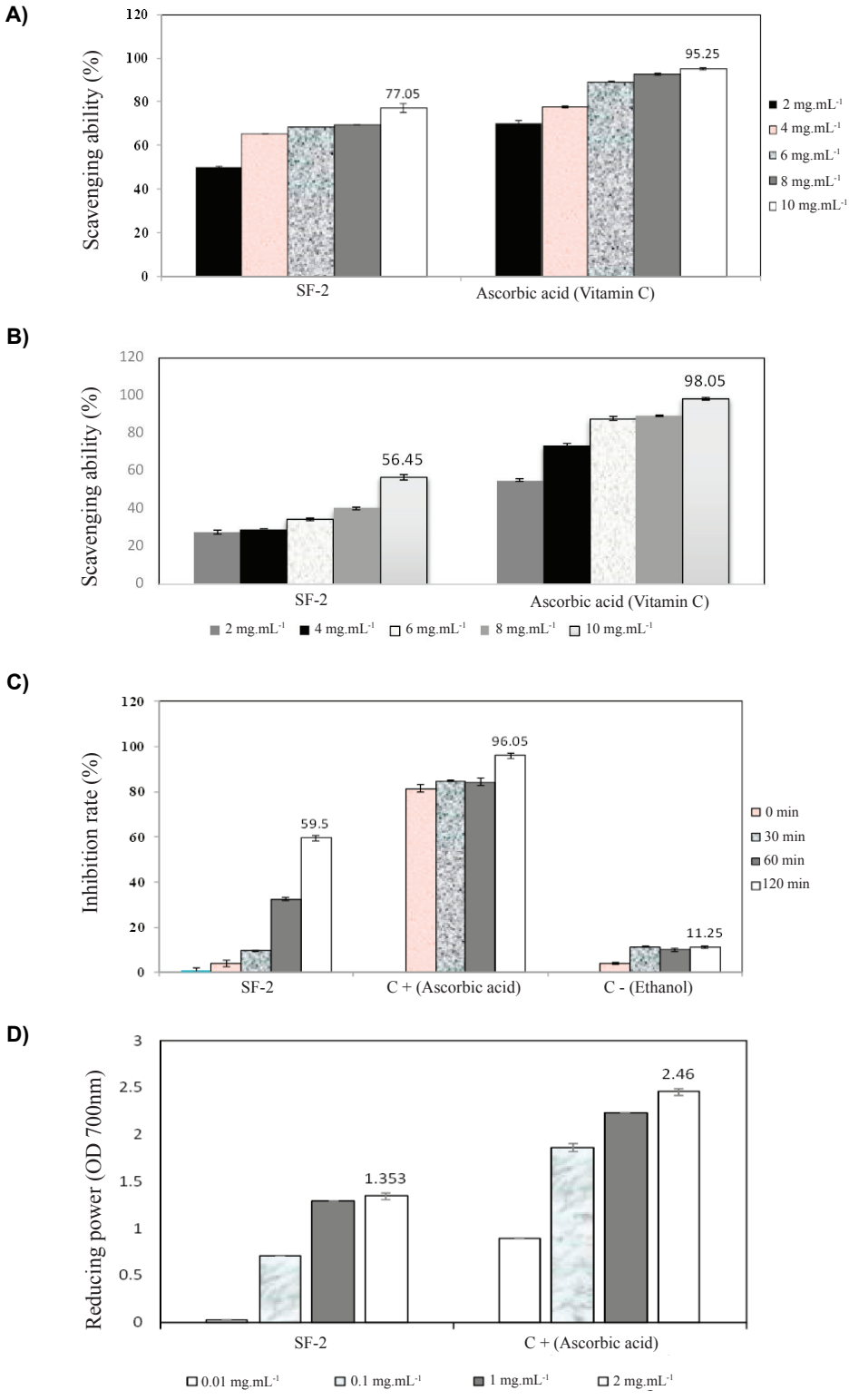

Figure 5. Scavenging capacity of Childinan SF-2 from *Daldinia childiae* using different assays; **A**) DPPH, **B**) Hydroxyl radical scavenging capability, **C**) bleaching assay, and **D**) Ferric reducing power. Ascorbic acid (Vitamin C) was used as a positive control. White column, grey column, white doted, grey doted and light horizontal columns showed extract concentrations of 2 $mg.mL^{-1}$, 4 $mg.mL^{-1}$, 6 $mg.mL^{-1}$, 8 $mg.mL^{-1}$ and 10 $mg.mL^{-1}$, respectively in DPPH and Hydroxyl radical methods. White, grey, white doted and grey doted columns revealed antioxidant protection ability of the samples versus time in β-carotene method and extract concentrations of 0.01 $mg.mL^{-1}$, 0.1 $mg.mL^{-1}$, 1 $mg.mL^{-1}$ and 2 $mg.mL^{-1}$, respectively in ferric reducing assay.

composition. The linkage and comprehensive structural information will be studied through methylation analysis in the future.

It is noteworthy that the increased proliferation and decreased cell death (apoptosis) are two major processes that contribute to the progression of tumor cell growth. The results obtained from treated cells with the highest concentration of Childinan SF-2 (5 $mg.mL^{-1}$), showed an increment in population of cells in G1 phase compared to that of the control group, which indicated that the fungal extracts arrested the cells in this phase. It is interesting to know that when the accumulation of cells increased in G1, it could not show the efficiency of the compounds for their anticancer activity. Hence, a remarkable bioactive compound should arrest cells at G2 phase, which showed that it was mediated by the mitotic division (32). The results proposed the induction of apoptosis of cells by Childinan SF-2. Furthermore, the biopolymer Childinan SF-2 prevented the cell division by blocking tumor cell cycle at G0/G1 stage. The results of the study appropriately were compatible with those obtained by Liu *et al*. (33) who observed the cytotoxic efficiency of the polysaccharide extracted from *Russula griseocarnosa* in HeLa and SiHa cells. They observed that a significant increment in both early and late apoptosis at cell lines in a dose-dependent manner. The study conducted by Li *et al*. (34) reported the antitumor activity of edible fungal polysaccharide Lentinan on MCF-7 cells. They reported new findings about the mechanisms of Lentinan-antitumor effect for development in functional foods and cancer therapy.

Fungal extracts have been shown to play a significant role as free radical scavengers for the prevention of oxidative damage in living organisms (20, 35, 36). The results of the study correlated well with those obtained by Ren and colleagues (37) who observed DPPH and hydroxyl radical scavenging activity of 75.4% and 68.5%, respectively for *Pleurotus abalonus* (PAP) extract. In the study conducted by Reis *et al*. (38) the DPPH activity of 17% was measured for methanolic extract from *Pleurotus ostreatus* that was much lower than Childinan SF-2 (77.5%). In contrast, *Penicillium flavigenum* CML2965 extract showed a strong DPPH activity of 98.2%. For hydroxyl radical scavenging capacity, Childinan SF-2 showed the scavenging power of 56.45% that was lower than those reported for *Daldinia pyrenaica* SF-1 (22) and *Neopestalotiopsis* SKE15 (16) extracts with 85.2% and 86.6% activity, respectively. In β-carotene bleaching assay, used as third method, the antioxidant capacity is evaluated by measuring the inhibition of the production of volatile organic compounds and the formation of C=C hydroperoxides arising from linoleic acid oxidation, which results in the discoloration of β-carotene (39). Bioactive components with different antioxidant effect can prevent or reduce the bleaching of β-carotene.

Regarding ferric reducing power that can help to improve oxidative stress, biopolymer Childinan SF-2 showed a significant activity (OD value of 1.35 nm) that was comparable with those obtained from methanolic extract of *Pleurotus ostreatus* (OD value of 1.96 nm) and *Lentinula edodes* (OD value of 1.98 nm) (37). It was noted that *P. flavigenum* CML2965 and *L. edodes* extracts exhibited significant antioxidant activities with 72.2% and 51% of β-carotene protection, respectively. These records were much higher than that of Childinan SF-2 (20.8%) in comparison to the Ascorbic acid (96.05%) and ethanol (11.25%) as positive and negative controls, respectively. Free radical scavenging capacity mostly evaluated by hydroxyl radical and DPPH assays, whereas the β-carotene-linoleic acid system assessment represents the protective features of the antioxidant activities and yet such evaluation is more specific for lipophilic compounds (38). Therefore, the results collectively showed versatility in antioxidant activity of Childinan SF-2 and dependence of its activity to the chemical composition of reactive compounds.

Resistance of bacterial strains to one or more antimicrobial agents usually increases through mutations and natural selection. Hence, exploring the alternative novel, effective and natural antimicrobial compounds has received much attention (39, 40). Although the biopolymer Childinan SF-2 did not entirely inhibit the growth of Gram-negative bacteria including *Klebsiella pneumonia* and *Escherichia coli*, it revealed a mild inhibitory effect on the growth of these bacteria with 36% and 45% absorbance reduction, respectively. Consequently, it causes the leakage of vital intracellular constituents and the impairment of the bacterial enzyme systems (41, 42). The antimicrobial activity of the biopolymer obtained from fungus *Cladosporium cladosporioides* was evaluated against various bacterial strains by Yehia *et al*. (43). The results showed *C. cladosporioides* biopolymer had the best antimicrobial activity causing a zone of inhibition ranging from 20.7 to 25.7 mm and a MIC value ranging from 3.90 to 15.62

μg.mL^{-1} against various tested bacterial phytopathogens (43). In the other study, the ethyl-acetate extract of the endophytic fungus *Penicillium* sp. (Stdif 9), exhibited the high inhibition against five Gram-positive bacteria including *Bacillus megaterium*, *Micrococcus luteus*, *Bacillus cereus*, *Bacillus subtilis* and *Micrococcus lysodeikticus*, as well as two Gram-negative bacteria of *Proteusbacillus vulgaris* and *Salmonella typhi* (44). Growth inhibition of *Staphylococcus aureus* is of high importance since methicillin has a widespread resistance to *S. aureus* and it is assumed that it potentially causes a serious public health threat worldwide (26).

Low viscosity of the biopolymer solution and high degree of reducing sugars, indicate that Childinan is an oligosaccharide. Probable pharmaceutical applications of the Childinan can be enhanced by fulfilment of structural characterization. The native structure gives a molecular model for new applications and chemical changes and production of semisynthetic designed products may result in more applicable products. Furthermore, Childinan may confer ecological advantages to the fungus. As it is produced along with growth and increasing of the biomass, it can be supposed that secretion of superficial exopolysaccharides protects the mycelia against invading organisms due to its antimicrobial activity and other mentioned bioactive properties. Furthermore, exopolysaccharide production may provide physical advantages such as better water adsorption from the environment and facilitated penetration as the result of its lubricating effect. This may control external microbial populations in the close peripheral region around hyphae. Although, we are not sure whether the same slime production and advantages really happened under natural condition; but it can be much more advanced process under natural condition and external slime may works much more complicated.

6. Conclusion

The novel bioactive compound, Childinan SF-2, was extracted from the fungal isolate *Daldinia childiae.* The *in vitro* study indicated that Childinan SF-2 could more effectively elevate the percentage of the apoptosis and necrosis of the cancer cells and block the cell cycle phase. Moreover, the extract showed considerable antioxidant activity via different evaluation methods. The partial characterization of the extract showed that purified Childinan SF-2 had a high content of total and reducing sugars. Further characterization of the chemical structure of Childinan SF-2 and fractionation to suprapure molecules are required to help to unravel the possible mechanism of action and designing of effective probably new pharmaceuticals against cancer.

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Conflict of interests

The author(s) have declared that there is not any conflict of interest.

References

1. Zhang L, Ren IB, Zhang J, Liu L, Liu J, Jiang G, *et al*. Anti-tumor effect of *Scutellaria barbata* D. Don extracts on ovarian cancer and its phytochemicals characterization. *J Ethnopharmacol*. 2017;**12**(206):184-192, doi:10.1016/j.jep.2017.05.032
2. Mahapatra S, Banerjee D. Production and structural elucidation of exopolysaccharide from endophytic *Pestalotiopsis* sp. BC55. *Int J Biol Macromol*. 2016;**82**:182-191, doi:10.1016/j.ijbiomac.2015.11.035
3. Maity p, Sen IK, Chakraborty I, Mondal S, Bar H, Bhanja SK, *et al*. Biologically active polysaccharide from edible mushrooms: A review. *Int J Biol Macromol*. 2021;**172**:408-417. doi:10.1016/j.ijbiomac.2021.01.081
4. Lojewska B, Swiątkiewicz S, Muszynska B. The use of Basidiomycota mushrooms in poultry nutrition-A review. *Anim Feed Sci Technol*. 2017;**230**:59-69. doi:10.1016/j.anifeedsci.2017.06.001
5. Xie L, Shen M, Hong Y, Ye H, Huang L, Xie J. Chemical modifications of polysaccharides and their anti-tumor activities. *Carbohydr Polym*. 2020;**229**:115436. doi:10.1016/j.carbpol.2019.115436
6. Yu Y, Shen M, Song Q, Xie J. Biological activities and pharmaceutical applications of polysaccharide from natural resources: a review. *Carbohydr Polym*. 2018;**183**:91-101. doi:10.1016/j.carbpol.2017.12.009
7. Sun Y, Zhang M, Fang Z. Efficient physical extraction of active constituents from edible fungi and their potential bioactivities: A review. *Trends Food Sci Technol*. 2019;**105**:468-482, doi.org/10.1016/j.tifs.2019.02.026.
8. Wu L, Zhao J, Zhang X, Liu SH, Zhao CH. Antitumor effect of soluble β-glucan as an immune stimulant. Int *J Biol Macromol*. 2021;**179**:116-124. doi:10.1016/j.ijbiomac.2021.02.207
9. Smirnou D, Knotek P, Nesporova K, Pavlik K, Franke L, Velebny V. Ultrasoundassisted production of highly-purified β-glucan schizophyllan and characterization of its immune properties. *Process Biochem.* 2017;**58**:313-319. doi:10.1016/j.procbio.2017.04.016
10. Muszynska B, Kisielewska A, Kala K, Argasinska JG. Anti-inflammatory properties of edible mushrooms: a review. *Food Chem*. 2018;**243**:373-381. doi:10.1016/j.foodchem.2017.09.149

11. Stadler M, Baumgartner M, Wollweber H. Three new *Daldinia* species with yellowish stromatal pigments. *Mycotaxon.* 2001;**80**:179-196. doi:10.1016/S1130-1406 (06)70037-7
12. Zhao ZH, Chen HP, Huang Y, Zhang SHB, Li ZH, Feng T, *et al*. Bioactive polyketides and 8, 14-seco-ergosterol from fruiting bodies of the ascomycete *Daldinia childiae*. *Phytochemistry*. 2017;**142**:68-75. doi:10.1016/j.phytochem.2017.06.020
13. Rogers JD, Ju YM, Watling R, Whalley AJS. A reinterpretation of *Daldinia concentrica* based upon a recently discovered specimen. *Mycotaxon*. 1999;**72**:507-520.
14. Gontia-Mishra I, Tripathi N, Tiwari SA. simple and rapid DNA extraction protocol for filamentous fungi efficient for molecular studies. *Indian J Biotechnol*. 2014;**13**(4):536-539. http://nopr.niscair.res.in/handle/123456789/30483
15. Kumar S, Stecher G. Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;**33**:1870-1874. doi:10.1093/molbev/msw054
16. Fooladi T, Soudi MR, Alimadadi N, Savedoroudi P, Heravi MM. Bioactive exopolysaccharide from *Neopestalotiopsis sp.* strain SKE15: Production, characterization and optimization. *Int J Biol Macromol*. 2019;**129**:127-139. doi:10.1016/j.ijbiomac.2019.01.203
17. Blumenkrantz N, Asboe-Hansen G. New method for quantitative determination of uronic acids. *Anal Biochem*. 1973;**54**(2):484-489, doi.org/10.1016/0003-2697(73)90377-1
18. Dodgson KS, Price RG. A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem J*. 1962;**84**(1):106. doi:10.1042/bj0840106
19. Jamshidian H, Shojaosadati SA, Vilaplana F, Mousavi SM, Soudi MR. Characterization and optimization of schizophyllan production from date syrup. *Int J Biol Macromol*. 2016;**92**:484-493. doi:10.1016/j.ijbiomac.2016.07.059
20. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *J Agric Food Chem*. 1992;**40**(6):945-948. doi:10.1021/jf00018a005
21. Zhang X, Guo J, Wang WN, Wei GX, Ma GY, Ma XD. Diversity and bioactivity of endophytes from *Angelica sinensis* in China. *Front Microbiol.* 2020;**11**:1489. doi:10.3389/fmicb.2020.01489
22. Fooladi T, Soudi MR, Hashemi SM, Antunes FAF, Abdeshahian P. Biological function and molecular properties of Pyrenaican SF-1 as biological macromolecule extracted from *Daldinia pyrenaica*. *Int J Biol Macromol.* 2020;**163**:298-308. doi:10.1016/j.ijbiomac.2020.06.242
23. Mayouf N, Charef N, Saoudi S, Baghiani A, Khennouf S, Arrar L. Antioxidant and anti-inflammatory effect of *Asphodelus microcarpus* methanolic extracts. *J Ethnopharmacol*. 2019;**239**(3):111914. doi:10.1016/j.jep.2019.111914
24. Sobeh M, Mahmoud MF, Abdelfattah MAO, Cheng H, El-Shazly AM, Wink M. Proanthocyanidin-rich extract from *Cassia abbreviata* exhibits antioxidant and hepatoprotective activities *in vivo*. *J Ethnopharmacol.* 2018;**213**:38-47. doi:10.1016/j.jep.2017.11.007
25. Sulej J, Osińska-Jaroszuk M, Jaszek M, Grąz M, Kutkowska J, Pawlik A, *et al*. Antimicrobial and antioxidative potential of free and immobilised cellobiose dehydrogenase isolated from wood degrading fungi. *Fungal Biol*. 2019;**123**(12):875-886. doi:10.1016/j.funbio.2019.09.007
26. Oh E, Bae J, Kumar A, Choi HJ, Jeon B. Antioxidant-based synergistic eradication of methicillin-resistant *Staphylococcus aureus* (MRSA) biofilms with bacitracin. *Int J Antimicrob* Agents. 2018;**52**(1):96-99. doi:10.1016/j.ijantimicag.2018.03.006
27. Kumar S, Stecher G. Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*. 2016;**33**:1870-1874. doi:10.1093/molbev/msw054
28. Stadler M, Læssøe T, Fournier J, Decock C, Schmieschek B, Tichy HV, *et al*. A polyphasic taxonomy of *Daldinia (Xylariaceae)*. *Stud Mycol*. 2014;**77**:1-143.doi:10.3114/sim0016
29. Belhaj D, Frikha D, Athmouni K, Jerbi B, Ahmed MB, Bouallagui Z, *et al*. Box-Behnken design for extraction optimization of crude polysaccharides from *Tunisian Phormidium* versicolor cyanobacteria (NCC 466): partial characterization, *in vitro* antioxidant and antimicrobial activities. *Int J Biol Macromol.* 2017;**105**(2):1501-1510. doi:10.1016/j.ijbiomac.2017.06.046
30. Li Y, You L, Dong F, Yao W, Chen J. Structural characterization, antiproliferative and immunoregulatory activities of a polysaccharide from Boletus *Leccinum rugosiceps*. *Int J Biol Macromol*. 2020;**157**:106-118. doi:10.1016/j.ijbiomac.2020.03.250
31. Wang T, Dong Z, Zhou D, Sun K, Zhao Y, Wang B, *et al*. Structure and immunostimulating activity of a galactofuranose-rich polysaccharide from the bamboo parasite medicinal fungus *Shiraia bambusicola. J Ethnopharmacol.* 2020;**257**:112833. doi:10.1016/j.jep.2020.112833
32. Martins-Gomes C, Souto EB, Cosme F, Nune FM, Silva AM. *Thymus carnosus* extracts induce anti-proliferative activity in Caco-2 cells through mechanisms that involve cell cycle arrest and apoptosis. *J Funct Foods.* 2019;**54**:128-135.doi:10.1016/j.jff.2019.01.010
33. Liu Y, Zhang J. Meng ZH. Purification, characterization and anti-tumor activities of polysaccharides extracted from wild *Russula griseocarnosa*. *Int J Biol Macromol*. 2018;**109**:1054-1060. doi:10.1016/j.ijbiomac.2017.11.093
34. Li WY, Wang J, Hub H, Li Q, Liu Y, Wang K. Functional polysaccharide Lentinan suppresses human breast cancer growth via inducing autophagy and caspase-7-mediated apoptosis. *J Funct Foods*. 2018;**45**:75-85. doi:10.1016/j.jff.2018.03.024
35. Shao LL, Xu J, Shi MJ, Wang XL, Li YT, Kong LM, *et al*. Preparation, antioxidant and antimicrobial evaluation of hydroxamated degraded polysaccharides from Enteromorpha prolifera. *Food Chem*. 2017;**237**:481-487. doi:10.1016/j.foodchem.2017.05.119
36. Chandra H, Kumari P, Prasad R, Gupta SCH, Yadav S. Antioxidant and antimicrobial activity displayed by a fungal endophyte *Alternaria alternata* isolated from *Picrorhiza kurroa* from Garhwal Himalayas, India. *Biocatal Agric Biotech*.2021;**33**:101955.
37. Ren D, Jiao Y, Yang X, Yuan L, Guo J, Zhao Y. Antioxidant and antitumor effects of polysaccharides from the fungus *Pleurotus abalones*. *Chem Biol Interact*. 2015;**237**:166-174. doi:10.1016/j.cbi.2015.06.017
38. Reis FS, Martins A, Barros L, Ferreira ICFR. Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between *in vivo* and in vitro samples. *Food Chem Toxicol*. 2012;**50**(5):1201-1207. doi:10.1016/j.fct.2012.02.013
39. Tavares DG, Barbosa BVL, Ferreira RL, Duarte WF, Cardoso PG. Antioxidant activity and phenolic compounds of the extract from pigment-producing fungi isolated from Brazilian caves.

Biocatal Agric Biotechnol. 2018;**16**:148-154. doi:10.1016/j.bcab.2018.07.031

40. Batiha GS, Hussein DE, Algammal AM, .George T, Jeandet P, EsmailAl-Snafi A, Tiwari A, Pagnossa JP, Lima CM, Thorat ND, Zahoor M, El-Esawi M, Dey M, Alghamdi S, Hetta HF, Martins N. Application of natural antimicrobials in food preservation: Recent views.*Food Control.* 2021;**126**:108066. doi: 10.1016/j.foodcont.2021.108066
41. Berri M, Slugocki C, Olivier M, Helloin E, Jacques I, Salmon H, et al. Marine-sulfated polysaccharides extract of *Ulvaarmoricana* green algae exhibits an antimicrobial activity and stimulates cytokine expression by intestinal epithelial cells. *J Appl Phycol.*2016;**28**:2999-3008. d doi:10.1007/s10811-016-0822-7
42. Kong Q, Yang Y. Recent advances in antibacterial agents. *Bioorg Med Chem Lett.*2021;**35**:127799. doi:10.1016/j.bmcl.2021.127799
43. Yehia RS, Osman GH, Assaggaf H, Salem R, Mohamed MSM. Isolation of potential antimicrobial metabolites from endophytic fungus *Cladosporium cladosporioides* from endemic plant *Zygophyllum mandavillei*. *S Afr J Bot.* 2020;**134**:1-7. doi:10.1016/j.sajb.2020.02.033
44. Wu H, Yan ZH, Deng Y, Wu ZH, Xu XL, Li X, *et al*. Endophytic fungi from the root tubers of medicinal plant *Stephania dielsiana* and their antimicrobial activity. *Acta* Ecologica *Sinica*. 2020;**40**(5):383-387. doi:10.1016/j.chnaes.2020.02.008