The Metabolic Profiling, Docking Studies and Antimicrobial Activity of the Endophytic Fungi, *Fomitopsis pinicola* (AVK1) isolated from the rhizome of *Zingiber officinalis*.

Krishnaveni Vunnam, Kousar Shaik Mahekal and Audipudi Amrutha V.*
Department of Microbiology, Faculty of Science, Acharya Nagarjuna University, Guntur, Andhra Pradesh, INDIA
*audipudiamrita@gmail.com

Abstract

Ginger, a medicinal herb and a spice and flavouring agent, is used to treat cancer, diabetes and infectious diseases. This study investigates the therapeutic efficiency of endophytic fungi (AVK1) isolated from ginger. Based on 18s rRNA partial gene sequencing and phylogenetic studies, AVK1 was identified as Fomitopsis pinicola and was submitted to GenBank with accession number (PP957928). F. pinicola was found to be an effective antimicrobial drug against various human pathogens, with the maximum zone of inhibition observed against Staphylococcus aureus and Salmonella typhimurium using agar well-diffusion assays. The MIC and IC50 values were 14.0 and 7.14 µg/mL respectively. The bioactive constituents present in ethyl acetate extract of AVK1 include gingerol (18.05%), paradol(14.81%), zingiberene(10.47%), shogaol(7.67%) and sesquiphellandrene (7.59%).

The anticancer properties of phytocompounds were assessed using molecular docking. In silico studies targeted overexpressed cancer proteins, confirming their potential as effective cancer treatments. Docking scores against drug target proteins were validated. The affinities of binding energies against the 1M17 target protein were shagaol (-6.5 kcal/mol) > gingerol (-6.2 kcal/mol) > zingiberene (-6.0 kcal/mol) and paradol (-5.8 kcal/mol). According to in vitro and silico studies, F. pinicola (AVK1) can be used as an efficient therapy against infectious diseases and cancers.

Keywords: Docking, metabolomic profiling, antimicrobial activity, antioxidant activity, anticancer.

Introduction

Ginger (*Zingiber officinale*), a spice and medicinal plant of the *Zingiberaceae* family, is renowned for its traditional use and pharmacological properties including anti-inflammatory, antioxidant and anticancer activities[14]. It has long been used in folk medicine in India and China. In the food industry, the wet and dry roots of ginger are used widely. Ginger stimulates the appetite and can also treat various infections. Endophytic fungi are symbiotic microorganisms that spend all or part of their life cycle living asymptomatically within plant tissues without causing any disease to plants[24]. Researchers have demonstrated that these microbes can produce a diverse array of valuable bioactive chemicals including anticancer drugs like camptothecin and paclitaxel[5,17,26]. However, only a small percentage of plant species' fungal endophytes have been examined,[23] despite their potential for antibiotic and anticancer properties.

A well-known ethnopharmacological tool, red ginger has a variety of medicinal benefits including antifungal[11], antihyperglycemic[8], antihypertensive[18], analgesic[3] and antibacterial[19]. In addition to having anti-biofilm properties, ginger rhizomes have an abundance of terpenoids in their essential oil, which is primarily composed of monoterpenes and also sesquiterpenes[21]. A prior study reveals that ginger extract contains cinnamic acids, ethyl cinnamate and gingerol derivatives[31]. Research on red ginger's endophytic fungus may provide more varieties of bioactive compounds influenced by plant-fungus interactions[2,24].

Despite the extensive characterisation of ginger's phytochemicals, limited attention has been given to its fungal endophytes. These endophytes may share or mimic host biosynthetic pathways, yielding similar or novel metabolites[2]. Prior studies have shown that endophytes can produce important secondary metabolites such as taxol, camptothecin and griseofulvin[17]. Endophytic microbes produce secondary metabolites with various medicinal properties including immunosuppressive, anti-inflammatory, anti-microbial, anticancer, antidiabetic and repellent properties[7,13]. This indicates that there is significant potential for discovering bioactive compounds through the exploration of plant-derived endophytic fungi. We examined fungal endophytes from *Zingiber officinale* in the current work as part of our ongoing effort to understand the antibacterial and cytotoxic compounds from endophytic fungi.

The present study aims to isolate and characterise endophytic fungi from *Z. officinale*, with a focus on the strain AVK1, identified as *Fomitopsis pinicola*. We evaluated its antimicrobial efficacy, enzymatic activity, metabolomic profile (via GC-MS) and anticancer potential using molecular docking studies.

Material and Methods

Collection of the sample: This present *in vitro* experimental study was conducted over 12 months from June 2023 to May 2024 at Acharya Nagarjuna University, Guntur, focusing on

the exploration of anticancer properties of ginger endophytes. In this study, fresh ginger rhizomes (Zingiber officinale) were collected from Mamillapalli village, Ponnuru Mandal, Andhra Pradesh, India, following strict inclusion criteria: healthy, undamaged rhizomes harvested within 24 hours and exclusion criteria: dried, contaminated and damaged rhizomes with pathogens. The selected rhizomes were transported to the Microbiology Laboratory, Acharya Nagarjuna University, Guntur, for isolation of endophytic fungi, *Fomitopsis pinicola* (AVK1) and further exploration of its bioactive constituents for the treatment of cancer.

Isolation and Purification of Endophytic Fungi, *Fomitopsis pinicola* (AVK1): To remove surface impurities, the obtained samples were washed and a surface sterilisation procedure was used to clean the sample surfaces. Following three rounds of sterile water rinsing on a sterile operating table, surface sterilization of ginger rhizomes was performed using sequential washes of 0.1% $HgCl_2$ (5 min), 8% NaOCl (5 min), 20% H_2O_2 (20 min), 20% formaldehyde (2 min) and 75% ethanol (5 min), followed by sterile water rinses[16,26]. The last aliquot is taken as a control. Prepare 0.1% NaCl in test tubes. Fill nine test tubes (10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}) with 9 mL of 0.1% NaCl each. After sterilisation, in a sterile setting, the rhizome (0.2 cm × 0.2 cm) piece is transferred to the mortar and crushed with 1 ml of 0.1% NaCl to create the sample. The 1ml sample is transferred to a 10^{-1} ml test tube, shaken and 1ml from 10^{-1}, 10^{-2}, 10^{-3} to 10^{-9} serially diluted.

Sabouraud's dextrose media is autoclaved at 20-25°C (68-77°F), after adding a pinch of streptomycin (100 µg/mL) to avoid bacterial contamination. After solidification, 0.1 mL of samples from 10^{-1},10^{-2},10^{-3},10^{-4},10^{-5},10^{-6},10^{-7},10^{-8},10^{-9} tubes were transferred and spread on a Petri plate using an L-shaped glass spreader and the fungal colonies were incubated in an incubator for 21 days by checking the plates daily during the incubation period. Once the new fungal strain was isolated, the pure cultures were prepared and maintained throughout the study and the mother culture was preserved under aseptic conditions for later use[12,16].

Morphological and molecular identification of *Fomitopsis pinicola* (AVK1): *F. pinicola* grows aerially by forming white mycelium with low density, resembling the characteristic features of the *Fomitopsidaceae* family. The spores are ellipsoid to cylindrical, smooth, hyaline. The basidia are clavate. The hyphae are hyaline, thin-walled. The strain's taxonomic status was ascertained using the "Fungal Identification Manual 1979".[25] The "Modern Medical Fungal Identification Manual"[30] and other relevant fungal taxonomic publications.

A genomic extraction kit was used to extract the endophytic fungus's genomic DNA and the universal primers ITS1 and ITS4 were used in PCR to amplify the Internal Transcribed Spacers (ITS) of the rRNA gene from fungal genomic DNA. A PCR reaction system includes 10 ng of DNA template, 200 µL of dNTP, 20 pmol of ITS4 and ITS5 primers, 0.25 U of Taq DNA polymerase, 1 × PCR buffer and 22 mmol/L $MgCl_2$. The ITS sequence was manually adjusted after being compared to the NCBI database's BLAST algorithm and correlated with numerous relatable sequences using the Clustal X 8.1 program. The N-J approach was used to create a phylogenetic tree.

Microscopical observation of *F. pinicola* (AVK1): Microscopically, the hyphae are cylindrical, septate and branched[28]. The spores are cylindrical to elliptical. Spores are smooth, hyaline, translucent and non-reactive with iodine. Cystidia are cylindrical to fusiform or spindle-shaped in structure. *Fomitopsis* species often have a dramatic hyphal system, meaning they have two types of hyphae: generative hyphae and skeletal hyphae. They may also have resinous or oily contents in their hyphae.

Screening of antimicrobial activity of endophytic fungal isolate *F. pinicola* (AVK1): The antimicrobial activity of ethyl acetate extracts of AVK1 was tested using agar well diffusion technique against pathogens purchased from NCIM including *Staphylococcus aureus* (NCIM 2079), *Salmonella typhimurium* (NCIM 2501), *Bacillus subtilis* (NCIM 2063), *Clostridium sporogenes* (NCIM 5113), *Pseudomonas aeruginosa* (NCIM 2200), *Aspergillus brasiliensis* (NCIM 1196) and *Candida albicans* (NCIM 3471). Streptomycin (100 µg/mL) serves as a positive control and DMSO serves as a negative control[27].

Antimicrobial assay by the Agar well diffusion method: The antimicrobial efficacy of an ethyl acetate crude extract of all 3 ginger endophytes, namely AVK1, AVK2 and AVK3, isolated from ginger, was tested against five pathogenic bacteria using the agar well diffusion assay[27] performed in triplicate and the findings were reported as mean ± S.D. The plates with antimicrobial activity were incubated for 48 hrs at 37°C. The zone of inhibition on the plate was measured after incubation. The potent Endophytic fungi were chosen for the optimisation of several physicochemical parameters such as incubation length, pH, temperature, carbon sources, nitrogen sources and amino acids. After optimisation for maximising the bioactivity of AVK1 is done, the MIC and IC50 values of ethyl acetate extracts of AVK1 against different human pathogens were determined using agar dilution and regression analysis[22].

Minimum inhibitory concentration (MIC) and IC^{50} values: The assessment of the lowest required concentration of test sample that under specific experimental conditions using agar dilution method, prevents the growth and survival of pathogenic bacteria, thus further affecting their growth visibility. MIC values are used to assess new antimicrobial agents' efficacy as well as to ascertain the drug susceptibilities of bacteria. Applying a predetermined number of cells to the agar plate's surface after varying the antimicrobial substance's concentration in a nutritional agar

medium is known as agar dilution[27]. Growth is evaluated following a specified duration of incubation (16–20 hours) and the MIC value is determined**.** According to Roshmi et al[22], the assessment of MIC value against every tested bacterium was carried out in triplicate.

Mycochemical analysis and Enzymatic assays: The fungal endophyte methanolic extracts were qualitatively evaluated for mycochemicals using the recommended standard mycochemical screening assays and (AVK1) *F. pinicola* was qualitatively screened for the production of different hydrolytic enzymes using various enzymatic plate assays[1]. Qualitatively, each test was represented as either positive (+) or negative (−) and the intensity was represented as +, ++, or +++.

Mycochemical Screening: Assay for qualitative mycochemical screening represents alkaloids, terpenoids, flavanoids, tannins, sterols, glycosides and phenols examined for the production by the endophytic fungal isolates.

Enzymatic assays: Assay for qualitative extracellular enzymes gives amylase, protease, lipase, cellulase, asparaginase, laccase, pectinase, L-glutaminase, gelatinase and catalase. These are some of the hydrolytic enzymes that were examined for production by the endophytic fungal isolates[4].

GC-MS metabolomic profile of AVK1 compounds: The chemical composition of methanol extracts of AVK1 mycelium was analysed using a GC-MSQP2010 (Shimadzu Europa GmbH, Dusseldorf, Germany) instrument with an RTX-5MS column. The injection volume was one microliter and the oven temperature was adjusted to 80°C. Helium was used as the carrier gas and the spectral mass was set to scan between 30 and 600 (m/z). The peaks obtained by the ethyl acetate extract were compared to the NIST database in the United States and compounds were summarised using the percentage relative peak area[10].

Molecular docking studies: The study uses computational techniques like molecular docking to understand target protein interactions. Phytocompounds from ginger endophyte (AVK1) were used as ligands in molecular docking studies[15]. The pharmacological target proteins were chosen based on literature survey of overexpressed proteins in cancer cells. The protein data bank (PDB) was used to retrieve three-dimensional structures of major therapeutic targets such as EGFR, HER2, EML4-ALK, KEAP1 (PDB Id: 1M17, 3PPO, 4Z55, 1X2J), CASP3, CASP8 (PDB Id: 3DEI, 3KJQ), CPPC paired disulphide rich peptide (DRPs) binding protein and- (PDB Id: 7W8O and 7W8K).

The auto ligand was energy-minimised and was used to identify the protein's active site. The 3-D geometries of gingerol, zingiberene, sesquiphellandrene, paradol and shogaol were then created using PyMol® software, which was then depicted using PyMol®. The molecular arrangements of natural inhibitors were constructed using Marvin Sketch Software version 20.11.0 and co-crystallised with the inhibitors. The study focuses on inhibiting target proteins related to different clinical forms of cancer.

Ligand and protein preparation: The study used RCSB ligand explorer software to retrieve target proteins' active sites for docking studies. Geometric optimisation was performed by eliminating ligands, heteroatoms, B and C side chains and water molecules. The structures were cleaned using Discovery Studio v4.5 and phytocompound structures were loaded into AutoDockTools using the "Sybyl mol20 format." The Lamarckian genetic algorithm (LGA) was used to dock therapeutic targets and to identify bioactive phytocompounds.

Molecular Dynamics Study: The Molecular Graphics Laboratory (MGL) was utilised to calculate macromolecule and ligand couplings, transforming PDB files into PDBQT files. Grid maps were created for amino acid residues in receptors and 10 conformers were confirmed using the Lamarckian genetic algorithm. Pharmacokinetic ADME predictions were assessed using Lipinski's rule of five.

The study used root mean square deviation (RMSD) to group molecules and examined docking sites for phytocompounds to bind to therapeutic targets. Molecular dynamics simulations were used to visualise intermolecular interactions, with the lowest energy indicating ease of binding. High binding affinity was found in phytocompounds. The OSIRIS property explorer tool was used to predict mutagenicity, tumorigenicity, irritating effects and reproductive toxicity. The optimal positions were examined using PyMOL software after docking.

Results and Discussion

Isolation of Endophytic Fungi: Microbial bioresources produce bioactive chemicals, which are unprocessed metabolites with significant healthcare applications. Scientists are exploring secondary metabolite compounds with active pharmacological properties in various microorganisms including enzymes, antibiotics, antioxidants, vitamins and anti-diabetic properties. Microorganisms may be a solution to antibiotic-resistant bacteria and diseases. The development of NGS technology and bioinformatics tools is expected to lead to the discovery of new biologics, as the pharmaceutical industry demands efficient, productive and environmentally friendly extraction methods for microbial-derived bioactive compounds. The present study focuses on AVK1, one of the three endophytic fungi, AVK1, AVK2 and AVK3, that were isolated from the ginger rhizome. Using an agar well-diffusion method, the antibacterial activity of each isolated fungal strain was evaluated.

Morphological and Microscopic Observation: Figure 1 depicts that the AVK1 isolate exhibited white, aerial mycelia

with cylindrical, hyaline spores. Figure 2 illustrates that *Fomitopsis pinicola* is a unique fungus with microscopic traits that distinguish it from other types. It grows to 1-2 cm in diameter in five days, with white spores, ellipsoid to cylindrical spores, basidia and thin-walled hyaline hyphae. Under a microscope, these microscopic traits allow for the identification of the fungus from other types.

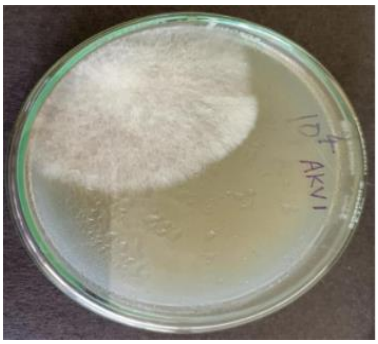


Figure 1: Macroscopic view of AVK1, *Fomitopsis pinicola.*

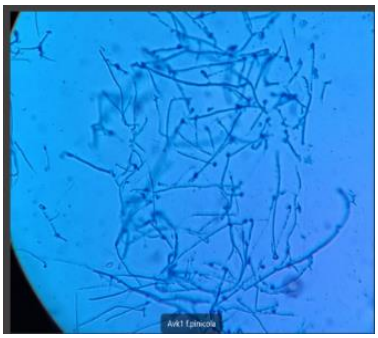


Figure 2: Microscopic view of AVK1, *Fomitopsis pinicola*

Molecular Identification of *Fomitopsis pinicola*: ITS sequencing revealed 99% similarity with *F. pinicola*, confirming its identity[9]. The BLAST tool to evaluate the similarity index was used to analyse the partial gene sequence of a fungus, revealing a 99.00% homology with *Fomitopsis pinicola* (Figure 3). The neighbour-joining approach was used to draw a phylogenetic tree and quantify evolutionary links. Based on microscopic features, colony morphology, sequence analysis and phylogenetic traits, the isolate was confirmed as *Fomitopsis pinicola* and added to GenBank with accession number PP957928 (Figure 3).

Antimicrobial Activity, MIC and IC^{50} values: The ethyl acetate extracts of all 3 fungal endophytes possessing bioactive secondary metabolites were tested for antibacterial activity using the agar well diffusion method as illustrated in figure 4. Results show impressive inhibition zones against all human pathogens, especially *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, measuring 30.00 mm, 30.00 mm and 20.05 mm respectively, with the highest zone of Inhibition among the pathogens.

AVK1 also possesses powerful antifungal activity against *Candida albicans*. As AVK1 shows the highest antimicrobial activity among all three fungal endophytes isolated from ginger, its potentiality of antimicrobial activity is further analysed by evaluating the MIC value of AVK1. The MIC of AVK1 is determined using the agar well diffusion method rather than the broth dilution method to prevent direct exposure to the human pathogens for a long period.

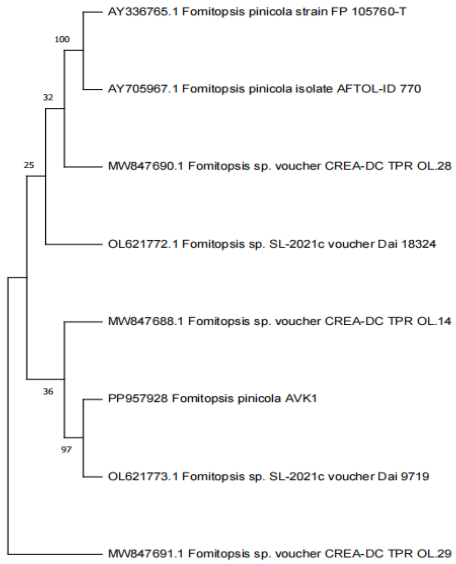


Figure 3: Dendrogram of AVK1, *Fomitopsis pinicola.*

The antimicrobial evaluation shows moderate effectiveness against *S. aureus* and *S. Typhimurium*, with strong MIC values of 14.05 µg/mL, as clearly illustrated in figure 5. The IC50 value of 7.14 µg/mL of ethyl acetate extract of AVK1 is clearly illustrated in figure 6. These findings highlight *Fomitopsis pinicola* (AVK1)'s potential as a bioactive chemical with strong antibacterial properties.

Fomitopsis pinicola, an endophyte with antibacterial properties, produces bioactive compounds like octadecane, benzaldehyde and griseofulvin. These compounds are used in the food industry to prevent oxidative lipid breakdown and preserve food's nutritional content. A study found 28 compounds in the methanol extract of *Fomitopsis pinicola*, with gingerol, paradol, zingiberene, shogaol, sesquiphellandrene, α-curcumin, bisabolene, caprinaldehyde and γ-cadinene as the major compounds. Sesquiterpenes like α-Zingiberene, α-Farnesene and curcumene were also found.

Mycochemical analysis and enzymatic screening: Ginger, a rhizome of *Zingiber officinale*, as a rudimentary medication and spice, has been used globally for its anti-inflammatory, anti-nausea and potentially anti-cancer properties. Its main active ingredients are gingerols and shogaols, with shogaols, produced through thermal processing, having stronger anti-carcinogenic properties. Studies have shown that gingerols and shogaols have greater anti-proliferative effectiveness against human lung and colon cancer cells[14]. Ginger contains compounds like

shogaol, citral, zingiberene and bisabolol, which have various therapeutic properties. To evaluate and analyse the metabolomic profile of *Fomitopsis pinicola* (AVK1) isolated from ginger, different screening procedures were employed.

Figure 4: Antimicrobial Activity of AVK1, *Fomitopsis pinicola.*

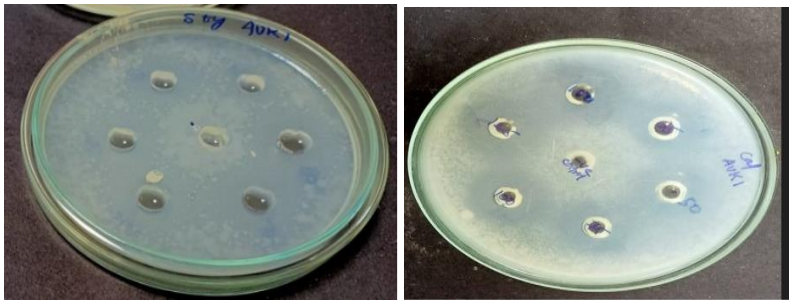
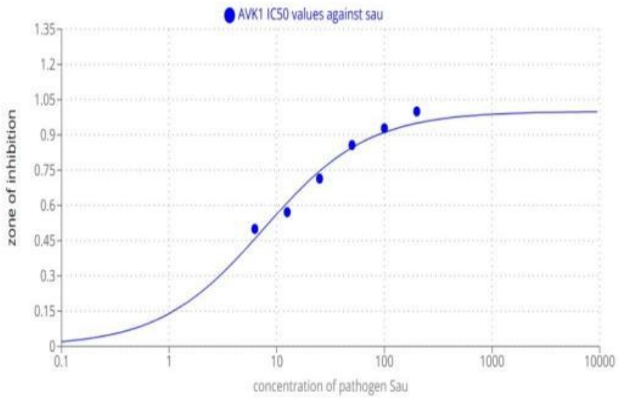


Figure 5: MIC of AVK1, *Fomitopsis pinicola.*



IC_{50} Regression Results [AVK1 IC50 values against sau]

Parameter	Value
IC_{50}	7.5401

IC_{50} Regression Results [AVK1 IC50 values against sau]

Parameter	Value
IC_{50}	7.5401
Equations	(show alternative)
Equation	$Y = 0 + \frac{1-0}{1+(\frac{X}{7.5401})^{-0.9004}}$
Equation Form	$Y = Min + \frac{Max-Min}{1+(\frac{X}{IC50})^{Hill\ coefficient}}$

Figure 6: IC50 value of AVK1, *Fomitopsis pinicola.*

Table 1
GC-MS bioactive compounds of AVK1 methanolic extract

S.N.	Peak#	R.Time	Area	Area%	Name	Common Name	Biological Activity
1	23	34.012	9054556	18.05	Gingerol	Gingerol	Anti-inflammatory, Antioxidant, Analgesic
2	15	19.006	7429173	14.81	4-(3-Hydroxy-2-Methoxyphenyl)-2-Butanone#	Paradol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
3	7	14.9	5250761	10.47	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-(R*,S*)]-	Zingiberene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
4	22	32.403	3846773	7.67	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one	Shogaol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
5	12	15.793	3805516	7.59	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-	Sesquiphellandrene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
6	6	14.446	3135784	6.25	1-(1,5-DIMETHYL-4-HEXENYL)-4-METHYLBENZENE	α-Curcumene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
7	10	15.179	2662840	5.31	α-Farnesene	α-Farnesene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
8	11	15.309	2029425	4.05	CYCLOHEXENE,1-METHYL-4-(5-METHYL-1-METHYLENE-4-HEXENYL)-,(S)-	Bisabolone	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
9	3	6.24	1898802	3.78	Decanal	Caprinaldehyde	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
10	9	15.078	1496905	2.98	Naphthalene,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-,(1.alpha.,4a.beta.,8a.alpha.)-	Y-Cadinene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
11	28	39.057	1038897	2.07	1-(4-Hydroxy-3-methoxyphenyl)tetradec-4-en-3-o	Shagoal	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
12	5	7.753	940312	1.87	Citral	Citral/Geraniel	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
13	1	3.06	582762	1.16	Octanal	Caprinaldehyde	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
14	24	35.488	576208	1.15	1-(4-Hydroxy-3-methoxyphenyl)-3,5-decanediol	Gingerdiol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
15	25	35.845	565374	1.13	1-(4-Hydroxy-3-methoxyphenyl)dodec-4-en-3-one	6-Shagaol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
16	4	7.026	535656	1.07	2,6-Octadienal,3,7-Dimethyl-	Citral/Geraniel	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
17	8	15.005	515888	1.03	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,8a-octahydronaphthalene	α-Selinene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
18	20	25.647	460602	0.92	m-Camphorene	Camphorene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial

19	13	17.008	440528	0.88	4-(1-Hydroxyallyl)-2-methoxyphenol	Hydroxyeugenol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
20	21	31.33	415650	0.83	3-Decanone,1-(4-hydroxy-3-methoxyphenyl)-	Gingerone	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
21	18	20.448	401958	0.8	2,6,10-Dodecatriene-1,12-diol,6-(hydroxymethyl)-10-methyl-2-(4-methyl-3-penten-1-yl)-,1-acetate	Gingerol Acetate	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
22	16	19.449	345827	0.69	2-((4aS,8R,8aR)-4a,8-Dimethyl-3,4,4a,5,6,7,8,8a-octahydronaphthalen-2-yl)propan-2-ol	Eudesm-6-en-11-ol	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial
23	14	18.361	318803	0.64	Cyclohexene,3-(1,5-dimethyl-4-hexenyl)-6-methylene-,[S-(R*,S*)]-	Sesquiphellandrene	Anti-inflammatory, Antioxidant, Analgesic, Anti-Cancer, Anti-Microbial

Table 2 depicts the mycochemical tests on *Fomitopsis pinicola* (AVK1) revealing an abundance of flavonoids, terpenoids, tannins, quinones, coumarins, glycosides, saponins and phenolic compounds in the methanolic extracts. Saponins were detected in trace amounts, while quinones, glycosides, coumarins and alkaloids were absent. Table 2 also shows the results of enzyme screening, confirming the production of hydrolytic enzymes from *Fomitopsis pinicola* (AVK1), assessed using a plate assay for cellulase, amylase and L-glutaminase, indicating potential for biopharmaceutical and food applications. Positive glutaminase activity was observed, suggesting potential anticancer properties, proving AVK1's remarkable enzymatic application in the field of biomedicine and also for industrial use.

GC-MS Results: Genomics has revolutionised microbial metabolite screening, offering an eco-friendly and biodegradable drug development approach. GC-MS analysis of *Fomitopsis pinicola* mycelium extracts revealed 28 bioactive chemicals, many of which are also present in ginger, suggesting metabolic convergence[10] as presented in figure 7. The analysis revealed the peaks associated with various compounds confidently identified utilising the NIST library databases, based on their relative peak areas. Table 1 clearly illustrates the main compounds identified in both extracts as gingerol, alpha-farnesene, curcumene, zingiberene, shogaol, paradol, sesquiphellandrene, bisabolene and cadinene.

The bioactive secondary metabolites produced from the endophytes isolated from the ginger share the metabolomic profile with that of the ginger, thus providing a strong base for further research on the biomedical applications of *F. pinicola*. The study found that raw ginger lacks antibacterial properties, but boiling or heating it converts gingerol to zingerone. Ginger bioactive compounds like zingerone and shogaol contribute to antimicrobial action. α-zingiberene, a key component, has antibacterial properties against certain bacteria. Gingerol, the main ingredient in ginger, has been shown to inhibit cell proliferation and platelet aggregation in pancreatic cancer cell lines. It also prevents the production of COX-2, an enzyme linked to inflammation and cancer development. Ginger contains paradol, a compound with strong anti-inflammatory, antioxidant and possibly anti-cancer effects. Paradol reduces oxidative stress and neuroinflammation in the central nervous system. It interacts with the epidermal growth factor receptor (EGFR) to prevent cancer cell development. Shogaol is known for its anti-inflammatory, antioxidant, anticancer and neuroprotective effects. Citral is a terpene with antimicrobial and anticancer properties.

Curcumin, a turmeric compound, has anti-inflammatory and antioxidant properties. Bisabolene, a plant compound, has antimicrobial, anti-cancer and anti-convulsant properties. Zingiberene, a ginger compound, has anti-inflammatory, antioxidant, anticancer and antidiabetic effects. A study found that 6-shogaol significantly suppressed cancer cell proliferation in various cancer types including lung, colon, ovarian and skin cancer cells, outperforming other ginger-derived compounds in mice[32]. Molecular docking, a virtual technique, simplifies drug discovery and development for cancer and viral infections like COVID-19. The study docked antigenic targets with ginger phytocompounds, revealing high binding energy and potential anticancer effects. ADME Tox predictions help analyse Shagaol's drug and non-potency properties.

The shagaol ligand with EGFR produces conformers with a 2.0 Å cluster in docking experiments, with a reference RMS value of 79.98 and a maximal binding energy of -5.31 kcal/mol. The ligand forms four hydrogen bonds with bond lengths of 2.03Å formed by the ligand at TYR54, GLU166, THR190 and GLN192 at the protein's active site. Studies on bioactive chemicals found in fungal species like *Fomitopsis officinalis* and *F. pinicola* show anticancer and apoptotic effects on various cancer cell lines[29].

F. pinicola methanol extract exhibits strong anti-cancer action, causing cell death and improving survival time in mice with sarcoma-180 tumours. This is the evidence of *F.*

pinicola's anti-tumour action in both laboratory and human clinical trials, potentially offering a novel alternative to existing anti-tumour medications[29]. This strong correlation suggests a shared biosynthetic pathway between the species, highlighting their intricate biochemical relationship.

Table 2
Metabolomic profiling of Fomitopsis pinicola AVK1

	Metabolites	**Result (AVK1)**
ENZYMATIC ASSAYS	Cellulase	(+)
	Protease	(-)
	Lipase	(-)
	Asparaginase	(-)
	Amylase	(+)
	Pectinase	(-)
	L-Glutaminase	(+)
	Gelatinase	(+)
	Catalase	(-)
PHYTOCHEMICAL SCREENING ASSAYS	ALKALOIDS	(++)
	FLAVANOIDS	(+++)
	TANNINS	(+)
	PHENOLS	(+++)
	SAPONINS	(-)
	PHYTOSTEROLS	(+)
	TERPENES	(+++)

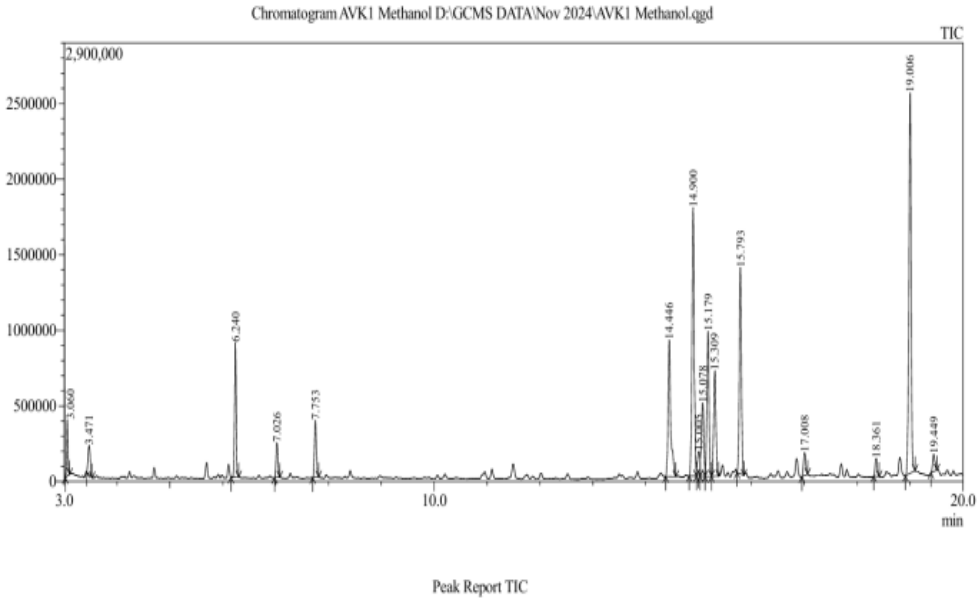


Figure 7: GC-MS Graph of AVK1, *Fomitopsis pinicola.*

Table 3
Binding Affinities of docking metabolites from the GC-MS Report

S.N.	**Bioactive Compound**	**Binding Affinity of Bioactive compound with Target PDB ID (Kcal/mol)**					
		1M17	3PPO	4Z55	1X2J	7W8O	7W8K
1	Gingerol	-6.2	-6.5	-6.0	-6.5	-28.0	-19.0
2	Zingeberene	-6.0	-5.8	-6.1	-6.1	-28.0	-12.0
3	Shogaol	-6.5	-5,9	-5.8	-5.6	-27.0	-54.6
4	Paradol	-5.8	-5.0	-6.0	-4.5	-24.5	-25.0
5	Sesquiphellandrene	-4.5	-4.6	-6.8	-5.0	-28.0	-20.0

Ligand and Protein preparations: The auto ligand program detected amino acids in a protein's active site, revealing potential anticancer drug properties. According to Lepinski's rule of five, shogaol, a valid pharmaceutical molecule, has four active torsion sites suitable for human usage.

Molecular Dynamic Study: The bioactive phytocompound shagaol, a bioactive compound, exhibits good drug-target interaction stability without drug binding displacement from the protein target domain, according to molecular dynamic data. The ligand Zingiberene's greatest interaction with the target protein is through hydrogen bonding, enhancing shagaol's overall binding affinity with the protein.

Molecular docking analyses: Table 3 demonstrates the molecular docking study using Autodock Vina software. The study was conducted on bioactive phytochemicals against overexpressed target proteins in malignant cells. Ten simulations were conducted to determine the optimal ligand-protein interaction. Shagaol, a bioactive phytoconstituent, had a higher docking score (-6.5 Kcal/mol) against the EGFR target protein than Gingerol (-6.2 Kcal/mol), Zingiberene (-6.0 Kcal/mol) and Paradol (-5.8 Kcal/mol). The target protein outperformed a standard drug, taxol (-4.9 Kcal/mol), making it a prominent drug molecule, supporting the *in vitro* results. RMSD and hydrogen bonding analyses confirmed strong ligand-protein interactions. These findings support the anticancer potential of AVK1-derived metabolites.

To protect cells from the harmful effects of reactive oxygen species, or free radicals, which result in oxidative stress and cellular damage, antioxidants are essential. These radicals are metastable substances that seize electrons from nearby molecules. These free radicals can be destroyed by the body's natural antioxidant defences which include glutathione and catalases. However, if these defences are compromised, natural exogenous antioxidants such as vitamin C, beta-carotene, vitamin E, flavones and chemicals originating from plants must be used.

Many substances found in plants, such as flavonoids, terpenoids, phenols and vitamins, have strong antioxidant properties and can scavenge free radicals[6,20], which are essential for preventing the onset of many diseases. All such useful bioactive constituents can also be produced from the endophytes of ethnomedicinal host plants, New biotechnological approaches and genomic manipulation can help in discovering novel and potent drug discovery.

Conclusion

The findings of this study look into the possibilities of employing *Fomitopsis pinicola* AVK1 methanol extracts from an endophytic fungus as potential inhibitors of recognised therapeutic targets in different types of cancer. According to molecular docking studies, gingerol, shogaol and zingiberene have the highest anticipated binding affinity, implying that they may play a role in inhibiting bacterial or microbial growth. The extracts' inhibitory effects are confirmed by antibacterial activity tests.

Based on the molecular docking results, it is possible to conclude that the herbal compound of ginger endophytes *Fomitopsis pinicola* AVK1 contains phytocompounds and has a higher interaction potential, which could be useful building blocks for developing new multi-targeting drugs that target various clinical forms of NSCLC, cervical cancer and all other cancers.

According to laboratory conditions and in bioinformatic-based simulation studies, the substantial antimicrobial, antioxidant, anti-inflammatory, anticancer cytotoxic and antiproliferative activities of AVK1 methanolic extract make it a promising treatment option for cervical cancer, lung cancer and all other cancers. So the fungal endophyte (AVK1) isolated from rhizome ginger which is used as food, serves as an antimicrobial, antioxidant and anticancer compound because of its potential bioactive compounds like gingerol, shogaol, zingiberene, paradol and sesquiphellandrene, which are revealed in GC-MS analysis.

Acknowledgement

The corresponding author is thankful for the financial assistance from the Seed grant (ANU/CIIPRT/PP/Sanction of Finance Assistance/2023) and RUSA –ANU Research Project (RUSA-ANU/ANU/RP-07/SO-2024) of Acharya Nagarjuna University, Guntur, A.P. We are also thankful for providing University Research Fellowship to UCS-ANU to carry this research to the first and second authors.

References

1. Campos R.P.C., Jacob J.K.S., Ramos H.C. and Temanel F.B., Mycopharmacological properties of endophytic fungi isolated from Cuban oregano (Plectranthus amboinicus Lour.) leaves, *Asian J Biol Life Sci*., **8(3)**, 103–110, https://doi.org/10.5530/ajbls.2019.8.17 **(2020)**

2. Caruso G., Abdelhamid M.T., Kalisz A. and Sekara A., Linking endophytic fungi to medicinal plants therapeutic activity: a case study on Asteraceae, *Agriculture*, **10(7)**, 286, https://doi.org/10.3390/agriculture10070286 **(2020)**

3. Fajrin F.A., The antioxidant activity of red ginger oil in aloxan-induced painful diabetic neuropathy in mice model, *Thai J Pharm Sci*., **43(2)**, 69–75 **(2019)**

4. Ganga Mani P. and Audipudi A., Penicillium citrinum AVGE1 an endophyte of Acorus calamus: its role in biocontrol and PGP in chilli seedlings, *Int J Curr Microbiol Appl Sci*., **5(5)**, 657–667, https://doi.org/10.20546/ijcmas.2016.505.066 **(2016)**

5. Gupta Aditi, Meshram Vineet, Gupta Mahiti, Goyal Sonia, Qureshi K.A., Jaremko M. and Shukla Kamalesh Kumar, Fungal endophytes: Microfactories of novel bioactive compounds with therapeutic interventions; a comprehensive review on the biotechnological developments in the field of fungal endophytic biology over the last decade, *Biomolecules*, **13(7)**, 1038–1038, https://doi.org/10.3390/biom13071038 **(2023)**

6. Hertog M.G.L., Feskens E.J.M., Kromhout D., Hollman P.C.H. and Katan M.B., Dietary antioxidant flavonoids and risk of coronary heart disease: the Zutphen Elderly Study, *Lancet*, **342(8878)**, 1007–1011, https://doi.org/10.1016/0140-6736(93)92876-u **(1993)**

7. Jalgaonwala R.E., Mohite B.V. and Mahajan R.T., A review: natural products from plant-associated endophytic fungi, *J Microbiol Biotechnol Res*., **1(2)**, 21-32 **(2011)**

8. Khafyah N., Dewi S.T.R. and Jumain J., The effectiveness of red ginger extract (Zingiber officinale var. rubrum) on decreased blood glucose levels in mice (Mus musculus), *Indones Health J*., **2(1)**, 16–21, https://doi.org/10.58344/ihj.v2i1.23 **(2023)**

9. Khan S.S., Kour D., Ramniwas S., Singh S., Kumar S., Kour S., Sharma R., Kour H., Rasool S., Rustagi S., Singh S., Chaubey K.K., Rai A.K. and Yadav A.N., Biotechnological potential of secondary metabolites: current status and future challenges, *J Appl Biol Biotech*., **11(6)**, https://doi.org/10.7324/jabb.2023.148341, 11-30 **(2023)**

10. Lutfia A., Munir E., Yurnaliza Y. and Basyuni M., Chemical analysis and anticancer activity of sesterterpenoid from an endophytic fungus Hypomontagnella monticulosa Zg15SU and its host Zingiber griffithii Baker, *Heliyon*, **7(2)**, https://doi.org/10.1016/j.heliyon.2021.e06292 **(2021)**

11. Marwan H., Hayati I. and Mulyati S., Effectiveness of biofungicide formula on rhizome rot disease of red ginger and its plant growth, *Biodiversitas*, **24(4)**, https://doi.org/10.13057/biodiv/d240425 **(2023)**

12. Mayila T., Tian X., An X., Feng Y. and Liu W., Isolation and identification of endophytic fungi from Alhagi sparsifolia Shap. and their antibacterial activity, *Heliyon*, **10(19)**, https://doi.org/10.1016/j.heliyon.2024.e39003 **(2024)**

13. Omojate G.C., Enwa F.O., Jewo A.O. and Eze C.O., Mechanisms of antimicrobial actions of phytochemicals against enteric pathogens: a review, *J Pharm Chem Biol Sci*., **2(2)**, 77-85 **(2014)**

14. Oyinlola K.A., Ogunleye E.G., Olutosin A.O., Adeyemo O.M. and Garuba E.O., Bioactive compound profiling and in vitro antimicrobial study of ginger (Zingiber officinale Roscoe) extract against pneumococcal bacteria, *Turk J Agric Food Sci Technol.*, **10(sp2)**, https://doi.org/10.24925/turjaf.v10isp2.2920-2925.5618, 2920–2925 **(2022)**

15. Padmini R., Uma Maheshwari V., Saravanan P., Lee W.K., Razia M., Alwahibi M.S., Ravindran B., Mohamed S.E., Kim Y.O., Kim H. and Kim H.J., Identification of novel bioactive molecules from garlic bulbs: a special effort to determine the anticancer potential against lung cancer with targeted drugs, *Saudi J Biol Sci*., **27(12)**, https://doi.org/10.1016/j.sjbs.2020.09.041, 3274–3289 **(2020)**

16. Petrini O., Endophytic fungi in British Ericaceae: a preliminary study, *Trans Br Mycol Soc*., **83(3)**, 510–512, https://doi.org/10.1016/s0007-1536(84)80050-9 **(1984)**

17. Rai N., Keshri P.K., Verma A., Kamble S.C., Mishra P., Barik S., Singh S.K. and Gautam V., Plant-associated fungal endophytes as a source of natural bioactive compounds, Mycology, **12(3)**, 139–159, https://doi.org/10.1080/21501203.2020.1870579 **(2020)**

18. Razali N., Dewa A., Asmawi M.Z., Mohamed N. and Manshor N.M., Mechanisms underlying the vascular relaxation activities of Zingiber officinale var. rubrum in thoracic aorta of spontaneously hypertensive rats, *J Integr Med*., **18(1)**, 46–58, https://doi.org/10.1016/j.joim.2019.12.003 **(2020)**

19. Rialita T., Nurhadi B. and Puteri R.D., Characteristics of microcapsule of red ginger (Zingiber officinale var. rubrum) essential oil produced from different Arabic gum ratios on antimicrobial activity toward *Escherichia coli* and *Staphylococcus aureus*, *Int J Food Prop*., **21(1)**, 2500–2508, https://doi.org/10.1080/10942912.2018.1528455 **(2018)**

20. Rice-Evans C., Miller N. and Paganga G., Antioxidant properties of phenolic compounds, *Trends Plant Sci*., **2(4)**, 152–159 **(1997)**

21. Rinanda T., Isnanda R.P. and Zulfitri Z., Chemical analysis of red ginger (Zingiber officinale Roscoe var. rubrum) essential oil and its anti-biofilm activity against *Candida albicans*, *Nat Prod Commun.*, **13(12)**, https://doi.org/10.1177/1934578x1801301206 **(2018)**

22. sRoshmi T., Soumya K.R., Jyothis M. and Radhakrishnan E.K., Effect of biofabricated gold nanoparticle-based antibiotic conjugates on minimum inhibitory concentration of bacterial isolates of clinical origin, *Gold Bull*., **48(1-2)**, 63–71, https://doi.org/10.1007/s13404-015-0162-4 **(2015)**

23. Strobel G., Daisy B., Castillo U. and Harper J., Natural products from endophytic microorganisms, *J Nat Prod*., **67(2)**, 257–268, https://doi.org/10.1021/np030397v **(2004)**

24. Strobel G., The emergence of endophytic microbes and their biological promise, *J Fungi*, **4(2)**, 57, https://doi.org/10.3390/jof4020057 **(2018)**

25. Wei J.C., Fungal identification manual, Shanghai, Shanghai Science and Technology Press, 1-645 **(1979)**

26. Wen J., Okyere S.K., Wang S., Wang J., Xie L., Ran Y. and Hu Y., Endophytic fungi: an effective alternative source of plant-derived bioactive compounds for pharmacological studies, *J Fungi*, **8(2)**, 205, https://doi.org/10.3390/jof8020205 **(2022)**

27. Wiegand I., Hilpert K. and Hancock R.E.W., Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances, *Nat Protoc*., **3(2)**, 163–175, https://doi.org/10.1038/nprot.2007.521 **(2008)**

28. Wieners M., Bässler C. and Scholler M., Mycoparasitism of Fomitopsis pinicola (Sw.) P. Karst. by Antrodiella citrinella Niemelä & Ryvarden, *Mycol Prog.*, **22(8)**, https://doi.org/10.1007/s11557-023-01906-4 **(2023)**

29. Wu H.T., Lu F.H., Su Y.C., Ou H.Y., Hung H.C., Wu J.S., Yang Y.C. and Chang C.J., *In vivo* and *in vitro* anti-tumour effects of fungal extracts, *Molecules*, **19(2)**, 2546–2556 **(2014)**

30. Wu S.X., Modern medical fungal identification manual. Beijing, Beijing Medical University and China Union Medical University Press, 1-503 **(1998)**

31. Yamauchi K., Natsume M., Yamaguchi K., Batubara I. and Mitsunaga T., Structure–activity relationship for vanilloid compounds from extract of Zingiber officinale var. rubrum rhizomes: effect on extracellular melanogenesis inhibitory activity, *Med Chem Res*., **28(9)**, https://doi.org/10.1007/s00044-019-02380, 1402–1412 **(2019)**

32. Zhu Y., Warin R.F., Soroka D.N., Chen H. and Sang S., Metabolites of ginger component [6]-shogaol remain bioactive in cancer cells and have low toxicity in normal cells: chemical synthesis and biological evaluation, *PLoS One*, **8(1)**, e54677–e54677, https://doi.org/10.1371/journal.pone.0054677 **(2013)**.

(Received 22nd May 2025, accepted 27th June 2025)