

# Antifungal abilities, and antitumor properties of ether extracts from *Scapania verrucosa heeg*

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## ABSTRACT

An endophytic fungus, *Chaetomium fusiforme*, was isolated from a liverwort known as *Scapania verrucosa*. A comparative analysis of the chemical constituents present in the ether extracts from both *S. verrucosa* and the culture of *C. fusiforme* were conducted using Gas Chromatography-Mass Spectrometry (GC/MS). The yield of ether extract, based on dried plant material, was determined to be 0.6%, and a total of 59 compounds were identified in *S. verrucosa*.

Among the characterized compounds in *S. verrucosa*, (+)-Aromadendrene (9.12%), hexadecanoic acid (6.92%), 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol (5.97%), *s*-tetrachloroethane (5.61%), and acetic acid (5.30%) were found to be

the most abundant components. These compounds collectively accounted for 84.64% of the total extract in *S. verrucosa*. In contrast, the constituents of the cultured endophyte extract were primarily composed of acetic acid (35.05%), valeric acid, 3-methyl-, methyl ester (21.25%), and butane-2,3-diol (12.24%).

Although the chemical composition of the extracts from *S. verrucosa* and its endophyte displayed little correlation, both extracts exhibited antifungal and antitumor activities. Moreover, *C. fusiforme* demonstrated a broader spectrum of activities. on biomaterial technology because these materials effectively support cell culture or cell transplantation with high cell viability or activity.

**Key Words:** *Scapania verrucosa*; *Chaetomium fusiforme*; GC/MS; Antifungal activity; Antitumor activity

## INTRODUCTION

Bryophytes are recognized for their rich repertoire of uncommon and innovative natural substances. Among these compounds, several demonstrate intriguing biological properties such as antimicrobial, cytotoxic, insect-repelling, muscle-relaxing, enzyme-inhibiting, apoptosis-inducing, lipoxygenase, calmodulin, hyaluronidase, cyclooxygenase, thrombin-inhibiting, and neuritic sprouting activities [1]. Liverworts, in particular, contain oil bodies containing a significant quantity of mono-, sesqui-, and diterpenoids, as well as aromatic compounds [1]. Notably, liverworts harbor unique compounds like pinguisane-type sesquiterpenoids, sacculatane-type diterpenoids, and bis(bi-benzyl) aromatic compounds that are not typically found in higher plants [2].

*Scapania verrucosa heeg*, a member of the *Scapaniaceae* family, is a liverwort predominantly found in the south-central regions of China, Nepal, and the Himalayan areas of Jammu and Kashmir. These liverworts, which are typically 10 mm–25 mm tall, thrive in forested areas, on rocks, and decaying wood [3]. Various species from the *Scapaniaceae* family, such as *S. aequiloba*, *S. ampliata*, *S. aspera*, *S. bolandeli*, *S. nemorea*, *S. ornithopodiodes*, *S. paludosa*, *S. parvixata*, *S. stephanii*, *S. subalpina*, *S. uliginosa*, and *S. undulate*, have been subjected

to chemical investigations in the past [3].

An endophyte, as per its definition, resides within the tissues beneath the epidermal cell layers of a plant host without causing apparent harm to it. Recent studies have revealed that fungal endophytes are prevalent across various plant species and engage in mutualistic relationships with their hosts. Some of these endophytes are believed to receive nutrients from the plant while providing the host with specific substances, including secondary metabolites, to defend against fungal, pest, and mammal threats. In line with this concept, endophytic metabolites have been reported to possess inhibitory properties against numerous microorganisms [4].

Endophytes, on one hand, can produce similar or identical biologically active compounds as their host, such as an endophytic fungus generating Taxol [5]. On the other hand, fungi serve as prolific sources of metabolites with substantial biological activities. Many critical anticancer, antifungal, and antibacterial therapeutic agents are either microbial metabolites or derivatives thereof. Investigating the metabolites produced by endophytic fungi increases the likelihood of discovering novel compounds. There is a growing interest in exploring endophytes from unique environments and habitats, driven by the realization that these organisms thrive in

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### CHEMICAL COMPONENTS OF THE PLANT AND THE ENDOPHYTE EXTRACTS COLLAGEN

The constituents present in the plant and endophyte extracts are:

#### Plant Extract:

The plant extract contains a variety of compounds, including mono-, sesqui-, and diterpenoids, as well as aromatic compounds.

Notable compounds found in the plant extract include (+)-Aromadendrene, hexadecanoic acid, 6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol, s-tetrachloroethane, and acetic acid. These compounds collectively make up a significant portion of the extract, representing approximately 84.64% of its composition.

#### Endophyte Extract:

The endophyte extract consists mainly of acetic acid, valeric acid (in the form of 3-methyl-, methyl ester), and butane-2,3-diol.

### ANTIFUNGAL ACTIVITY

Compared to DOX, the host plant exhibited moderate inhibitory effects on all tested tumor cells. However, the fungus isolated from *S. verrucosa* demonstrated significantly stronger cytotoxic activity when compared to the host plant, with most of its IC<sub>50</sub> values falling below 50 µg/mL.

As the effectiveness of many antibiotics has been compromised due to drug resistance and severe adverse reactions, there is an urgent demand for the discovery of new antibiotics. Recent studies have highlighted the isolation of novel compounds with antimicrobial properties from cultured endophytic fungi [5]. Additionally, reports have emerged regarding the isolation of endophytic fungi from Thai medicinal plants that exhibit antimicrobial, anticancer, and antimalarial activities [5]. This study underscores that endophytic fungi obtained from *S. verrucosa* also possess a diverse range of antifungal and antitumor activities. Although these activities were not exceptionally strong, they outperformed those of the host plant. Notably, despite differences in their chemical compositions, we have revealed a microbial source of the valuable phytochemical known as "succedaneum.

### ISOLATION OF STRAINS

The endophytic fungal strain was isolated from *S. verrucosa* using the procedure outlined by Schulz [6]. To achieve this, *S. verrucosa* underwent the following steps:

1. It was washed with distilled water.
2. It was sterilized with 75% ethanol for 1 minute, followed by treatment with 2.5% sodium hypochlorite for 15 minutes.
3. Afterward, it was rinsed three times with sterile water.
4. The sterilized segments had both ends removed, and the remaining portion was incubated at 28±1 °C on PDA medium. This medium was supplemented with ampicillin (200 µg/mL) and streptomycin (200 µg/mL) to prevent bacterial growth until mycelium or colonies developed from the injured surface.
5. The mycelium was purified and cultivated under the same conditions.
6. A separate segment from the same source, without surface

sterilization, was cultured as a negative control to assess the presence of any surface contaminants or microbes.

7. The isolated endophytic fungi were assigned numerical identifiers and individually transferred to new Potato Dextrose Agar (PDA) slants. Subsequently, these cultures were stored at 4 °C after being incubated at 28 ± 1 °C for a duration of 7 days.

### FERMENTATION AND EXTRACTION OF THE ENDOPHYTIC FUNGUS

To prepare the inoculum, the outer edges of 7-day-old Petri dish cultures of the endophyte were transferred into 250 mL flasks containing a broth (100 mL) composed of potato (200 g), dextrose (20 g), and water (1000 mL). These flasks were then subjected to continuous shaking at 280 rpm for 7 days at a temperature of 28 ± 1 °C. Following fermentation, the broth produced by the endophyte was subjected to filtration [7].

The filtrate was then subjected to extraction using diethyl ether, and the resulting extracts were concentrated using a rotary evaporator at 45 °C. To confirm whether any of the isolates obtained in this study were present in the PDB extract in the substrate, an EtOAc extract of the sterile medium treated in the same manner but without the introduction of the microorganism was used for a GC-MS comparison [8]. This comparison revealed that all the isolates were indeed produced by the aforementioned endophytic fungus.

### ESSENTIAL OIL ANALYSIS

Chromatography was conducted utilizing a DB-Wax capillary column with dimensions of 30 meters in length, 0.25 mm in Internal Diameter (ID), and a film thickness of 0.25 µm. The electron impact technique was employed at 70 eV. Helium was employed as the carrier gas, flowing at a rate of 1.0 mL/min, and a 1 µL sample was introduced. The injector and detector temperatures were maintained at 230 °C and 200 °C, respectively [9].

The column oven was programmed as follows: an initial temperature of 60 °C was held for 2.0 minutes, followed by a program rate of 10 °C/min, leading to a final temperature of 250 °C, which was maintained for 10 minutes. The sample was dissolved in CH<sub>2</sub>Cl<sub>2</sub>, and a split injection technique was employed. The compounds were identified based on their Retention Indices (RI), which were determined using n-alkanes (C11-C31), and their retention times. Further confirmation was achieved by comparing their mass spectra with those in the NIST/NBS-Wiley library and literature data. Relative percentage quantities were computed from the Total Ion Chromatogram (TIC) by computer analysis [10].

### IDENTIFICATION OF THE ENDOPHYTE

The endophytic fungal strain was identified by the morphological method. The morphological examination was performed by scrutinizing the fungal culture, the mechanism of spore production, and the characteristics of the spores. All experiments and observations were repeated at least twice.

#### Antifungal activity test

The antifungal properties of the essential oils obtained from both the host and its endophyte were evaluated against *Candida albicans*,

*Cryptococcus neoformans*, *Trichophyton rubrum*, *Aspergillus fumigatus*, and *Pycnicularia oryzae*[10]. For fungal growth, Sabouraud Dextrose Agar was utilized. Dilutions of both *S. verrucosa* and its cultured endophyte extract were prepared in Dimethyl Sulfoxide (DMSO). These extract solutions were then serially diluted (4:1) within 96-well plates. Organisms, with a concentration of approximately  $1.5 \times 10^3$  colony forming units (CFU/ml), were subsequently added to each well.

#### Antitumor activity test

The test cells were cultured in RPMI 1640 medium supplemented with 100 units/mL penicillin, 100 µg/ml streptomycin, and 15% Newborn Bovine Serum (NBS). These cultures were maintained at 37°C in a 5% CO<sub>2</sub> atmosphere. For the experimental phase, exponentially growing cells ( $4 \sim 6 \times 10^4$ ) were employed.

Subsequently, the cells were exposed to samples dissolved in DMSO at a concentration of 1000 µg/mL for a duration of 72 hours at 37°C. Following this incubation period, the sample solution was removed, and the cells were washed with phosphate-buffered saline (pH 7.4). Next, 10 µl of a 0.5% solution of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) in phosphate-buffered saline was added to each well. After an additional 4 hours of incubation, 0.04 M HCl was introduced.

The viability of the cells was assessed by measuring the absorbance at 570 nm. This procedure was conducted three times, and the concentration required for a 50% Inhibition of Cell Viability (IC<sub>50</sub>) was determined graphically.

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