

Isolation and Identification of Endophytic Fungi from *Hypocrea pachybasioides* and Investigation of the Fungi Cytotoxic Activities

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Abstract

Background and Objective: In the present study, a wild fungus, *Hypocrea pachybasioides*, has molecularly identified using ribosomal ITS5-ITS4 region as well as analysis of chemically functional groups. This fungus grows on rotten woods. This study is the first report on chemical and pharmacological activities of the fungus. The *Hypocrea* genus has been linked to another fungus such as *Trichoderma* spp., which makes it important to identify this species. There are no studies on the fungus chemical and pharmacological characteristics. The aim of the study was to identify a wild fungus through molecular methods, as well as its chemical and pharmacological identification.

Material and Methods: Compounds from methanolic extract of the mycelium and those secreted in the culture broth were assessed. First, fungal deoxyribonucleic acid was extracted for molecular identification. Qualitative assessments were carried out for compounds such as tannins, saponins and coumarins, as well as quantitative assessments for total phenols and flavonoids. High-pressure liquid chromatography analysis was carried out for organic acids. Furthermore, antiproliferative assessments were carried out using sulforhodamine B method.

Results and Conclusion: Assessments carried out on both fractions showed that compounds such as alkaloids and saponins included the highest quantities in the suggested hedonic scale. In contrast, anthraquinones were detected in lower quantities, while coumarins and tannins were not detected. Methanolic extract from mycelia showed cytotoxic activity in HeLa cell line. Therefore, *Hypocrea pachybasioides* can be addressed as a candidate for further pharmacological studies based on the criteria from the National Institutes of Health of the United States. This is suggested as a potentially biotechnological model for identifying various metabolites with therapeutic characteristics.

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1. Introduction

Hypocrea is a genus of fungi belonging to the class of *Ascomycetes*. Its species are saprophytes and predators of other fungi. There are 509 species belonging to *Hypocrea* genus, most of them identified by their morphological characteristics. These fungi are widely distributed and grow in tropical, subtropical, moist, arid, temperate and boreal forests. They are brightly or slightly colored and can

develop on rotten woods in the form of a shelf. These fungi have been associated with other species of *Ascomycota* and *Basidiomycota* of the anamorphic type [1]. Furthermore, *Hypocrea* (*H.*) is linked to the anamorphic fungi of *Trichoderma* spp. since all species have been reported as phylogenetically relatives [1, 2]. All these characteristics make difficulties to identify several species belonging to

Hypocrea [2]. Thus, molecular methods are useful tools for the identification of these fungi with phenotypic and genotypic complexities within the *species of Hypocrea/Trichoderma*. Molecular identification is important since several species of *Hypocrea* (anamorph to *Trichoderma*) have shown activities against pathogenic fungal species such as *Phytium* spp., *Rhizoctonia solani* and *Verticillium dahliae*. Moreover, they can act on *Botrytis cinerea*, which causes the root rot of forest species, suggesting protective roles to avoid decay in fruit storage [3]. Biological control by *Hypocrea* spp. was verified in the treatment of pathogenic species such as *Fusarium* spp. of tomato crops [4]. Similarly, it has been demonstrated that the fungi synthesize chemical compounds that accelerate growth of various vegetable crops. Lo and Lin (2002) [5] reported positive effects on soil using *Trichoderma* (*T.*) *harzianum* for the cultivation of squash and cucumbers while Keswani et al. (2014) reported that *Trichoderma* spp. secreted several compounds that acted as growth regulators for horseradish, tomato, rice and tobacco [6]. In contrast, studies on compounds secreted by *Hypocrea* spp. have not been carried out. Since other genera such as *Trichoderma* are anamorph, it has been suggested that *Hypocrea* spp. possibly produce various compounds with simple or complex molecular structures and various chemical-biological characteristics. Secondary metabolites such as lignans, flavonoids, phenols, terpenes, sterols, alkaloids, coumarins and antibiotics with various biological characteristics can provide novel uses in various sectors of food, agrochemical and pharmaceutical industries [7].

In the present study, a wild fungus was isolated and identified as *H. pachybasioides* by molecular approaches. Contents of chemically functional groups such as alkaloids, saponins, anthraquinones, coumarins and tannins were analyzed as well. Moreover, total phenols and flavonoids were assessed in extracts from mycelia and those secreted into the culture broth. The cytotoxic effects of these metabolites were also investigated in three different human tumor cell lines. These data may be useful for further isolations of *H. pachybasioides* metabolites as potential biotechnological compounds.

2. Materials and Methods

2.1. Fungus collection

Fungi used in this study were collected from the Sierra de Tequexquahuac, Texcoco, Estado de Mexico. Taxonomic identification was carried out in the Fungi Laboratory of the Universidad Autonoma Chapingo.

2.2. Culture conditions

Collected fungi were disinfected in 1% sodium hypochlorite (v/v) solution for 10 min and then transferred into sterile distilled water (DW) (30 ml) to remove excess

sodium hypochlorite. A slice of the fungi was transferred into a Petri dish with potato dextrose agar (PDA) media and incubated at 25 °C for a week (w). After growing the fungi on the plate, a mycelial disk was inoculated into 100 ml of potato dextrose broth (PDB) media and incubated at 25 °C for a week (w). Mycelia were separated from the culture media via filtration and incubated at 35 °C for 3 d. Dehydrated mycelia were pulverized using mortar and then 20 mg of the sample were used for the isolation of DNA.

2.3. DNA extraction

Briefly, DNA extraction was carried out using AxyPrep multisource genomic DNA miniprep kit (cat. AP-MN-MS-GDNA-50, Axygen, Union City, CA, USA), based on the manufacturer's instructions. The DNA integrity was visualized on 0.7% (w/v) agarose gels stained with GelRed (Nucleic Acid Gel, Biotium; Hayward, CA, USA) using MultiDoc-It (UVP; Upland, CA, USA).

2.4. Amplification of the ITS region using PCR

The PCR reactions were prepared using the primer pair of *ITS4* (TCCTCCGCTTATTGATATGC) and *ITS5* (GGAAGTAAAAGTCGTAACAA) [9]. The reaction contained 40 ng of genomic DNA, Vent enzyme buffer (1×), MgCl₂ (1.5 mM), dNTPs (0.2 mM), primers (each 0.2 μM) and 1.25 U of Vent polymerase enzyme (BioLabs, New England Biolab, Ipswich, MA, USA) in a final volume of 50 μl. Amplification of the genes was carried out using Axygen thermal cycler (Axygen MaxyGene II Thermal Cycler, CA, USA) under the following conditions of one cycle at 94°C for 5 min, 30 cycles at 94°C for 30 s; 5 °C for 45 s and 72°C for 1 min and then one cycle at 72 °C for 5 min. Amplicons were electrophoresed on 2% (w/v) agarose gels.

2.5. Molecular identification of *Hypocrea pachybasioides*

The amplified PCR products of the ITS region were cloned into the vector of pJET1.2/blunt (Thermo Scientific, USA) to achieve a complete sequence of the analyzed and unknown regions. Vectors with the inserts were transformed into competent *Escherichia coli* Top 10F' cells (Invitrogen™, Waltham, MA, USA). Plasmid DNA was extracted using the GeneJET plasmid miniprep kit (Thermo Scientific, Waltham, MA, USA) based on the manufacturer's instructions. Positive clones were verified through digestion with BglII to release a 550-bp fragment. In addition, clones were sequenced using primers of pJET forward (CGACTCACTATAGGGAGAGCGGC) and pJET Reverse (AAGAACATCGATTTTCCATGGCAG), which was carried out at the Instituto de Biología of the Universidad Autonoma Nacional de Mexico. The sequence was analyzed through sequence comparisons of the GenBank database (NCBI) using Blast algorithm [10].



2.6. Extracellular and intracellular fungal extracts

To assess presence of chemically functional compounds e.g. alkaloids, anthraquinones, tannins, volatile coumarins and saponins. Methanol was used to achieve two extracts from the collected fungi, one from the concentrated culture broth (extracellular) and the other one from the mycelia (intracellular). Total phenolics and flavonoids were quantified in the two samples. Moreover, methanol was used to isolate the metabolites. Erlenmeyer flasks with 100 ml of PDB media were inoculated with fungal mycelia and incubated at 25 °C for 10 d. Then, mycelia were separated from the culture media by filtration. Mycelia and filtered broth were dried in an oven at 35 °C for 3 d. Dried mycelia were pulverized using mortar and transferred into 50 ml of methanol for 1 w. Methanolic extract was concentrated under decreased pressure using rotary evaporator.

2.7. Detection of alkaloids and anthraquinones

Thin layer chromatography (TLC) was carried out using silica gel plaques with dimensions of 3 × 5 cm (60F254) and results revealed presence of alkaloids and anthraquinones using Dragendorff reagent and UV light, respectively. An aliquot (0.5 µl) of the extract was transferred onto a plate and set in a solvent system of dichloromethane-methanol (95:5). Alkaloids were verified by the appearance of brown-red spots using Dragendorff reagent. Anthraquinones were investigated with the typical yellow or red fluorescent coloring after exposing the plates to UV light [11].

2.8. Detection of tannins and saponins

To analyze tannins in the extracts, three various solutions (10 mL) were prepared, including % (w/v) Tube 1, 1% gelatin solution; Tube 2, 1% gelatin solution and 10% NaCl; and Tube 3, 10% NaCl. Then, 2 ml of the extract were transferred into each tube and vigorously mixed using vortex. White precipitate in Tubes 1 and 2 indicated presence of tannins [11]. To assess saponins in the fungal extract, 2 ml of the samples were transferred into 10 ml of water. Tubes were heated 30 min at 80 °C and then set to cool down at room temperature (RT) and then stirred vigorously. Presence of stable foams within 15–20 min indicated presence of saponins [11].

2.9. Quantification of total phenols

To assess total phenols in the extracts, Folin-Ciocalteu reagent was used based on a protocol by [12]. Results were expressed as mg gallic acid/g dry extract (mgGAE/g) [12].

2.10. Quantification of total flavonoids

The flavonoid content was assessed using standard quercetin following the protocol [12]. Results were reported in mg quercetin/g dry extract (mgQE/g) [12].

2.11. Analysis of organic acids

Briefly, D,L-malic, oxalic and tartaric organic acids were detected in the fungal extracts using a methodology developed by [13]. Technically, 20 µl of three various samples were analyzed, including mycelia, filtered broth or the organic acid standards, which were injected into HPLC of Agilent Technology model 1260 equipped with a quaternary pump (Agilent Technology, California, USA) with a multiple wavelength detector (MWD). The column included an X-Terra MS C18 column, 5 µm (4.6 × 250 mm) and the mobile phase consisted of phosphate buffer (50 mM, pH 2.8) in isocratic mode. The flow was adjusted to 0.7 ml/min. Results were recorded at 210 nm and used in a standard curve. Results were expressed as parts per million.

2.12. Antiproliferative activity assay

To assess cytotoxic effects of *H. pachybasioides* extracts in human epithelial carcinoma (HeLa). Cell line was incubated at 37 °C in a humidified atmosphere of 5% CO₂ and 100% air in complete RPMI 1640 media. Cells in the log growth phase were treated with three various concentrations of methanolic extract from the mycelia in a range of 0.032–20.0 µg/ml and incubated at 37 °C for 72 h under similar culture conditions. Each experiment was carried out in triplicate and colchicine positive control was used as an inhibitor of cell division. Cell concentration was assessed using colorimetric method and sulforhodamine B. Results were expressed as percentage of cell growth using the formula of cellular growth (%) = $(At - Ab) / (Ac - Ab) \times 100$. Where, At was the average OD of the treatment, Ac was the average OD of the control and Ab was the average of the initial growth OD (blank) [14].

2.13. Statistical analysis

Linear regression analysis of the semi-logarithmic graphs between the concentrations and percentages of the cell growth was used. Effective concentration of the compound needed to inhibit cell proliferation by 50% (IC₅₀ in µg/ml) was assessed using Sigma plot software [15].

3. Results and Discussion

3.1. Fungus molecular identification

Molecular identification of the fungi usually uses nuclear DNA markers (18S and 28S ribosomal genes), spacer regions such as ITS 1 and ITS 2, external ETS and IGS intergenic [16]. Although, most of the reports on fungi identification have used the ITS region. Several genomic regions within the ITS region have been standardized and are now referred to as DNA barcodes. These barcodes are short regions, typically 400–800 base pairs (bp) [17], facilitating molecular identification of fungi. This includes anamorphic fungi, which can particularly be challenging to identify [18]. In this study, the ITS4-ITS5 region was used, including ribosomal gene region of 5.8S (Fig. 1A).



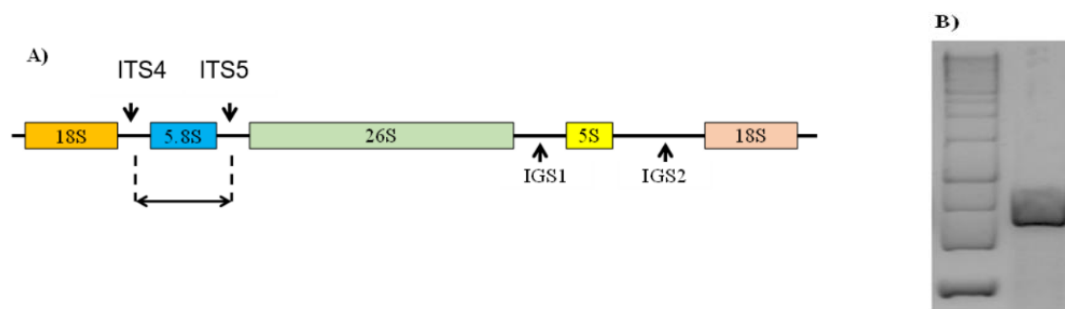


Figure 1. Example of the molecular identification of a wild fungus collected and identified as *Hypocrea pachybasioides*. A) Ribosomal regions used for molecular identification in fungi, and B) amplification of the fragment corresponding to ITS 4-ITS 5 region using PCR endpoint.

Fragment was achieved using ITS 4 forward and ITS 5 reverse primers, resulting in an amplicon of 450 bp within the expected range (Fig. 1B) [17].

Gimenez-Pereira et al. reported use of the ITS region to molecularly identify *H. pseudokoningii* within other filamentous fungi [19]. Then, the band obtained was cloned into the pJet 1.2 vector and sequenced using pJet forward and pJet reverse primers. Then, data were analyzed using Gen Bank database of the National Center for Biotechnology Information (NCBI), showing 99% identity and value of 0.0 with the sequence corresponding to *H. pachybasioides* (GU062213.1). This result helped molecular identification of the collected strain and a phylogenetic tree was constructed to show proximity of *H. pachybasioides* to other strains from similar species, demonstrating its close relationship majorly with other fungi of *Trichoderma* spp. (Fig. 2).

3.2. Chemically functional groups

Compounds such as saponins, tannins, coumarins, alkaloids, anthraquinones in the methanolic extracts from the mycelia and filtered culture media of *H. pachybasioides* were assessed. All these compounds have been investigated in various organisms because they include pharmacological activities. For example, alkaloids have been used as anticancer, antimicrobial, antiparasitic, antidepressant, antimalarial, anticonvulsant and antineurodegenerative agents. Anthraquinones are other molecules associated to antiviral, analgesic, diuretic, anticancer, anti-inflammatory, antimicrobial, antiparasitic, vasorelaxant and cathartic activities [20]. Furthermore, tannins may show antitumor, antimicrobial and antioxidant activities [21]. Coumarins have been reported to include anticancer, coagulant, antiedema, anti-hypertensive, anti-hypercholesterolemic, anticoagulant, antimicrobial, antiviral and antihypertensive activities [22]. Saponins have shown anticancer, antidiabetic, antiviral, antiallergic, antihypercholesterolemic, antimicrobial and antiparasitic activities [23]. Presence of these compounds in the methanolic extracts allowed the current authors to report the first approach on the chemical

compounds produced by this fungus. In mycelia, presence of alkaloids was detected at a moderate concentration, while saponins and anthraquinones were detected at lower concentrations. However, the broth extract included a large quantity of saponins and alkaloids (Table 1). Presence of the chemically functional groups highly suggests that some might present a therapeutic activity. Organic acids such as ascorbic, D,L-malic, oxalic and tartaric were enriched in the broth extract, compared to the mycelia, and presented a similar magnification as the juice extracted from *Stenocereus stellatus* [13] [24].

3.3. Total phenols and flavonoids quantifications

Total phenols and flavonoids were quantified, which might include significant effects on cells [24]. Quantities of total phenols and flavonoids were lower in culture broth, compared to the extract from mycelia, both from a methanolic extract (Table 1).

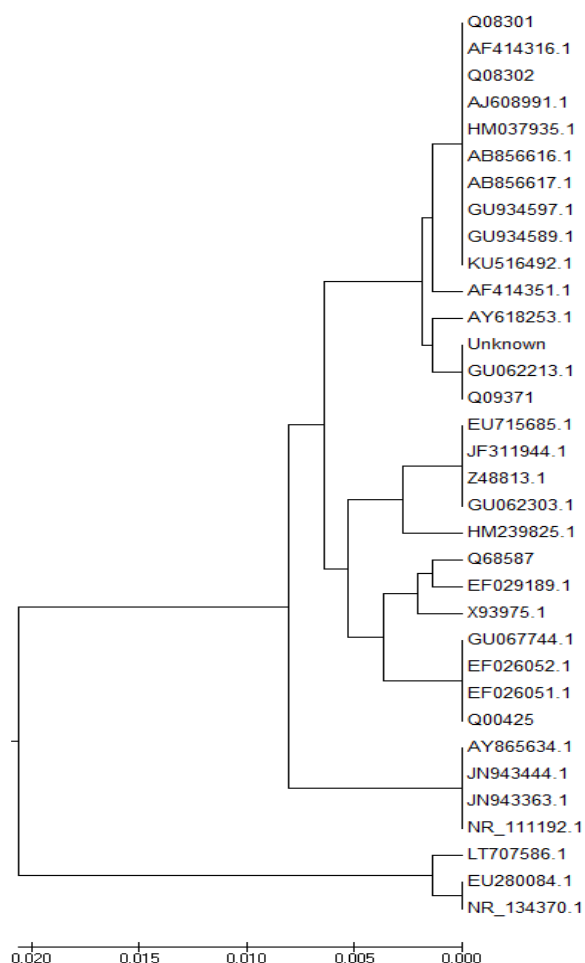
3.4. Cytotoxic analysis

To assess pharmacological activities, cytotoxic assessments were carried out using the methanolic extract from the mycelia on cell lines of MCF-7, HeLa and HCT15. So, the half maximal inhibitory concentration (IC_{50}) was calculated by fitting curves of data from the sulforhodamine B assay (Table 1), where the antiproliferative effect was reported. Colchicine was used as control. Results showed that HeLa was the most sensitive cell line to the extract with an IC_{50} of 12.9 ppm while no activity was detected in MCF-7 and HCT15 cells, less than 20 ppm in the assessed range. The IC_{50} was established as a criterion for cytotoxic activity of the crude extracts by the US national cancer institute; in which, range of 0-20 ppm is cytotoxic [25].

3.5. Relationships between the chemical compounds and cytotoxic assay

Use of fungi for the extraction of various compounds of nutritional and medicinal importance has extensively been studied. Although fungi are primary microorganisms producing metabolites, a few studies have focused on the analysis of chemically functional groups in the fungi.





Phylogenetic tree was constructed using neighbor-joining method and MEGA 3.0. GU062213.1 (*H. pachybasioides*), KU516492.1 (*T. polysporum*), GU934589.1 (*H. pachybasioides* Strain 850), GU934597.1 (*H. pachybasioides* Strain 862), GU067744.1 (*H. pachybasioides* Isolate F30 18S), AB856617.1 (*T. polysporum*), AB856616.1 (*T. polysporum*), DQ093710.1 (*H. pachybasioides* Isolate aurim1204), EF026052.1 (*Vouchered mycorrhizae*), AY865634.1 (*T. minutisporum* Strain DAOM 167069), HM037935.1 (*T. album* Strain wxm157), AJ608991.1 (*T. album*), HM239825.1 (uncultured Ascomycota Clone 1147), EF026051.1 (*Vouchered mycorrhizae* (fungi) Clone KSC 10022), JN943444.1 (*H. minutispora* Strain NBRC 9062), GU062303.1 (*Hypocrea* sp. I348), DQ004258.1 (Ascomycete sp. KS10021), DQ083026.1 (*T. croceum* Strain DAOM 167068), JQ685872.1 (*T. foliicola* Strain Hypo 650 voucher WU 31612), DQ083011.1 (*T. stellatum* Strain GJS 99-222), Z48813.1 (*H. pilulifera*), X93975.1 (*T. hamatum* Strain ATCC 28012), JN943363.1 (*T. minutisporum* Strain NBRC 101779), EU715685.1 (*H. pilulifera* Strain SGLMf38), AF414316.1 (*T. croceum*), AF414351.1 (*T. album* Strain CCRC33638), EF029189.1 (*T. hunua* Culture-collection ICMP:14388), AY618253.1 (*T. album*), NR_111192.1 (*T. minutisporum* DAOM 167069), EU280084.1 (*H. crassa* Strain DAOM 233775), NR_134370.1 (*T. crassum* DAOM 164916), JF311944.1 (*H. pilulifera* Strain P156_D1_4) and LT707586.1 (*T. virens*).

Figure 2. Phylogenetic analysis constructed using ITS sequence from a wild fungus identified as *Hypocrea pachybasioides*

Compounds with pharmacological activity have already been identified and characterized. Saponins are used as compounds with antifungal activity such as strobilurins and oudesmansins present in basidiomycetes and ascomycetes.

Endophytic fungi of medicinal plants such as *Nectria*, *Aspergillus*, *Fusarium*, *Verticillium*, *Engyodontium*, *Plectosphaerella*, *Penicillium* and *Cladospori* include these compounds, showing antifungal and antibacterial activities. Furthermore, these fungi are industrial materials to produce saponins with antimicrobial activity for use in health and agricultural sectors [26]. It is noteworthy that these treatments are mostly used as mixtures. It is possible to improve effects of nano transporters [27].

It is well known that phenolics include antioxidant capacity. In this study, total phenolic compounds and flavonoids were assessed and the quantity was higher in mycelia than broth. In plants, most of the phenolics are in detected in the cytosol. This explains differences between the mycelia and broth extracts since the mycelial extract suffers cell disruption. It is useful to carry out studies on the

antioxidant capacity of *H. pachybasioides*, as reported by Zeng et al., 2011 in two *Hypocrea* spp. [28]. Alkaloids include pharmacological activities such as antimicrobial, insecticidal, cytotoxic, vasoconstrictive, anti-hemorrhagic and anticancer activities. For example, ergometrine and ergoline from *Claviceps* spp. have shown vasoconstricting and anti-hemorrhagic activities and are used for migraine treatment. Oxalin extracted from *P. oxalicum* is an alkaloid with strong cytotoxic activity similar to taxol that is reported to arrest the cell cycle in M phase [29]. Reports of anthraquinones with pharmacological effects on fungi are little; however, tetrahydro-anthraquinone of *Alternaria* sp. XZSBG-1 has shown cytotoxic activity on cell lines of HCT-16 and HeLa while 6-8-di-O-methylveranthine isolated from *A. versicolor* EN-7 has shown antibacterial effects on *E. coli* and *Staphylococcus aureus*. Furthermore, *T. aureoviride* PSU-F95 produces two anthraquinones: coniotrantra-quinone-1 and emodine, which inhibit the growth of resistant strains of *S. aureus* [30].



Table 1. Qualitative and quantitative chemical analyses of the functional groups from the mycelia and filtered broth of *Hypocrea pachybasioides*.

Analyses	Extract (<i>Hypocrea pachybasioides</i>)	
	Mycelium	Filtered broth
Chemical qualitative analyses		
Saponins	+	+++
Coumarins	-	-
Anthraquinones	+	+
Alkaloids	++	+++
Tannins	-	-
Chemical quantitative analyses		
Total phenols (mgGAE/g)	0.22±0.05	0.07±0.01*
Total flavonoids (mgQE/g)	0.07±0.01	0.04±0.01*
Organic acids (mg ac./mL extract)		
Ascorbic	0.57±0.01	0.16±0.04*
D,L-Malic	3.80±0.01	15.59±0.01*
Oxalic	0.17±0.01	0.26±0.01*
Tartaric	0.53±0.01	1.75±0.01*
Cytotoxic assay**		
MCF-7	> 20	> 20
HCT15	> 20	> 20
HeLa	12.9	> 20

Qualitative scale: - No presence was observed. Presence: + low, ++ moderate, +++ high. Methods used as formerly described. *Student’s t test ‘*’ indicates differences between Mycelium vs Filtered broth. $\alpha = 0.05$. **IC₅₀ in ppm

These studies support results of the current study because with the extract from the mycelia, it was possible to report important cytotoxic activities for HeLa cell lines. Further studies are recommended to isolate specific metabolites in *H. pachybasioides*. Compounds with higher cytotoxic activities isolated from fungi are polysaccharides and heteropolysaccharides, which include high solubility in water and alcohols. These present better results when assessed with *in vitro* models on cancer cell lines [31]. The current results are aligned with this idea because it has been observed that methanolic extract of the *H. pachybasioides* mycelia includes antiproliferative activity for HeLa cell line. *Trichoderma* spp. produce such effects in Hela and HCT-7 cell lines [32]. Another important example is lipopeptaibols isolated from *T. strigosum* that have shown activity against various cell lines, including HCT-116 [33]. Cytotoxic activity of *H. pachybasioides* mycelia might be associated to high phenol contents, as occurs in *S. stellatus* extracts previously achieved in the current authors’ laboratory. It is suggested that phenols enhance antioxidant activity in cells. Correlations have been made between the cytotoxic effects and total phenols, antioxidant activities and cytotoxicity using the following formula: “Antioxidant capacity = a * phenols + b * D_malic + c * glucose; where, a = 0.54, b = 0.56, c = 0.28 (standard errors = 0.19, 0.20 and 0.21, respectively) and R = 0.76” [22]. This correlation with data was achieved from peels of tropical fruits: *Annona squamosa* L. (purple sugar apple), *A. reticulata* L. (custard apple), *Chrysophyllum cainito* L. (green star apple) and *Melicoccus bijugatus* Jacq. (mamoncillo) with a correlation with $r^2 = 0.97$ ($p = 0.05$) with total phenols [24,34].

4. Conclusion

In this study, a wild fungus was identified as *H. pachybasioides* using molecular approaches. A general study was carried out to assess various metabolites. It has been shown that methanolic extract from the mycelia caused inhibition in cell growth and substantial cytotoxic effects on HeLa cell line. This effect can be attributed to polyphenolic compounds. It is noteworthy that a few reports are available on this fungus and these data are the first data on *H. pachybasioides* pharmacological activity. Further studies are recommended on the purification, identification and characterization of therapeutic compounds as well as other compounds of biotechnological interest.

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6. Conflict of Interest

The authors report no conflict of interest.

7. Authors Contributions

All authors reviewed the results and approved the final version of the manuscript. Study conception and design, JMQ, JAME and ESP.

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جداسازی و شناسایی قارچ‌های اندوفیت از هیپوکرا پاچی‌بازیدیویدس و بررسی فعالیت‌های سیتوتوکسیک قارچ‌ها

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چکیده

سابقه و هدف: در مطالعه حاضر، قارچ وحشی هیپوکرا پاچی‌بازیدیویدس با استفاده از ناحیه ریبوزومی ITS5-ITS4 و همچنین تجزیه و تحلیل گروه‌های عملکردی شیمیایی در حد مولکولی شناسایی شده است. این قارچ روی چوب‌های پوسیده رشد می‌کند. این مطالعه اولین گزارش از فعالیت‌های شیمیایی و دارویی این قارچ است. جنس هیپوکرا با جنس تریکودرما مرتبط شده است که شناسایی این گونه را مهم می‌کند. هیچ مطالعه‌ای در مورد ویژگی‌های شیمیایی و دارویی قارچ وجود ندارد. هدف از این مطالعه شناسایی یک قارچ وحشی از طریق روش‌های مولکولی و همچنین شناسایی شیمیایی و دارویی آن بود.

مواد و روش‌ها: ترکیبات حاصل از عصاره متانولی میسلیم و ترکیبات ترشح شده در محیط کشت بررسی شد. ابتدا، اسید دئوکسی ریبونوکلیک قارچی برای شناسایی مولکولی استخراج شد. ارزیابی‌های کیفی برای ترکیباتی مانند تانن‌ها، ساپونین‌ها و کومارین‌ها و همچنین ارزیابی‌های کمی برای کل فنل‌ها و فلاونوئیدها انجام شد. تجزیه و تحلیل کروماتوگرافی مایع با فشار بالا برای اسیدهای آلی انجام شد. علاوه بر این، ارزیابی ضد پرولیفراتیو با استفاده از روش سولفورودامین B انجام شد.

یافته‌ها و نتیجه‌گیری: ارزیابی‌ها نشان داد ترکیباتی مانند آلکالوئیدها و ساپونین‌ها بیشترین مقدار را در مقیاس هدونیک نشان دارند. در مقابل، آنتراکینون‌ها در مقادیر کمتر شناسایی شدند، در حالی که کومارین‌ها و تانن‌ها اصلاً شناسایی نشدند. عصاره متانولی میسلیم فعالیت سیتوتوکسیک را در رده سلولی هلا نشان داد. بنابراین، هیپوکرا پاچی‌بازیدیویدس را می‌توان به عنوان یک کاندید برای مطالعات فارماکولوژیک بیشتر بر اساس معیارهای مؤسسه ملی بهداشت ایالات متحده در نظر گرفت. این به عنوان یک مدل بالقوه زیست‌فناورانه برای شناسایی متابولیت‌های مختلف با ویژگی‌های درمانی پیشنهاد می‌شود.

تعارض منافع: نویسندگان هیچ گونه تضاد منافع را اعلام نمی‌کنند.

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