*Uttar Pradesh Journal of Zoology*

***Volume 45, Issue 4, Page 55-67, 2024; Article no.UPJOZ.3264***
***ISSN: 0256-971X (P)***

# Isolation of Endophytic Fungi from *Adhatoda vasica* and Assessing its Antibacterial Efficacy and Cytotoxic Impact on the A549 Cancer Cells

**Jenifer Jincy. T [a++], Biji C. P [b#*] and A. Vijaya Chithra [c†]**

*[a] Bharathiar University, Coimbatore, India.*
*[b] Department of Zoology, Mercy College, Palakkad, Kerala, India.*
*[c] Department of Microbiology, Sri Ramakrishna College of Arts and Science for Women, Coimbatore, India.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.56557/UPJOZ/2024/v45i43907

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3264

***Original Research Article***

***Received: 11/12/2023***
***Accepted: 16/02/2024***
***Published: 16/02/2024***

## ABSTRACT

The current study investigated the anti-bacterial and anti-cancer properties of endophytic fungi obtained from medicinal plant *Adhatoda vasica*. We isolated and identified the endophytic fungi *Humicola fuscoatra*. by examining their morphological characteristics and microscopic structures. We then extracted their secondary metabolites using ethyl acetate. The ethyl acetate extracts of the

*[++] Research Scholar;*
*[#] Assistant Professor;*
*[†] Associate Professor;*
**Corresponding author: Email: bijicp@gmail.com;*

fungus *H. fuscoatra* have shown promising effectiveness against human pathogens, including *Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*. A strain of *H. fuscoatra* exhibited significant antibacterial activity. In addition, we have assessed the effects of ethyl acetate extracts from the fungus *H. fuscoatra* on the growth of the human lung cancer cell line A549 in vitro assays. Using ethyl acetate extracts from the fungus *H. fuscoatra* effectively suppressed the growth, survival, migration, and invasion of cancer cells. In this paper we studied in detail the identification of isolated endophytic fungi *H. fuscoatra* from *Adhatoda vasica* and characterized its active metabolite compounds. The ethyl acetate-extracted secondary metabolites from the fungal culture supernatant were analyzed using gas chromatography and mass spectrometry, which detected the presence of Hexadecanoic Acid, Hexadecane, Heptadecane, Octadecane, Octadecanoic acid, phytochemicals. The results suggest that the endophytic fungus *H. fuscoatra* found in medicinal plants has the potential to produce bioactive compounds. They could also be used as a biocontrol agent against bacterial pathogens and for therapeutic purposes in the A549 lung cancer cell line.

*Keywords: Humicola fuscoatra, Secondary metabolites, Antibacterial activity, Anticancer activity, GC-MS analysis.*

## 1. INTRODUCTION

The rising prevalence of health issues caused by cancer, drug-resistant bacteria, parasitic protozoans, and fungi is a matter of concern [1]. Cancer has emerged as a significant health concern due to its substantial impact on morbidity and mortality. Severe side effects related to many anticancer drugs and the emergence of drug-resistant cells pose significant challenges to their use and effectiveness in treatment [2]. Antibiotic resistance is a significant concern due to the widespread use of antibiotics to treat common bacterial infections. The rise of antimicrobial resistance is increasing the harmfulness and potency of infectious microbes [3]. Therefore, it is essential to find effective medications for diseases caused by microbial infections and for the treatment of human illnesses. A thorough exploration is currently in progress to discover advanced and more efficient substances to address these disease issues. Endophytes have emerged as a promising reservoir of potentially valuable medicinal compounds [4].

Endophytic fungi, despite being underutilized, have proven to be a valuable source of unique and potent secondary metabolites. The endophytic fungi found in medicinal plants that have therapeutic value have attracted the interest of research groups worldwide. They are being studied for their potential to produce host-associated or new lead molecules [2]. The range of bioactive endophytic compounds is extensive, encompassing various properties such as anticancer, antioxidant, antibacterial, antifungal, viral, and antidiabetic effects, among others [5,6]. Medicinal plant-associated microbial endophytes have generated around 20,000 organic derivatives. These derivatives encompass a wide range of compounds such as phenol, phenolic acids, indole, isocoumarin, lactones, polysaccharides, amines and amides, phenylpropanoids, chlorinated metabolites, and xanthones [7].

In our continuous search for antimicrobial and anticancer drugs from natural sources, we targeted one endophytic fungus, *Humicola sp.,* harbored in the leaves of the *Adosa* medicinal plant, (Acanthaceae). It is widely used in traditional Ayurveda for a variety of purposes. *A. vasica* is widely recognised for its efficacy in the treatment of cancer. The leaf of *A. vasica* exhibited a stimulating effect on the cancer cells. The leaf of *A. vasica* stimulates cancer cells and possesses several antispasmodic and expectorant effects, providing a valuable therapy for asthma, bronchitis, and other respiratory conditions [8].

The *Humicola* species exhibits substantial biotechnological and industrial potential. The Humicola genus, which is a member of the Chaetomiaceae family, is known for its abundant production of diverse and structurally complex metabolites that possess a wide array of biological activities. Moreover, *Humicola* species have attracted considerable attention because of their remarkable ability to produce thermally stable enzymes that have both biotechnological and economic importance [9].

Various secondary metabolites were extracted from the mycelia of *Humicola fuscoatra* (Traaen) KMM 4629, which is commonly found in

association with the Kuril colonial ascidium. These metabolites include fuscoatrol A, 11-epiterpestacin, and β-nitropropionic acid. The compounds were isolated from the ethyl acetate extract. Fuscoatrol A exhibited antimicrobial properties against *Staphylococcus aureus* and *Bacillus subtilis*, while also showing harmful effects on the developing eggs of the sea urchin *Strongylocentrotus intermedius*. Furthermore, 11-epiterpestacin exhibited the ability to eliminate *S. aureus* and *B. subtilis,* while *S. aureus* was effectively targeted by β-nitropropionic acid [10]. "In addition, *Humicola fuscoatra*, an endophytic fungus found in halophytic plants, produces a wide range of metabolites that have been shown to possess antifungal, antibacterial, and antiproliferative properties against various plant pathogens, including Arthrobotrys conoides Drechsler, *Pyrenophora graminea* S. Ito & Kurib., and *Pyricularia grisea* Cooke ex Sacc., as well as bacteria like *Agrobacterium tumefaciens, Pseudomonas syringae,* and *Xanthomonas oryzae"* [11].

The primary objective of this study was to evaluate the cytotoxic and antibacterial attributes of secondary metabolites obtained through ethyl acetate extraction from *Humicola* fungi isolated from the healthy leaves of *A. vasica*. In this report, we present a comprehensive analysis of their antibacterial efficacy against *K. pneumoniae, E. coli, B. subtilis,* and S. aureus, along with their cytotoxic effects on A549 (lung cancer cell lines) cells.

## 2. MATERIALS AND METHODS

### 2.1 Isolation and Identification of Endophytic Fungi

Samples of *Adhatoda vasica* nees were collected from the medicinal garden at Mercy College, located on the campus in Palakkad, Kerala, India. Plants were identified at the at the Department of Botany, Mercy College, Palakkad, Kerala. The samples went through three washes with tap water and were then surface sterilized using the methods outlined by Zeng et al. [12]. "In short, the leaves were immersed in 70% ethanol for 2 minutes, followed by a 4-minute treatment with 0.1% mercuric chloride. Following that, the samples were washed three times with sterilised distilled water. The samples were cut to 3.0 × 3.0 cm to eliminate any residual surface chemicals. Following that, the specimen was cut into small portions measuring 1.0 × 1.0 cm using a sterile blade. The pieces were subsequently placed in Sabouraud dextrose agar (SDA) medium, which had been supplemented with chloramphenicol (50 µg/mL). The samples were kept at a temperature of 25 °C ± 2°C for two weeks, and the plates were observed daily. The mature mycelia were delicately transferred to fresh SDA plates to obtain a more purified form of fungal isolates" [13]. Fungi were identified in their sporulation state through the use of lactophenol blue staining. The fungal isolates were identified by analyzing their colony colour, morphology, hyphal structure, spore size, and spore-bearing structures. These characteristics were then compared to the standard manuals of endophytic fungi [14].

### 2.2 Extraction of Secondary Metabolites from Isolated Endophytic Fungus

"The fungi, *H. fuscoatra* was grown in potato dextrose broth (PDB) and later transferred to a 500-mL Erlenmeyer conical flask containing broth. The incubation period for the flask was 14 days at a temperature of 28 °C ± 2 °C. After incubation, we filtered the mycelium using Whatman No. 1 filter paper. The mycelia were mixed with an ethyl acetate solvent for maceration. After a week, the mixture underwent filtration using Whatman No. 1 filter paper, and this procedure was repeated twice. The concentrated extracts were obtained by using a rotary evaporator under reduced pressure. The crude extracts were dissolved in dimethyl sulfoxide (DMSO) and studied for their potential anticancer and antibacterial effects" [4].

### 2.3 Determination of Antibacterial Activity of Fungal Crude Extracts

"The antibacterial activity of secondary metabolites extracted from *H. fuscoatra* was tested against Human bacterial pathogens, including *K. pneumoniae* (ATCC 13883), *E.coli* ( ATCC 25922), *B. Subtilis* (ATCC 6633), and *S. aureus* (ATCC 25923) using the agar well diffusion method. Microbes were distributed on Muller-Hinton agar (MHA) plates. Then wells were bore on the agar plates and three concentration of ethyl acetate extracted from *H. fuscoatra* were poured in separate wells 25, 50, 75 and 100 µg/ml. Antibacterial activities were observed following an incubation period of 24–48 hours at 37 degrees Celsius. The presence of a zone of clearance on the plates indicated the strain's bioactive nature. Streptomycin was used as a positive control, while DMSO served as the negative control. Each antibacterial activity test

was conducted with three replicates with modification" [4].

## 2.4 Anticancer Activity of Ethyl Acetate Extracts of the fungus *H. fuscoatra* as a Potential Tool against Human Lung Cancer A549 Cell Line

### 2.4.1 Cell culture

The A549 human lung cancer cell line was obtained from the National Centre for Cell Sciences (NCCS) in Pune, India. The cancer cells were cultured in a medium containing various components, such as L-glutamine, sodium pyruvate, glucose, and HEPES. Foetal bovine serum was also added to support cell growth. The penicillin and streptomycin were adjusted to a volume of 1 ml. The cells were kept at a temperature of 37°C in a $CO_2$-rich environment with 5% $CO_2$ and humidity.

### 2.4.2 MTT assay

To assess the cytotoxicity of secondary metabolites extracted from *H. fuscoatra*, the following experiments were conducted: The A549 cell monolayers were cultured in 96-well tissue culture plates. The cells were treated with varying concentrations of ethyl acetate extracts obtained from the fungus *H. fuscoatra*. Next, we investigated cell viability by assessing the cells' capacity to metabolise the tetrazolium salt MTT. This process involves the action of the mitochondrial enzyme succinate dehydrogenase, resulting in the formation of a formazan crystal. Three replicates were performed for each concentration. Regression analysis determined the $IC_{50}$, the concentration at which cell viability is reduced by 50%. A formula was used to calculate the percentage viability based on the OD value.

% of viability= OD value of experimental sample / OD value of experimental control × 100:

### 2.4.3 Morphological study

The human cancer cells grown on coverslips ($1 \times 10^5$ cells/coverslip) were incubated for 24 hours with ethyl acetate extracts from the fungus *H. fuscoatra* at various concentrations (25 µg/ml, 50 µg/ml, 100 µg/ml). Afterward, they were fixed in an ethanol-acetic acid solution (3:1; v/v). The coverslips were carefully placed on glass slides for the morphometric analysis. Micrographs were taken of three monolayers in each experimental group. The morphological changes of the cells were examined using Nikon's bright field inverted light microscopy at 40x magnifications.

### 2.4.4 Fluorescence microscopic analysis of apoptotic cell death

A microliter of the dye mixture, consisting of acridine orange (AO) and ethidium bromide (EtBr) in distilled water, was combined with the cell suspension on clean microscope coverslips. Various concentrations of ethyl acetate extracts from the fungus *H. fuscoatra* treated the cancer cells. After treatment, the cells were washed with phosphate-buffered saline (PBS) at pH 7.2 and then stained with 10µL of AO/EtBr. Following a 2-minute incubation period, the cells were rinsed twice with PBS and observed using a fluorescence microscope (Nikon Eclipse, Inc., Japan) at a magnification of 40x, utilising a 480 nm excitation filter. The experiment involved carefully positioning the cells on a glass coverslip within a six-well plate and exposing them to varying concentrations of secondary metabolites derived from ethyl acetate extracts of fungi for 24 hours. The cells were fixed using a solution of methanol and acetic acid (3:1, v/v), followed by a wash with PBS. The cells were then stained with 10 µL of DAPI for 20 minutes in the dark and observed under fluorescence microscopy (Nikon Eclipse, Inc., Japan).

## 2.5 Detection of Bioactive Compounds by GC-MS Analysis

The analysis of ethyl acetate extracts of *H. fuscoatra* was performed using an Agilent 6890 gas chromatography instrument (Agilent, USA) fitted with a fused silica capillary column PAS-5MS (30 m×0.32 mm×0.25 µm film thickness). The carrier gas used was helium, flowing at a rate of 1 ml/min in pulsed splitless mode. The solvent delay took place precisely at the 3-minute mark, while the injection volume amounted to 1 µl. The mass spectrometric detector was used in electron impact ionization mode, with an ionisation voltage of 70 electron volts (eV), encompassing the mass-to-charge ratio (m/z) range from 50 to 500. The electromagnetic voltage was adjusted to 1650 V. The ionisation took place at a temperature of 230°C. The routine started at an initial temperature of 60°C for 2 minutes and thereafter rose to 280 °C at a pace of 5 °C per minute. The injector and detector were maintained at constant temperatures of 250 and 280°C, respectively. A comparison of their mass spectral patterns with those stored in the WILEY/NIST mass spectral

database enabled the identification of the chemicals.

### 2.6 Data Analysis

The analyses were conducted using version 16.0 of the SPSS software package. The data on cell apoptosis and growth inhibition were analysed using probit analysis to calculate the $IC_{50}$ values. The cell viability data was converted into arcsine H proportion values and then analysed using One way ANOVA.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation of Endophytic Fungi

The endophytic fungus isolates were collected from the leaves of *Adhatoda vasica* in the presence of 13 isolates. Endophytic fungi commonly inhabit different parts of plants [15]. Endophytic fungi can enter the plant through various pathways, such as stomata, lenticels, natural wounds, roots, or sprouting radicles. The fungus infects the plant at the site of entry and subsequently spreads to all parts of the plant via the xylem. According to Khiralla et al. [16], there is a significant presence of endophytic fungus in both temperate and tropical rain forests. More than 300,000 plant species have been discovered to host endophytic fungi. The point of this study is to look into antibacterial and anticancer secondary metabolites are that are extracted from *Humicola* fungal isolates using ethyl acetate. The emphasis is placed on the effect that their presence has on bacterial pathogens and human lung cancer cells.

### 3.2 Morphological Identification of Endophytic Fungi

Thirteen strains of endophytic fungi extracted from *Adhatoda vasica* leaves were classified taxonomically as members of the genus *Humicola* using morphological identification methods. The fungal culture grown on SDA media has a green colour, characterized by filamentous mycelia with undulating edges. After a week, the culture undergoes a change in appearance and turns white (Fig. 1a). Coloured crystals were observed in the slide cultures obtained from this fungus, along with septate hyphae (Fig. 1b). *Humicola* is a highly adaptable fungal species that can be found in various environments, including wood, soil litter, grass, forest soil, seawater, and compost. The shape of the outcomes leads to the production of numerous small conidia, allowing them to spread over long distances and increase their population [17]. The genus *Humicola* displays unique traits, such as a colony with a dark green hue, spherical to semi-spherical conidia, and elongated, unbranched conidiophores with thick walls [18].

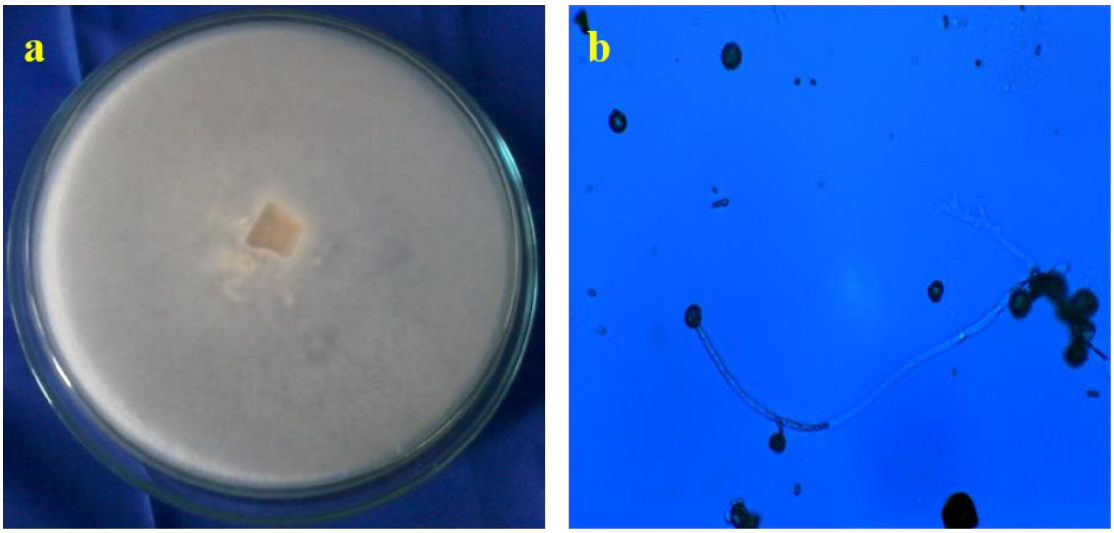


**Fig. 1. Morphology of endophytic fungi from *Adhatoda vasica* leaves Humicola (a and b)**

### 3.3 Production and Extraction of Secondary Metabolites

The isolates have been used for the production and extraction of secondary metabolites. The isolates were obtained using ethyl acetate as the extraction solvent.

### 3.4 Antimicrobial Assay

Ethyl acetate extracts of *H. fuscoatra* were tested against *K. pneumoniae, E. coli, B. subtilis,* and *S. aureus* on nutritive agar medium at various doses. The screening 25, 50, 75 and 100 µg/ml per well, with higher concentrations resulting in stronger antibacterial effects. The investigation also showed a zone of inhibition against *K. pneumoniae, E. coli, B. subtilis,* and *S. aureus* (Fig. 2). Research found that ethyl acetate extracts from the fungus *H. fuscoatra* at 100 µg ml effectively destroyed all examined bacterium species. The most inhibitory zones were *B. subtilis* (17.20 mm), followed by *K. pneumoniae* (14.13 mm), *E. coli* (12.25 mm) and *S. aureus* (9.85 mm). Ethyl acetate preparations of *H. fuscoatra* were antimicrobial. Many investigations have shown that fungal crude extract has antimicrobial action [19,20]. Extracellular and intracellular metabolites from endophytic *H. fuscoatra* isolates were antifungal and antibacterial against many plant diseases [11].

### 3.5 Anticancer Activity of Ethyl Acetate Extracts of the Fungus *H. fuscoatra* as a Potential Tool Against Human Lung Cancer A549 Cell Line

#### 3.5.1 Cytotoxic assay

The effect of ethyl acetate extracts of the fungus *H. fuscoatra* on the Human cancer cells by using the MTT assays.

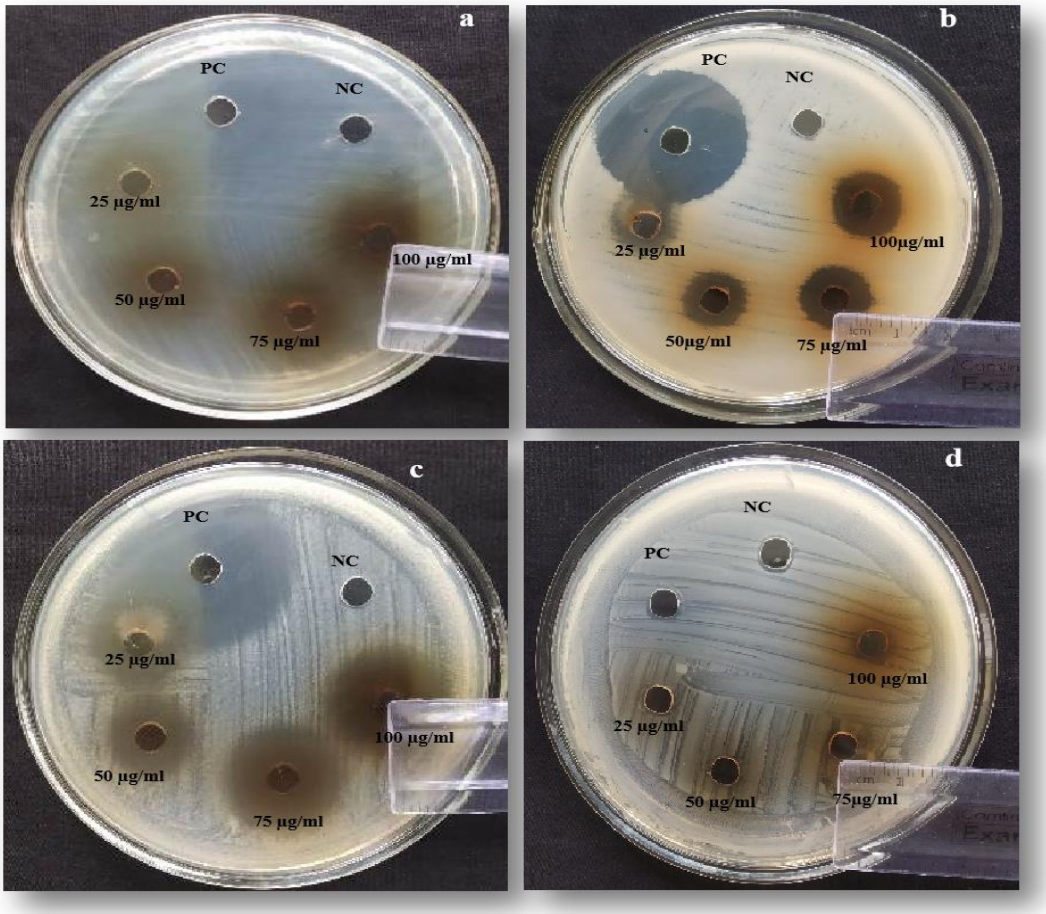


**Fig. 2. Antibacterial activity of ethyl acetate extracts of *H. fuscoatra* against the tested microbial species . *pneumoniae,* (b) *E. coli,* (c) *B. subtilis,* and (d) *S. aureus***

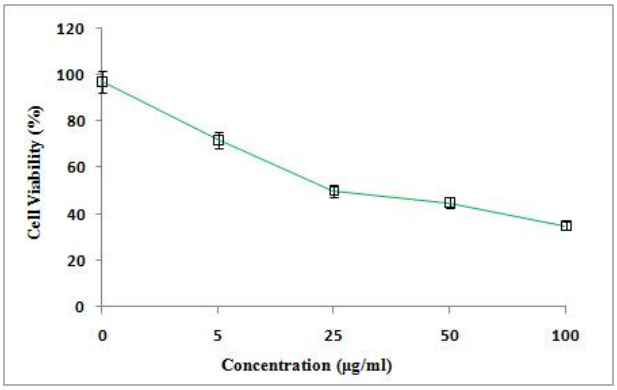


**Fig. 3. MTT analysis of ethyl acetate extracts of the fungus *H. fuscoatra***

**Table 1. Cytotoxic activity of Complexes (µg/ml)**

| Extract | A549 ($IC_{50}$) |
|---|---|
| Ethyl acetate extracts of fungi | 26± 1.2 |

*$IC_{50}$ – Values of respective Compounds (at 24 hrs)*

The cytotoxicity effects of the ethyl acetate extracts from the fungus *H. fuscoatra* were evaluated on A549 human lung cancer cells using the MTT bioassay for 24 hours (Fig. 3). "The resulting $IC_{50}$ value is presented in Table 1. Fig. 3 shows that the ethyl acetate extracts of the fungus *H. fuscoatra* inhibited the growth of A549 human lung cancer at specific doses. The $IC_{50}$ values for the ethyl acetate extracts of the fungus *H. fuscoatra* against A549 human lung cancer cell lines were 26 µg/ml. The ethyl acetate extracts of the fungus *H. fuscoatra* exhibited significant inhibitory activity against A549 human lung cancer cell lines. The compounds found in the fungal extracts can be attributed to the observed cytotoxicity in this study. It has been widely acknowledged by researchers that extracts from endophytes are highly effective in producing potent cytotoxic metabolites" [21].

### 3.5.2 Morphological analysis

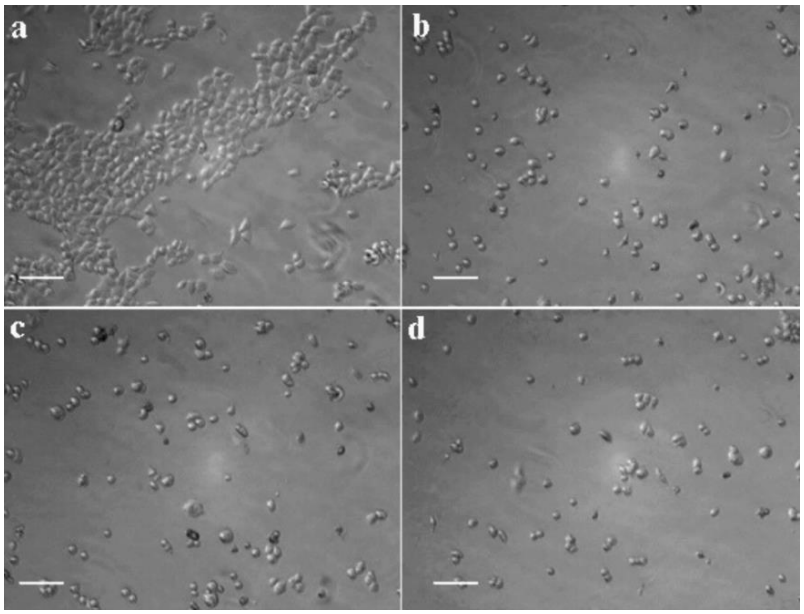


**Fig. 4. Morphological analysis of ethyl acetate extracts of the fungus *H. fuscoatra*-treated A549 cells for 24 hrs (a) Control (b) 25 µg/ml (c) 50 µg/ml (d) 100 µg/ml**

Fig. 4 demonstrates the morphological change of A549 cells observed after treatment for 24 hrs with the $IC_{50}$ concentration of the ethyl acetate extracts of the fungus *H. fuscoatra*. "The change in the morphology of treated cells can be seen in a dose-dependent way. Cytotoxicity was increased with increased ethyl acetate extracts of the fungus *H. fuscoatra*. This extract incited cell shrinkage, adjusted the cell, and reduced the number of feasible cells. These progressions demonstrate that ethyl acetate extracts of the fungus *H. fuscoatra* actuated apoptosis in A549 cells (Figs. 4b, c, and d). On the other hand, untreated control cells did not show any adverse effects (Fig. 4a). Chemotherapeutic drugs kill tumour cells by activating a cascade of events resulting in apoptosis" [22].

### 3.5.3 Fluorescence microscopic analysis of nuclear fragmentation - AO/EtBr Staining

Fluorescence microscopy was used to analyze the impact of ethyl acetate extracts from the fungus *H. fuscoatra* on cancer cells, with a specific focus on their apoptogenic activity. There was no aggregation of the ethyl acetate extracts from the fungus *H. fuscoatra* observed during the assay, as the majority of the extracts dissolved at these low concentrations. Following the application of $IC_{50}$ concentrations of ethyl acetate extracts from the fungus *H. fuscoatra*, the apoptosis induction in A549 cells was evaluated using fluorescence microscopy with acridine orange/ethidium bromide (AO/EtBr) staining. The results indicated that the live cells exhibited a green fluorescence, while the dead cells displayed an acridine orange fluorescence. Fig. 5 (a) shows that the untreated control cells had a significant number of live cells. Meanwhile, the A549 cells treated with ethyl acetate extracts of the fungus *H. fuscoatra* showed a higher number of apoptotic cells and apoptotic bodies. These cells displayed characteristics such as nuclear shrinkage, nuclear damage, and blebbing, which were observed as orange-coloured bodies in Fig. 5(b, c & d). It has been recently reported that in vitro studies have shown that endophytic fungi isolated from *Ziziphus mauritiana* exhibit a promising cytotoxicity effect against the HeLa cell line. The bioactive compounds of 3-beta-hydroxyurs-12-en-28-oic acid have been found to be effective in treating cervical cancer [23].

### 3.5.4 Fluorescence microscopic analysis of nuclear fragmentation - DAPI Staining

In addition, we assessed the ethyl acetate extracts of the fungus *H. fuscoatra* using the DAPI staining method. Fig. 6 displays the fluorescence microscopic description of the cells stained with DAPI after 24 hours, both with and without the ethyl acetate extracts of the fungus *H. fuscoatra*. Fig. 6 (a) indicates that there were no significant changes observed in the cells. However, when the cells were treated with ethyl acetate extracts of the fungus *H. fuscoatra* (Fig. 4 b, c, and d), there was a noticeable increase in brightness, suggesting the presence of condensed chromatins and nuclear fragmentations in the A549 cells. The fluorescence microscopic analysis findings suggest that the ethyl acetate extracts of the fungus *H. fuscoatra* have the potential to be a valuable therapeutic option for cancer treatment.

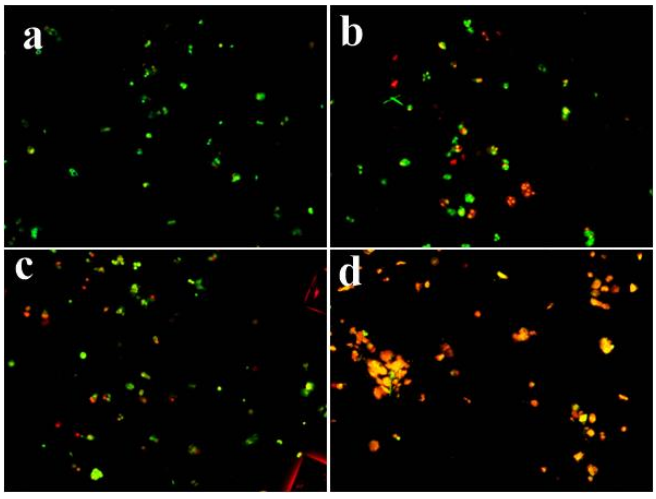


**Fig. 5. AO/EtBr staining assay of ethyl acetate extracts of the fungus *H. fuscoatra*- treated A549 cells (a) Control (b) 25 µg/ml (c) 50 µg/ml (d) 100 µg/ml**

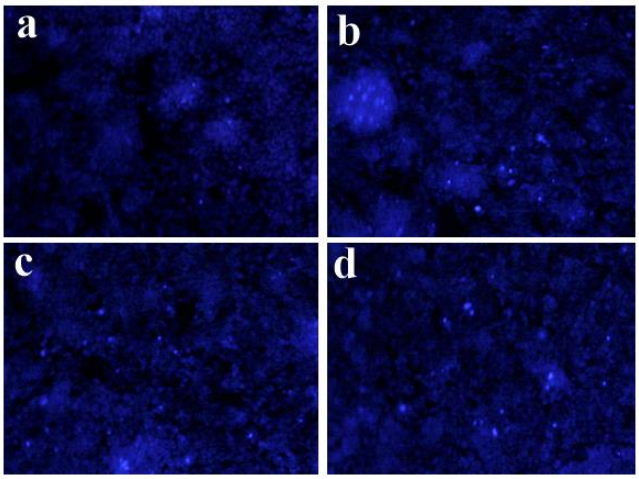


**Fig. 6. DAPI staining assay of ethyl acetate extracts of the fungus *H. fuscoatra*- treated A549 cells (a) Control (b) 25 µg/ml (c) 50 µg/ml (d) 100 µg/ml**

The current investigation demonstrates that the ethyl acetate extracts of the fungus *H. fuscoatra* exhibit noteworthy cytotoxic effects on the A549 cancer cell line. Similarly, findings were observed in the extract of *Penicillium sclerotiorum*, an endophytic fungus, which was tested for in vitro cytotoxicity against five different cancer cell lines. HeLa showed the highest sensitivity among the tested cell lines, with an $IC_{50}$ of 7.75 µg/ml, followed by A549 with an $IC_{50}$ of 10 µg/ml. A431 and U251 showed moderate sensitivity, with $IC_{50}$ values of 20 and 32 µg/ml, respectively. On the other hand, MCF7 cells did not respond to the treatment [24]. Chandra [25] states that numerous endophytic fungi have the potential to be a valuable source of anticancer drugs. Various secondary metabolites derived from endophytic fungi exhibit promising anticancer properties [25-27]. These studies provide evidence that the bioactive compounds produced by endophytes have the potential to be alternative approaches in the search for new anticancer drugs [27,28]. According to recent reports, extracts from an endophytic fungus called Alternaria have demonstrated significant effectiveness in combating lung (A549) and breast (MCF-7) cancer cells. The results showed that the crude extract demonstrated a strong ability to inhibit the tested cancer cell lines, as confirmed by the MTT technique employed in this study. In a study conducted by Al-Keridis et al .[29], it was found that the crude extract demonstrated $IC_{50}$% values of 46.69 mg/mL for MCF-7 and 23.71 mg/mL for A549.

In a previous study, Figueiredo et al. [30] discovered cytotoxic activities in *Stenotrophomonas maltophila*. The bacterial isolate showed $IC_{50}$ values of 17.2µg/ml, 26.5µg/ml, and 35.5µg/ml in the EAC, SiHa, and Hep G2 cancer cell lines, respectively. In a study conducted by Ravikumar et al. [31], it was found that when $IC_{50}$ values are below 30µg/ml in cancer cell lines, there is a strong indication of the potential effectiveness of anticancer medications. Avand et al. [32] reported that the bacteriocin produced by *lactococcus lactis* has demonstrated cytotoxic effects on MCF7 breast cancer cell lines.

## 3.6 Detection of Bioactive Compounds by GC-MS Analysis

GC-MS analysis was conducted on the partially purified crude sample, which led to the identification and recording of the retention time, area percentage, and peak height of various chemicals (Table 2). The gas chromatography results of the fungal ethyl acetate extract show that *H. fuscoatra* contains numerous significant bioactive compounds. The chemicals Hexadecanoic Acid, Hexadecane, Heptadecane, Octadecane, Octadecanoic acid, and Indolizine exhibited the highest area percentages (Fig. 7) and possessed antibacterial and cytotoxic properties. The study conducted by Theantana et al. [33] and Guo et al. [34] revealed that the endophytic fungus derived from five Thai medicinal plants, commonly utilised in Thai traditional medicines, contained significant amounts of hexadecanoic acid and octadecanoic acid methyl ester. According to a study conducted by Nascimento et al. [35], two substances were identified: methyl esters of (9Z)-

octadecenoic acid and (9Z,12Z)-octadecadienoic acid. The compounds were derived from the fungal endophyte *Fusarium oxysporum*. The compounds showed promising cytotoxicity against various cancer cell types. A beneficial microorganism was isolated from the medicinal plant *Smallanthus sonchifolius* (Poepp.).

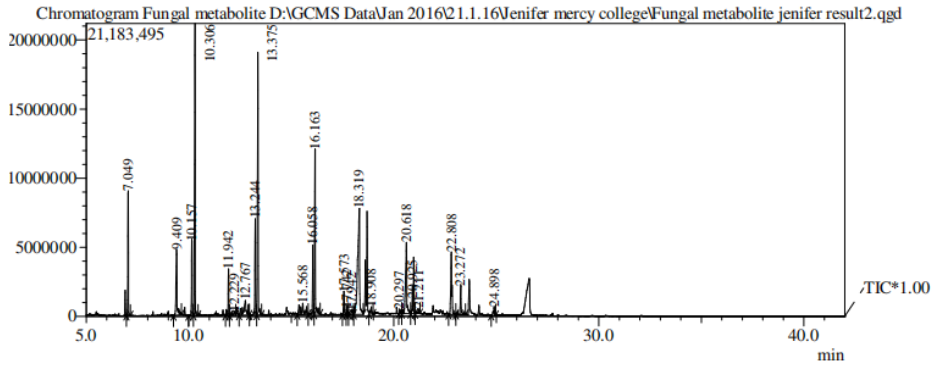


**Fig. 7. GC-MS chromatogram of ethyl acetate extracts of the fungus *H. fuscoatra***

**Table 2. GC–MS analysis of ethyl acetate extract of the endophytic fungi *H. fuscoatra***

| R. Time | Area | Area% | Height | Height% | Name | Base m/z |
|---|---|---|---|---|---|---|
| 7.049 | 6962260 | 5.05 | 7284196 | 6.88 | DODECANE | 57.10 |
| 9.409 | 6407156 | 4.88 | 4564705 | 4.31 | 4-NITROBENZALDEHYDE | 51.05 |
| 10.157 | 11341065 | 3.38 | 5424243 | 5.12 | 1-PENTADECENE | 41.05 |
| 10.306 | 6148964 | 13.74 | 19961991 | 18.85 | HEXADECANE | 57.10 |
| 11.942 | 7495631 | 2.23 | 3070905 | 2.90 | PHENOL, 2,4-BIS(1,1-DIMETHYLETHYL)- | 191.15 |
| 12.229 | 1819255 | 0.54 | 276056 | 0.26 | Benzoic acid, 4-ethoxy-, ethyl ester | 121.10 |
| 12.767 | 9617273 | 2.86 | 971984 | 0.92 | DODECANOIC ACID | 60.05 |
| 13.244 | 14311001 | 4.26 | 6146854 | 5.80 | Cetene | 41.05 |
| 13.375 | 43293118 | 12.89 | 17627838 | 16.64 | HEPTADECANE | 57.10 |
| 15.568 | 8233090 | 2.45 | 716210 | 0.68 | TETRADECANOIC ACID | 43.10 |
| 16.058 | 0761235 | 3.20 | 4983902 | 4.71 | 1-Nonadecene | 43.05 |
| 16.163 | 7991322 | 8.33 | 10406207 | 9.82 | OCTADECANE | 57.10 |
| 17.573 | 4339990 | 1.29 | 1659587 | 1.57 | 7,9-DITERT-BUTYL-1-OXASPIRO[4.5]DECA-6,9-DIENE-2,8-DIONE | 57.10 |
| 17.742 | 1463293 | 0.44 | 559547 | 0.53 | Hexadecanoic acid, methyl ester | 74.05 |
| 17.942 | 1973765 | 0.59 | 250767 | 0.24 | Nonadecane, 9-methyl- | 43.10 |
| 18.319 | 0637790 | 15.07 | 7644236 | 7.22 | HEXADECANOIC ACID | 43.05 |
| 18.908 | 2026115 | 0.60 | 488166 | 0.46 | OCTYLCYCLODECANE | 55.05 |
| 20.297 | 1131583 | 0.34 | 269809 | 0.25 | NONADECANE, 9-METHYL- | 57.10 |
| 20.618 | 1593325 | 6.43 | 4655426 | 4.40 | Octadecanoic acid | 43.05 |
| 20.925 | 11630896 | 3.46 | 1552896 | 1.47 | 1-Docosene | 43.05 |
| 21.211 | 1180790 | 0.35 | 309155 | 0.29 | (1-PROPYLNONYL)CYCLOHEXANE # | 83.15 |
| 22.808 | 6406264 | 4.88 | 4417006 | 4.17 | INDOLIZINE | 70.05 |
| 23.272 | 6967864 | 2.07 | 2107340 | 1.99 | ACETAMIDE, 2,2-DICHLORO-N-[2-HYDROXY-1-(HYDROXYMETHYL)-2-(4-NITROPHENYL)ETHYL]-, [R | 117.05 |
| 24.898 | 2194517 | 0.65 | 570256 | 0.54 | Hexacosane | 57.10 |
| | 5927562 | 100.00 | 105919282 | 100.00 | | |

## 4. CONCLUSION

The present study evaluated the antibacterial and anti-cancer properties of an endophytic fungus derived from *Adhatoda vasica*. The fungal species in this study were identified through an analysis of their culture morphology. The ethyl acetate extracts from the fungal isolates showed strong antibacterial activity against common human bacterial pathogens such as *Klebsiella pneumoniae, Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus*. A study was conducted to evaluate the cytotoxic effects of an endophytic fungus on the A549 cell line, which led to an enhancement in the metabolic activity of the cells. The extracts from the endophytic fungi also showed a significant amount of various phytochemicals. The presence of bioactive compounds was identified through the utilization of GC-MS, which revealed the presence of multiple antibacterial compounds. The study highlighted the practical application of ethyl acetate extracts obtained from the fungus. *H. fuscoatra* provides alternative methods for the discovery of natural product drugs that are reliable, affordable, and environmentally friendly.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES


1. Strobel GA. Endophytes as sources of bioactive products. Microbes and infection. 2003:5(6):535-44.
2. Geethanjali D. New Anticancer Secondary Metabolites from an Endophytic Fungus Chaetomium Nigricolor Isolated from *Catharanthus Roseus*: Structural Elucidation and Molecular Mechanism Underlying Anticancer Effects (Doctoral dissertation).
3. Eshboev F, Karakozova M, Abdurakhmanov J, Bobakulov K, Dolimov K, Abdurashidov A, Baymirzaev A, Makhnyov A, Terenteva E, Sasmakov S, Piyakina G. Antimicrobial and Cytotoxic Activities of the Secondary Metabolites of Endophytic Fungi Isolated from the Medicinal Plant *Hyssopus officinalis*. Antibiotics. 2023;12(7):1201.
4. Sharma D, Pramanik A, Agrawal PK. Evaluation of bioactive secondary metabolites from endophytic fungus *Pestalotiopsis neglecta* BAB-5510 isolated from leaves of *Cupressus torulosa* D. Don. 3 Biotech. 2016;6(2):210.
5. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: a review. Applied Biochemistry and Microbiology. 2008;44:136-42.
6. Agrawal S, Samanta S, Deshmukh SK. The antidiabetic potential of endophytic fungi: Future prospects as therapeutic agents. Biotechnology and Applied Biochemistry. 2022;69(3):1159-65.
7. Kumar A, Banjara RA, Aneshwari RK, Chandrawanshi NK. Microbial Endophytes of Medicinal Plants as an Emerging Bioresource for Novel Therapeutic Compounds. InMicrobial Products 2022;(pp. 235-248). CRC Press.
8. Nikhitha JN, Swathy KS, Chandran RP. In vitro anticancer activity of ethanol extract of *Adhatoda vasica* Nees on human ovarian cancer cell lines. Journal of Genetic Engineering and Biotechnology. 2021;19:1-9.
9. Ibrahim SR, Mohamed SG, Altyar AE, Mohamed GA. Natural products of the fungal genus Humicola: Diversity, biological activity, and industrial importance. Current Microbiology. 2021;78(7):2488-509.
10. Joshi BK, Gloer JB, Wicklow DT. Bioactive natural products from a sclerotium-colonizing isolate of *Humicola fuscoatra.* Journal of Natural Products. 2002;65(11):1734-7.
11. Hosseyni Moghaddam MS, Safaie N, Soltani J, Pasdaran A. Endophytic association of bioactive and halotolerant *Humicola fuscoatra* with halophytic plants, and its capability of producing anthraquinone and anthranol derivatives. Antonie Van Leeuwenhoek. 2020;113:279-91.
12. Zeng ZQ, Yu HB, Xu HR, Xie YQ, Gao J. Fast training support vector machines using parallel sequential minimal optimization. In2 008 3rd international conference on intelligent system and knowledge engineering. IEEE. 2008;1:997-1001.
13. Li B, Shan CL, Zhou Q, Fang Y, Wang YL, Xu F, Han LR, Ibrahim M, Guo LB, Xie GL, Sun GC. Synthesis, characterization, and antibacterial activity of cross-linked chitosan-glutaraldehyde. Marine Drugs. 2013;11(5):1534-52.
14. Kanjana M, Kanimozhi G, Udayakumar R, Panneerselvam A. GC-MS analysis of

bioactive compounds of endophytic fungi *Chaetomium globosum, Cladosporium tenuissimum* and *Penicillium janthinellum*. J. Biomed. Pharm. Sci. 2019;123.

15. Fouda AH, Hassan SE, Eid AM, Ewais EE. Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). Annals of Agricultural Sciences. 2015;60(1):95-104.
16. Khiralla A, Spina R, Yagi S, Mohamed I, Laurain-Mattar D. Endophytic fungi: occurrence, classification, function and natural products. Endophytic fungi: diversity, characterization and biocontrol. 2016;1-9.
17. Gandjar I, Samson RA, van den Tweel-Vermeulen K, Oetari A, Santoso I. Introduction of general tropical fungi. University of Indonesia, Depok. [Indonesian]; 1999.
18. Elfiati D, Syafitri M, Hidayat A. Exploring endophytic fungi from *Rhizophora spp*. leaves for improving plant growth. InIOP Conference Series: Earth and Environmental Science. 2022;977(1):012018. IOP Publishing.
19. Garcia A, Rhoden SA, Bernardi-Wenzel J, Orlandelli RC, Azevedo JL, Pamphile JA. Antimicrobial activity of crude extracts of endophytic fungi isolated from medicinal plant. Journal of Applied Pharmaceutical Science. 2012;2(10):035-40.
20. Idris AM, Al-tahir I, Idris E. Antibacterial activity of endophytic fungi extracts from the medicinal plant *Kigelia africana*. Egyptian Academic Journal of Biological Sciences, G. Microbiology. 2013;5(1):1-9.
21. Wang FW, Jiao RH, Cheng AB, Tan SH, Song YC. Antimicrobial potentials of endophytic fungi residing in *Quercus variabilis* and brefeldin A obtained from Cladosporium sp. World Journal of Microbiology and Biotechnology. 2007;23:79-83.
22. Shalom J, Cock IE. *Terminalia ferdinandiana* Exell. fruit and leaf extracts inhibit proliferation and induce apoptosis in selected human cancer cell lines. Nutrition and Cancer. 2018:70(4):579-93.
23. Sheeba H, Ali MS, Anuradha V. *In-vitro* anti-cancer activity of endophytic fungi isolated from *Ziziphus mauritiana* in cervical cancer cell line. European Journal of Medicinal Plants. 2020;31:38-48.
24. Kuriakose GC, Arathi BP, Rs HK, Th AK, Ananthaswamy K, Jayabhaskaran C. Extract of Penicillium sclerotiorum an endophytic fungus isolated from *Cassia fistula L.* induces cell cycle arrest leading to apoptosis through mitochondrial membrane depolarization in human cervical cancer cells. Biomedicine & Pharmacotherapy. 2018;105: 1062-71.
25. Chandra S. Endophytic fungi: Novel sources of anticancer lead molecules. Applied microbiology and biotechnology. 2012 Jul;95:47-59.
26. Tan RX, Zou WX. Endophytes: a rich source of functional metabolites. Natural product reports. 2001;18(4):448-59.
27. Guo B, Wang Y, Sun X, Tang K. Bioactive natural products from endophytes: A review. Applied Biochemistry and Microbiology. 2008;44:136-42.
28. Selim KA, El-Beih AA, AbdEl-Rahman T, El-Diwany AI. Biology of endophytic fungi. Current Research in Environmental & Applied Mycology. 2012;2(1): 31-82.
29. Al-Keridis LA, Al-Mekhlafi FA, Al-Shuraym LA, Wadaan MA, Al-Khalifa MS. GC-MS profile of extracts of an endophytic fungus Alternaria and evaluation of its anticancer and antibacterial potentialities. Open Chemistry. 2023;21(1):20230145.
30. Figueirêdo PM, Furumura MT, Santos AM, Sousa AC, Kota DJ, Levy CE, Yano T. Cytotoxic activity of clinical *Stenotrophomonas maltophilia.* Letters in applied microbiology. 2006;43(4): 443-9.
31. Ravikumar S, Fredimoses M, Gnanadesigan M. Anticancer property of sediment actinomycetes against MCF–7 and MDA–MB–231 cell lines. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(2):92-6.
32. Avand A, Akbari V, Shafizadegan S. *In vitro* cytotoxic activity of a *Lactococcus lactis* antimicrobial peptide against breast cancer cells. Iranian Journal of Biotechnology. 2018;16(3).
33. Theantana T, Kanjanapothi D, Lumyong S. In vitro inhibition of lipid peroxidation and the antioxidant system of endophytic fungi from Thai medicinal plants. Chiang Mai J Sci. 2012;39(3):429-44.
34. Guo L, Wu JZ, Han T, Cao T, Rahman K, Qin LP. Chemical composition, antifungal and antitumor properties of ether extracts of *Scapania verrucosa Heeg.* and its endophytic fungus *Chaetomium fusiforme*. Molecules. 2008;13(9):2114-25.

35. Nascimento AM, Conti R, Turatti IC, Cavalcanti BC, Costa-Lotufo LV, Pessoa C, Moraes MO, Manfrim V, Toledo JS, Cruz AK, Pupo MT. Bioactive extracts and chemical constituents of two endophytic strains of *Fusarium oxysporum*. Revista Brasileira de Farmacognosia. 2012; 1276-81.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*https://prh.mbimph.com/review-history/3264*