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RESEARCH ARTICLE

Bioprospecting of Medicinal Mushroom - *Inonotus vitis* from Western Ghats of India

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Abstract

Background: Fungi, particularly mushrooms contain a diverse range of bioactive compounds are used in the pharmaceutical and nutraceutical industries. *Inonotus* P. Karst., a genus within the *Hymenochaetaceae* family *(*order *Hymenochaetales*), is a polypore fungus characterized by its pileate growth habit and pigmented basidiospores. **Aim:** The present investigation focused on the bioprospecting of medicinal mushroom. **Methods:** Mushroom *Inonotus vitis* collected from Pune region of Western Ghats of India. The morphological and microscopic trait analysis, ITS regionamplification by PCR, and molecular phylogenetic analysis was performed. **Results:** Following morphological and microscopic trait analysis, ITS region was amplified by PCR, and by using molecular phylogenetic analysis identity of *I. vitis* was confirmed. The methanolic extract of *I. vitis* demonstrated biological activity against *Vibrio cholerae, Bacillus subtilis,* and *Propionibacterium acnes.* An increase in antioxidant capacity was observed with higher extract concentrations. Furthermore, the extracts exhibited anticancer activity against MCF-7 cell lines, with an IC_{50} value exceeding 80 µg/mL. Metabolic profiling using HR-LCMS identified 22 bioactive metabolites in the extract. **Conclusion:** Based on the experimental findings, it is suggested that the fungus holds potential for applications in the healthcare and nutraceutical sectors.

Major Findings: The molecular phylogeny of *I. vitis* from Western Ghats of Maharashtra was reported for the first time in this study. The gram-positive and gram-negative bacteria were both significantly inhibited by the extracts made from the *I. vitis*. The MCF7 and Hela cell lines have been used to test the anticancer potential and showed significant activity. The *I.* vitis could be used by the pharmaceutical sector to produce bioactive compounds for the treatment of a number of diseases.

Keywords: Antibacterial, Anticancer Activity, Antioxidant, Metabolites Profiling, Molecular Phylogeny

1. Introduction

Fungi represent second largest kingdom of life and are important to humans[1]. They are among the most diverse and significant groups of organisms on the Earth, valued not only for their medicinal and nutritional benefits but also their critical roles in the ecosystem functioning. Fungi contribute to addressing global challenges and improving resource efficiency through their roles as pathogens, nutrient providers, and decomposers. Several investigations have shown that fungi, particularly mushrooms as most significant sources of bioactive chemicals[2]. In recent decades, there has been remarkable advancement in the utilization of fungi for innovative processes, products, and solutions. These advancements have significantly enhanced our

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conceptual understanding of the fungal kingdom on broad scale[3]. Estimates of global fungal diversity vary widely, with projections ranging from 500,000 to 10 million species. Hawksworth and Lücking[4] estimate 2.2–3.8 million species, of which only approximately 120,000 have been formally described. This suggests that mycologists have identified roughly 8% of the predicted total fungal diversity, leaving much to be explored[5].

According to Chang and Wasser[6], there are an estimated 150,000–160,000 species of mushrooms on Earth; however, only around 14,000 have been identified so far, and of those, about 7000 exhibit varied edibility. Among these, approximately 3,000 species, belonging to 21 genera, are considered mostly suitable for consumption. Furthermore, while an estimated 2,000 species are believed to have medicinal value, only 270 species have been scientifically documented for their medicinal potential[7]. Clinical reports have demonstrated the potential of mushrooms as a source of therapeutic agents for both infectious and non-infectious diseases. People widely recognize and consume certain mushrooms for their nutritional and therapeutic qualities. Bioactive polysaccharides isolated from basidiocarp, mycelium, and submerged broth of certain mushroom belonging to basidiomycetes possesses bioactivity and exhibit antioxidant, anticancer and antibacterial activity[8].

The real number of fungal species in the world is likely much higher than currently estimated. Many species of Basidiomycota, particularly from the class Agaricomycetes, produce visible fruiting bodies, or basidiomes, and have been used as a source of food since the dawn of human civilization, their full diversity remains underexplored. The genus *Inonotus* belongs to the class *Agaricomycetes,* order *Hymenochaetales,* and family *Hymenochaetaceae*. Recently, six new species of *Inonotus* were identified in India, further highlighting the genus's diversity[9]. *Inonotus* P. Karst. (*Hymenochaetales* Oberwinkler; *Hymenochaetaceae* Donk) was first proposed in 1879 to classify polypores with a pileate habit and pigmented basidiospores[10]. Subsequently, the genus was expanded to include all species exhibiting the brown background coloration and pigmented basidiospores characteristic of the type species, *I. cuticularis* (Bull.) P. Karst.[11]. Morphological characteristics such as the type and pigmentation of spore wall, habit of basidiocarp, the shape and color of the basidiospores, and presence or absence of setal components are used to differentiate species within the genus. Various features, including external and internal characters, reproductive structures, nuclear behavior, pigmentation, and ecological attribute, suggest that *Inonotus* is heterogeneous[12-16]. Among the various *Inonotus* species, *I. obliquus* is renowned for its wide range of therapeutic properties[17,18]. These include immunomodulatory, anti-inflammatory, antiviral, antidiabetic, antioxidant, antiparasitic, neuroprotective, and, more recently, antifatigue activities[17-20]. However, the chemical characterization of many *Inonotus* species remains incomplete. In these studies we examined bioactive metabolites, metabolic profiling, antioxidant, antibacterial, and anticancer properties, morphological studies and molecular phylogeny of *I. vitis*.

2. Materials and Methods

2.1 Collection

A sample of *I. vitis* anamorphic stage was obtained from *Tamarindus indica* plant from Aundh area of Pune, India's Western Ghats region (Lat. 18.5631^0, long. 73.817706^0). The fruit body was collected in polypropylene bag in winter season (month of October) (Figure 1A). Color, form, size, and morphological characteristics of the delicate fruiting body were examined in the field and laboratory. The sample was authenticated from the Agharkar Research Institute, Pune.

2.2 Morphological and Microscopic Observations

Delicate fruiting body was air dried for 48 hours before being pulverized into a fine powder and used[21]. The spores were observed under a 10X compound microscope (TRHL-66) after 0.1 gm of powder was suspended in a drop of lactophenol cotton blue dye (Figure 1B). The spores were later examined using a scanning electron microscope (FEI Nova NanoSEM 450) (Figure 1C). Using Sharma *et al.*[9] criteria, the sample was identified morphologically and anatomically based on the observed morphological, anatomical, and biochemical features.

2.3 Molecular Phylogenetic Analysis

DNA was extracted from a powdered sample of *I. vitis* using a fungal genomic DNA extraction procedure[22]. Fungal Mycelium were homogenized (FastPrep-24TM 5G Instrument) in 2 ml Eppendorf tubes with 0.17 g of glass beads (425-600 µm in size) and a ¼-inch ceramic sphere[23]. The fungal isolates were identified, amplifying the internal transcribed spacer region (ITS) ribosomal DNA. The PCR was carried out by standard techniques with the following cycling program: initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 1min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 10min and hold on 20°C.The amplification was done using primers ITS1and ITS4[24] Remove excess primers and unincorporated nucleotides from the PCR product by using ExoSAP-IT™ Express reagent kit. The Staden package was used for sequence assembly and editing[25]. Sequences for phylogenetic reconstruction were primarily obtained from publications[26-29]. The Maximum Likelihood (ML) approach was employed to study the evolutionary journey of the fungi based on the Hasegawa-Kishino-Yano model with bootstrap value 1000to describe the substitution pattern the best of MEGA 11 software[30].

2.4 Preparation of Fungal Crude Extracts

A 250 mL flask containing approximately 100 mL of aseptically sterilized Potato Dextrose Broth (PDB) was used to suspend the pure culture of the sample. Additionally, the flask was incubated at 25±2°C for six days while being held in a rotating shaker. Flasks were incubated in a constant condition after six days. After 30 days, complete growth and mat development were seen in the cultured flasks. Dried *I. vitis* mats were macerated in water and methanol solvents to create the extracts. About 4 g of mats were macerated in 100 mL of total solvent for methanol extracts, and they were then incubated on a rotary shaker at room temperature for 5 days.

In order to prepare the hot water extract, 4 grams of mat were macerated in 100 milliliters of distilled water. The resulting liquid was then placed in a hot water bath set at 80 degrees Celsius for two hours. To obtain a concentrated extract, each extract was filtered through cellulose paper and vacuum-evaporated following the incubation period. While complete solvents were evaporated in the methanol extract to prevent solvent interference, 10 mL of concentrate was recovered following evaporation in the hot water extract[31].

2.5 FTIR Analysis

To investigate the compound makeup, FTIR (Bruker 3000 Hyperion Microscope equipped with a Vertex 80 FTIR System) analysis was performed on the *I. vitis* aqueous extract. The FTIR spectra were collected within the 4000 - 450 cm^{-1} range (Bruker, Germany)[32].

2.6 Metabolic Profiling by HR-LCMS (High Resolution Liquid Chromatography and Mass Spectrometry)

The HR-LCMS analysis of active methanolic extract were performed. The extracts chemical fingerprints were created using Agilent Technologies USA's model-G6550A TOF/Q-TOF mass spectrometer, which has a 0.01% mass resolution. MS with a minimum and maximum range of 120 (M/Z) and 1200 (M/Z) respectively, and a scanning rate of one spectrum/second was selected as the acquisition method. Gas chromatography was kept running at 250°C and 13 psi per minute. Hip sampler model G4226A was utilized, with 100 µL/min auxiliary speed, 100 µL/min ejection speed, 5 µL flush out factor, and 3 µL injection volume. Used for HR-LCMS, the binary pump model G4220B has a 0.300 mL/min high pressure limit of 1200 bar and a column comp model G1316C with a 40 °C temperature. The solvent composition A: B percent was 95:5 with a flow of 0.300 mL/min in the first minute of the 35-minute collection period. Using the DAD model-G4212B spectrum range from 190 nm to 640 nm, the peak width was smaller than 0.10 min (2.0 s response time). The components were separated and identified using experimental m/z, database difference (library), and MS/MS fragments[33,34].

2.7 Antioxidant Activities

The antioxidant potential was assessed by DPPH (2,2-Diphenyl-1-picrylhydrazyl) method[35]. After dissolving the 5gm powder of the *I. vitis* specimen in 20 mL of methanol, the sample was incubated for 24 hours. The filtrate was subjected to a Rota evaporator (Rotary Vacuum Evaporator, Equitron) after being filtered via Whatmann paper no. 4. Prior to analysis, evaporated extract was further diluted to 1: 1000by

methanol. The 20, 40, 60, 80, and 100 µL extract and 1 mL DPPH (0.1mM) were incubated in the absence of light for 30 min. The 96-well plate was used for reaction mixture with 270 µL of methanol containing DPPH radicals (6×10^{-5} mol/L) and 30 µL of an extract concentration range. An absorbance was recorded at 517 nm. The following formula was used to compute the percentage of antioxidants or %RSA = [(Ac−As) ÷Ac] × 100; where: Ac–Control reaction absorbance; As–Testing specimen absorbance.

2.8 Antimicrobial Activities

For antimicrobial investigation Human pathogenic gram-positive bacteria *P. acne*, *S. mutans*, and *B. subtilis*, and gram-negative *Escherichia coli*, *Proteus mirabilis*, and *V. cholera* were employed.

The 4–5 isolated colonies of bacteria were added to 5 mL of tryptone soy broth using a loop, and the mixture was incubated at 35–37 °C for 2–8 hours until the turbidity reached the required level of 0.5 McFarland/0.1 O.D. at 620 nm. The culture was then removed and diluted. The 0.25mL of standardized inoculum was added to 25mL of heated, melted Mueller Hinton Agar and put into a sterile plate. Permit the plate to set. A sterile cork borer was used to prepare the wells in the plate. Six test samples, a positive control (chloramphenicol), and a negative control (distilled water) are all included on each plate. Each sample was loaded into the wells in a volume of 0.1 mL. The plates were incubated at 30°C for 48 hours without inverting the plate. Zones exhibiting total inhibition were measured, and their sizes were recorded to the nearest millimeter, using a calibrated instrument zone scale[33,36].

2.9 Anticancer Activities

The *I. vitis* sample's *in vitro* cytotoxicity was assessed using the SRB assay[35]. The HeLa and MCF-7 cell lines were culture and treated with 10, 20, 40 and 80 µg/mL drug concentration as per the method described by Thakare *et al.*[33]. The Adriamycin (ADR) was used as a positive control. The cell growth inhibition activity was captured using research microscope Nikon with a magnification of 20 and the image processing programme NIS-Elements was used to capture photographs of the sample examined[34,37-39].

2.10 Statistical Analysis

The experiments were conducted in triplicate to examine the anticancer and antioxidant properties. The one-way analysis of variance was evaluated using statistical software SPSS 19.0. A sample means was compared by Duncan's multiple range tests at a significance threshold of 0.05.

3. Results

Chlamydospores of *I. vitis* are soft and spongy, dark brown, globose to spherical, and have thick walls. They measure 100 - 120 mm in width, 120–170 mm in length, and up to 20 mm in thickness. When harvested, the annual pileate plant was delicate and dark brown after air drying (Figure 1A). When the pore surface is young, it is soft and pubescent, mostly with an agglutinated cuticle, concentrically zoned. The margin is thin to thick, slightly lobed, and the pore size is irregular, ranging from 2 to 5 mm. As it ages and approaches the base of the basidiocarp, a thin, faint black zone that separates the upper, golden-brown portion from the bottom part becomes more noticeable. The bottom portion is radially fibrous and glossy. Spores that were nearly uniform in size and shaped were seen using a compound microscope with a low power (Figure 1B). Using a scanning electron microscope, disc-shaped spores were observed with a diameter of 8.5 to 9.6µm (Figure 1C).

Inonotus vitis (GenBank Accession No-PP275109) showed the highest similar to the ITS sequence of *I. vitis* (GenBankNR168218) in the NCBI Gene Bank database. ABLASTn search revealed 100% query cover and 100% identity. *I. andersonii* (GenBank: AM269781) and *I. krawtzewii* (GenBank KF446599) displayed a max score of 649, total score of 711, and 95% identity. The taxa that clustered together were supported by bootstrap analysis, conducted with 1,000 repetitions. The bootstrap values, expressed as percentages, are indicated on the respective clades in the tree. The scale bar on the phylogenetic tree represents the evolutionary distance used to construct the tree. These distances, expressed as the number of base substitutions per site, were calculated using the Hasegawa-Kishino-Yano model. The Hasegawa-Kishino-Yano (HKY) model was employed because it provides a computationally

efficient yet physiologically realistic method for analysing the evolution of DNA sequences by taking into account transition-transversion bias and uneven base frequencies. This guarantees phylogeny inference that is correct and customized for the dataset. *Fulvifomes fastuosus* was used as the outgroup taxon for the analysis. The evolutionary position of *I. vitis* is shown in Figure 2.

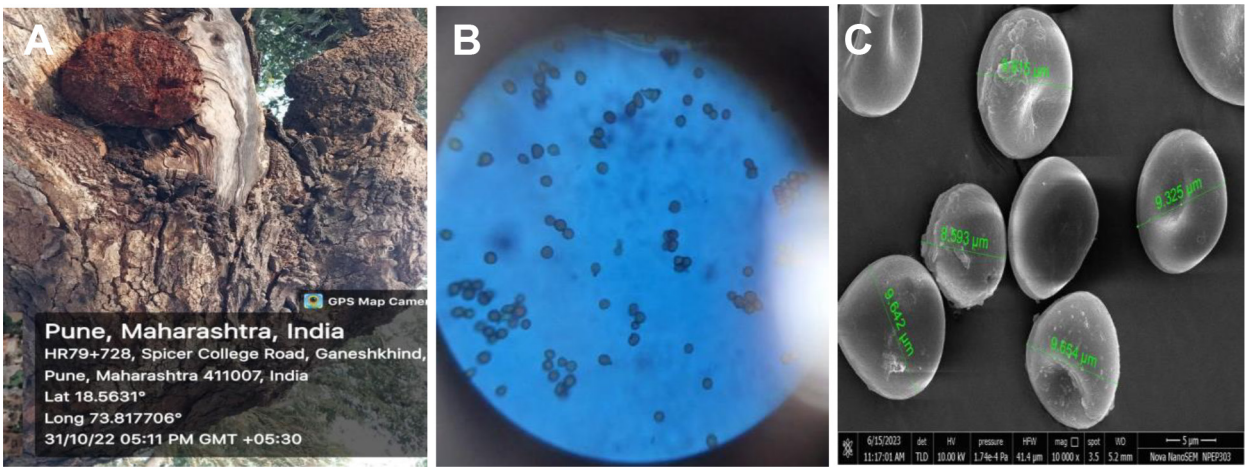


Figure 1. *I. vitis:* **(A).** host and fruiting body; **(B).** compound microscope image of spores; **(C).** SEM image of spores.

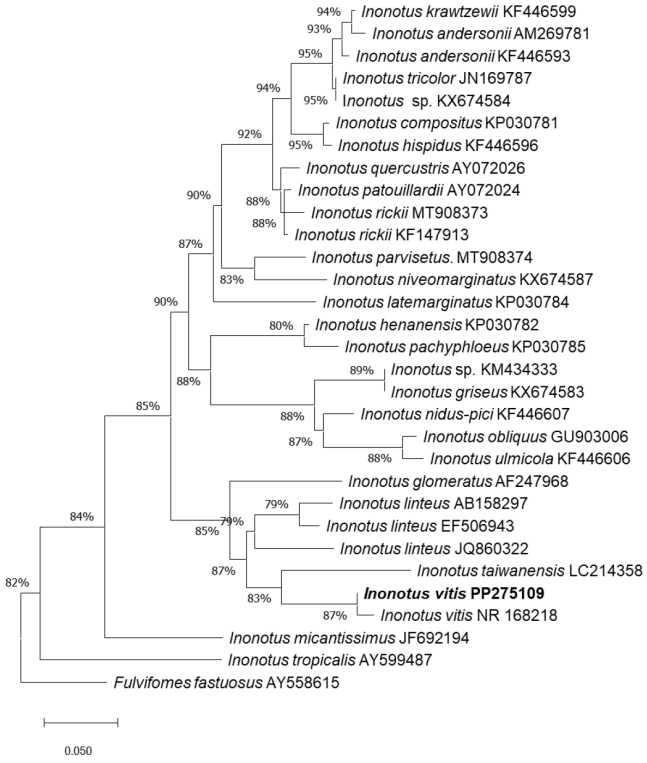


Figure 2. Phylogenetic tree constructed using ITS sequences of *I. vitis* (PP275109) and related species rooted with *F. fastuosus*. The maximum likelihood (ML) analysis was performed using MEGA XI Branches are labelled with ML bootstrap1000 replicates. The novel species are bold and highlighted

To investigate the compound makeup of the *I. vitis*, FTIR analysis was carried out. FTIR spectra (Figure 3) showed the existence of polysaccharide (C-O-C) in the region of 990–1200 cm^{-1}. The stretching of the glycosidic bond is indicated by the peaks at 1150–1160 cm^{-1} (H–N–C). Moreover, additional peaks located at 1377.77 cm^{-1} suggest the existence of β-D glucan. Protein vibration is represented by the peak at 1653 cm^{-1} (H-N-CO). The C-H asymmetrical and symmetrical stretching vibrations include additional peaks at 2857.06 and 2924 cm^{-1}, respectively. In our investigation the N-H stretching vibration peaks were located in the range of 3101–3648 cm^{-1}. While polysaccharide-bound proteins were indicated by the amide (N-H stretching) detected at 3415 cm^{-1}.

A variety of bioactive compounds were identified, as shown in Figure 4. These compounds were characterized by comparing their mass spectra with primary libraries using high-resolution liquid chromatography. The extract's m/z values ranged from 150.11 to 496.34. A total of 22 compounds were identified and are listed in Table 1.

In the current study, the radical scavenging activity (DPPH assay) was found to be positively correlated with the concentration of the methanolic extract of *I. vitis*. Various extract concentrations were tested,

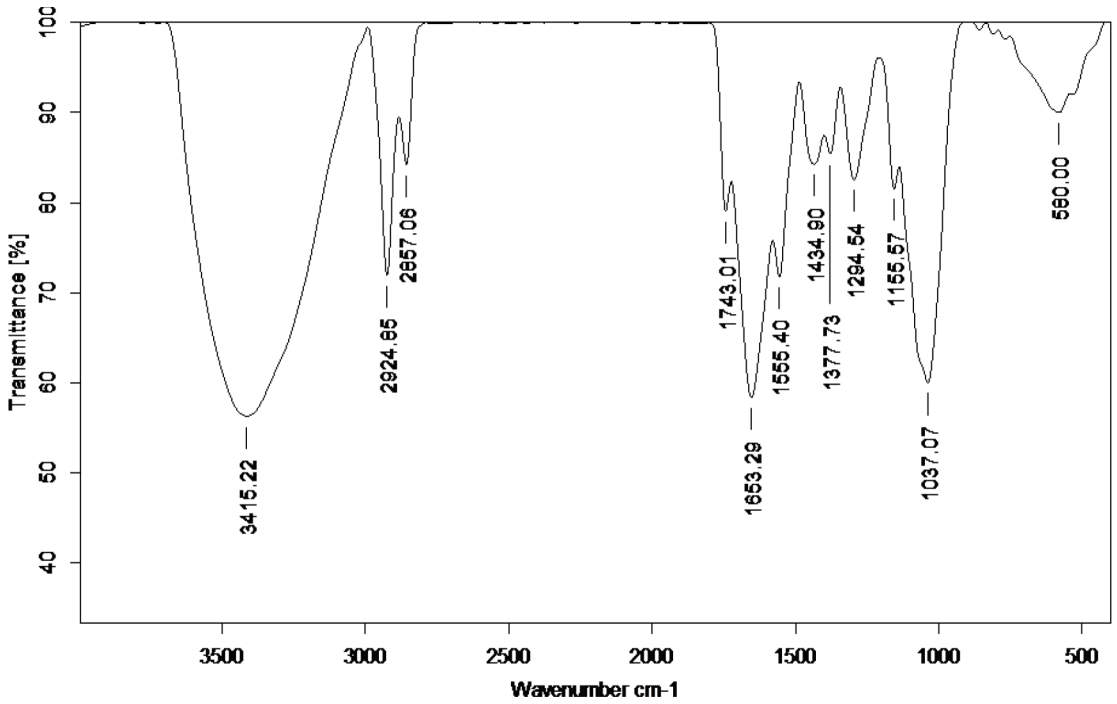


Figure 3. *I. vitis* aqueous extract FTIR spectra.

Table 1. HR-LCMS based metabolites elucidation of *I. vitis*

Sr. No.	Name	Formula	Mass
1	Trolamine	$C_6H_{15}NO_3$	149.1048
2	D-Arabitol	$C_5H_{12}O_5$	152.0675
3	3beta,6beta-Dihydroxynortropane	$C_7H_{13}NO_2$	143.0942
4	Anthranilic acid	$C_7H_7NO_2$	137.0468
5	Miserotoxin	$C_9H_{17}NO_8$	267.0953
6	(R)-(Homo)2-citrate	$C_8H_{12}O_7$	220.0573
7	(E)-10-Hydroxy-2-decene-4,6-diynoic acid	$C_{10}H_{10}O_3$	178.0618
8	2,3′,4,6- Tetrahydroxybenzophenone	$C_{13}H_{10}O_5$	246.0521
9	(-)-Dioxibrassinin	$C_{11}H_{12}N_2O_2S_2$	268.034
10	Isopimpinellin	$C_{13}H_{10}O_5$	246.052
11	2′,4′-Dihydroxy-2-biphenylcarboxylic acid	$C_{13}H_{10}O_4$	230.0569
12	Manumycin A	$C_{31}H_{38}N_2O_7$	550.2602
13	Dihydrodeoxystreptomycin	$C_{21}H_{41}N_7O_{11}$	567.2868
14	C16 Sphinganine	$C_{16}H_{35}NO_2$	273.2654
15	6″-Malonylcosmosiin	$C_{24}H_{22}O_{13}$	518.1188
16	Olprinone	$C_{14}H_{10}N_4O$	250.0851
17	Valdiate	$C_{17}H_{26}O_5$	310.1765
18	4-O-Methylmelleolide	$C_{24}H_{30}O_6$	414.2023
19	Eugenol	$C_{10}H_{12}O_2$	164.0836
20	1-(5-Methyl-3-pyridinyl)-1-decanone	$C_{16}H_{25}NO$	247.1928
21	Diethyltoluamide	$C_{12}H_{17}NO$	191.1305
22	2-Methyl-1-phenyl-2-propanyl butyrate	$C_{14}H2_0O_2$	220.1451

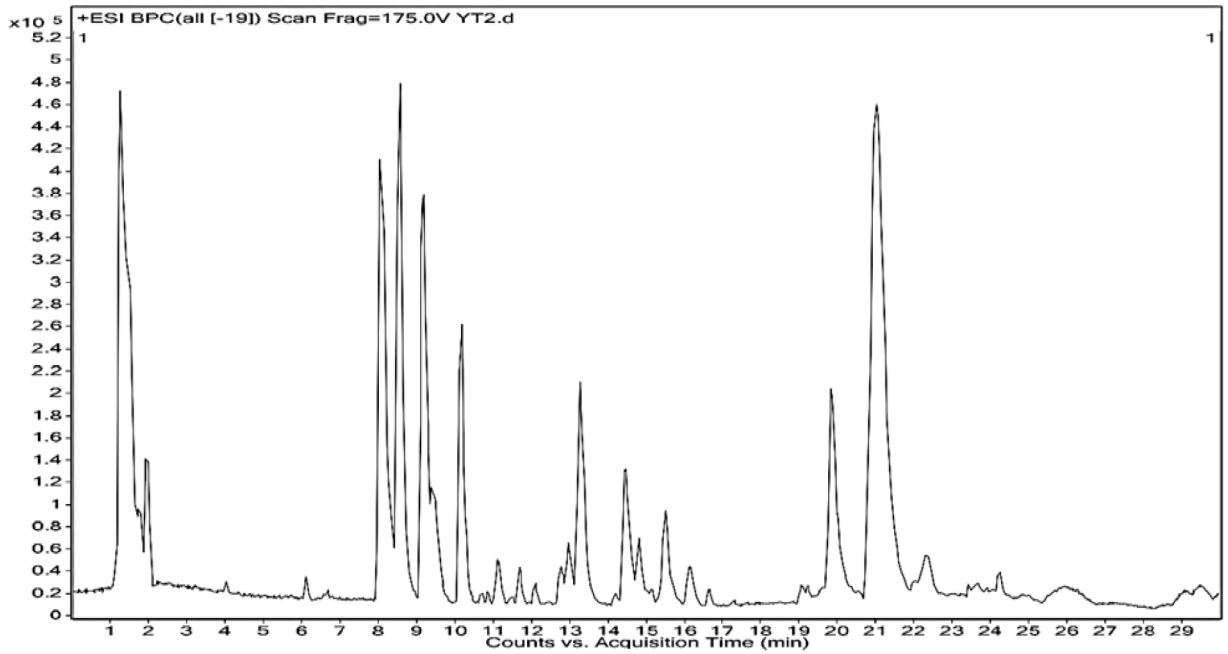


Figure 4. Chromatogram of HRLC-MS of *I. vitis* extract. The peaks in chromatogram represent the presence of various metabolites.

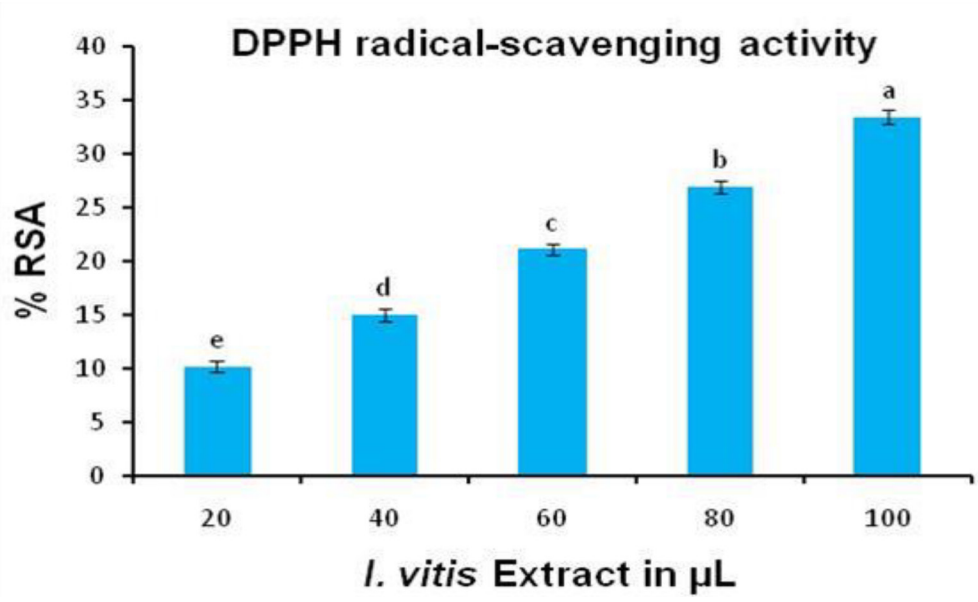


Figure 5. *I. vitis* extract radical scavenging activity. The small letters on the bar indicate the significance difference in radical scavenging activity between applied concentrations at 0.05 significance level.

including 20, 40, 60, 80, and 100 µL. The percentage radical scavenging activity (% RSA) observed at 100 µL was 33.33%. The IC_{50} value of the extract, representing the concentration required to inhibit 50% of free radicals, was determined to be 0.158 µg (Figure 5).

The current study assessed the antibacterial properties of *I. vitis* methanolic extract against both gram +Ve and gram -Ve bacteria. Zones of Inhibition (ZOI), measured in millimeters, were observed for the sample extracts, the positive control (chloramphenicol), and the negative control (sterile distilled water) (Figure 6).

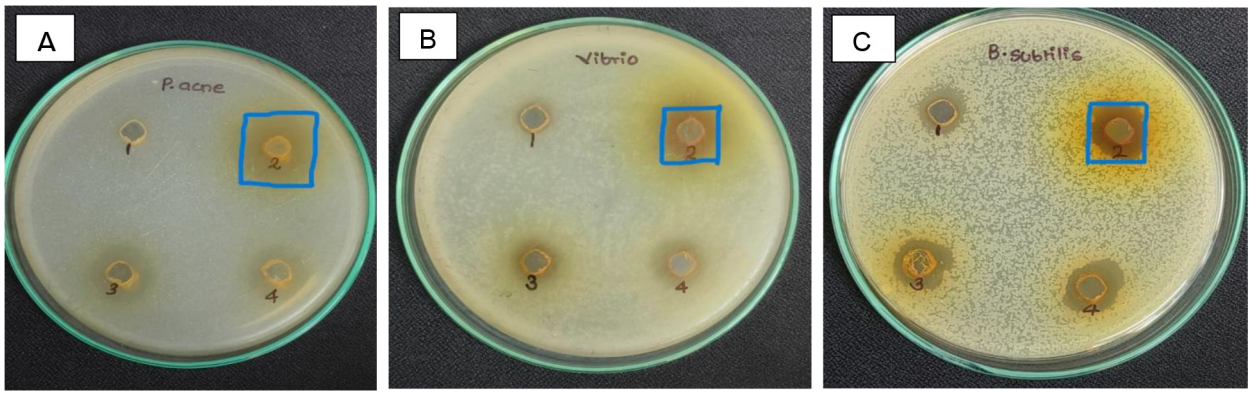


Figure 6. *I. vitis* extract inhibition of A) *P. acne* B) *V. cholerae* C) *B. subtilis* bacteria.

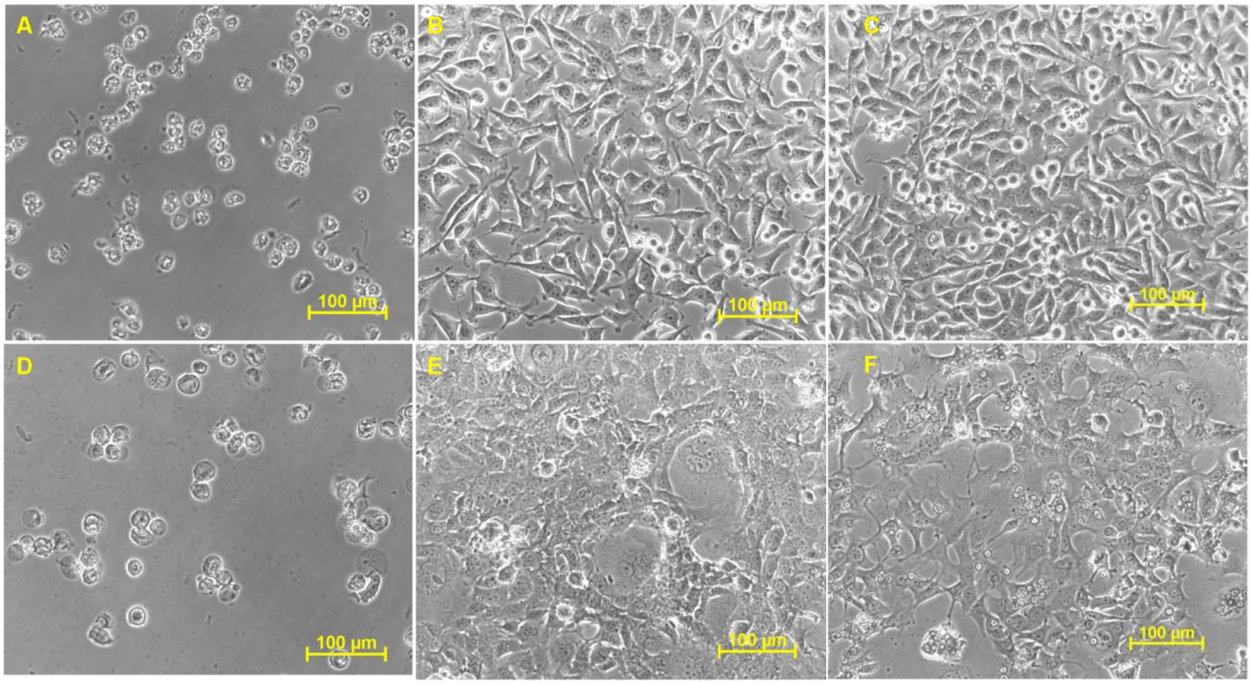


Figure 7. *I. vitis* extract cytotoxicity activity against HeLa cells treated with A) Adriamycin (ADR) B) control C) 80 µg/mL *I. vitis* extract and MCF-7 cells treated with D) Adriamycin (ADR) E) control F) 80 µg/mL *I. vitis* extract.

Table 2. Zone of inhibition of *I. vitis* extracts against various microorganisms

Sr. No.	Test Organism	ZOI by *I. vitis*	ZOI by PC in mm	ZOI by NC in mm
1	*Escherichia coli*	-	38	-
2	*Proteus mirabilis*	-	23	-
3	*Propionibacterium acne*	20	38	-
4	*Streptococcus mutans*	-	15	-
5	*Vibrio cholerae*	15	35	-
6	*Bacillus subtilis*	14	34	-

On Mueller-Hinton Agar plates, the *I. vitis* extract exhibited ZOI values of 20 mm against *P. acnes*, 15 mm against *V. cholerae*, and 14 mm against *B. subtilis* (Table 2). In comparison, the positive control showed ZOI values of 38 mm, 35 mm, and 34 mm for the respective microorganisms.

The *I. vitis* extract was evaluated for its anticancer activity against HeLa and MCF-7 cell lines. As shown in Figures 7A, B, C, and 8A, the extract had no significant effect on the HeLa cancer cell line. However, a dose-dependent increase in anticancer activity was observed against the MCF-7 cell line, with higher drug

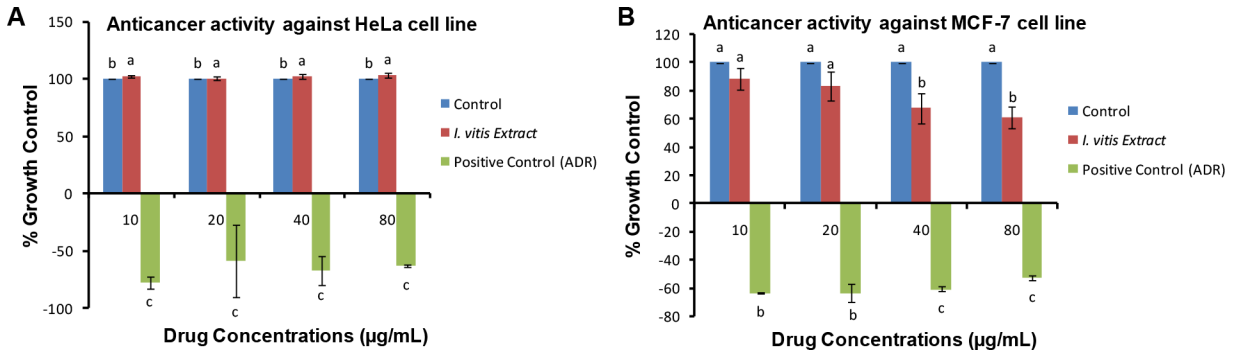


Figure 8. *I. vitis* extract anticancer activity against A) HeLa cell line and B) MCF-7 cell line. The small letters on bars indicate the significance difference in anticancer activity between applied drugs compared with control at the 0.05 significance level.

concentrations showing greater efficacy compared to the control (Figures 7D, E, F, and 8B). The IC_{50} value for the MCF-7 cell line was determined to be greater than 80 µg/mL.

4. Discussion

Identification of fungi is aided by morphological and microscopic characteristics[40]. The fragile brown fruiting bodies of *I. vitis* has a spherical, thick-walled, globose to spherical, dark brown form. Disc-shaped chlamydospores were observed, with an average diameter ranging from 8.5 to 9.6 µm. A crucial aspect of fungal taxonomy is the accurate identification of all fungal species and their relatives. However, the reconstruction of the Fungal Tree of Life (FTOL) remains significantly incomplete, with only approximately 5% of the estimated 2.2–3.8 million fungal species worldwide having been identified[41]. The morphological and molecular phylogeny study of *I. vitis* was studied.

Understanding phylogeny, or the evolutionary history of each organism within the Fungal Tree of Life (FTOL), is crucial. The ongoing task of fungal taxonomy involves reconstructing the FTOL, where fungal species are ideally well-defined, thoroughly characterized, and accurately placed in their respective evolutionary contexts. Advances in DNA sequencing have facilitated the development of phylogenies based on multiple genes rather than solely on morphological traits[42].

Molecular systematics has become an indispensable tool in modern fungal taxonomy. The nuclear ribosomal DNA (rDNA) consists of two Internal Transcribed Spacer (ITS) regions and three ribosomal RNA genes. Due to their high variability, ITS sequences are widely used to distinguish closely related taxa within the same genus. For over two decades, primers targeting the fungal nuclear ribosomal operon have been employed to identify fungi through molecular data[24]. Currently ITS1, 5.8S, ITS4, nrLSU-26S or 28S and nrSSU-18S were commonly utilized for fungal identification[39-40]. Different rates of evolution have been observed for these three distinct regions, leading to different levels of genetic variation. The ITS evolves most quickly and has the greatest difference between taxa, while SSU evolves most slowly[42,43]. The regions with unclear alignment were manually eliminated from this dataset due to the substantial deviation in the uneven regions of ITS1 and ITS2. 1443 bp was the ultimate alignment length. To reconstruct the tree, 29 specimens with *I. vitis* ITS sequences are included in the ITS dataset. *F. fastuosus* (AY 558615) ITS sequence was employed as an out-group taxon. It showed that the strain in the sample was the same as *I. vitis* (Figure 2).

For the purpose of characterizing and identifying substances present in an unidentified combination of material, FTIR has shown to be a useful instrument[46]. To investigate the chemical makeup of the *I. vitis* extract, FTIR analysis was done. Polysaccharide (C-O-C) is present in the range of 990 -1200 cm^{-1} in the FTIR spectra (Figure 3). The stretching of the glycosidic bond is indicated by the peaks at 1150–1160 cm^{-1} (H–N–C). Moreover, additional peaks located at 1377.77 cm^{-1} suggest the existence of β-D glucan. Protein vibration is represented by the peak at 1653 cm^{-1} (H-N-CO). The peak at 2857.06 are C-H

asymmetrical and peaks at 2924 cm-1 are symmetrical stretching vibrations[46]. In current studies the peaks for N-H stretching vibration were found between 3101–3648 cm^{-1}. The polysaccharide bound proteins were indicated by amide (N-H stretching) at 3415 cm^{-1} [46,47].

Finding the extract's metabolite makeup requires HR-LCMS analysis[47]. HR-LCMS study of *I. vitis* methanolic extract revealed the occurrence of several bioactive components. In our extract, most of the m/z values ranged from 150.11 to 496.338. Table 1 shows list of all 22 reported compounds. The majority of these substances exhibit biological potential as neuroprotective, antidiabetic, antiparasitic, antifatigue actions[17-20].

The extremely reactive element oxygen is harmful to living organisms because it generates reactive oxygen species[48]. When a substance transfers an electron or hydrogen to an oxidizing agent, a chemical reaction known as oxidation takes place. Oxidation activities can result in the production of free radicals. These radicals can then set off chain reactions that, when they occur inside of a cell, can cause damage or even death. Antioxidants break these chain events and inhibit more oxidative processes by removing free radical intermediates[49].

Reducing agents like ascorbic acid, thiols, or polyphenols are commonly used as an antioxidants[50]. Antioxidant systems therefore serve to maintain oxidants at their ideal level rather than to completely eliminate them. The body's defense mechanisms against illnesses caused by the assault of free radicals are controlled by antioxidants[51,52]. Accordingly, antioxidant supplementation can prevent degenerative illnesses like cancer, Parkinson's disease, Alzehimer's disease, and atherosclerosis that are brought on by oxidative stress[53]. *Inonotus obliquus* was also found to exhibit antioxidant activity[35,54]. According to a recent study, *I. vitis* extract has a strong correlation with its ability to scavenge radicals (Figure 5).

International healthcare researchers are making the quest for novel or alternative antimicrobials a top priority in order to eradicate this critical problem. Asexual forms of Ascomycota are the source of some common antibiotics, such as penicillin, cephalosporins, and griseofulvin, but very few commercial antimicrobial medicines are derived from basidiomycetous fungus. But basidiomycota have garnered considerable attention recently as a potential source of novel antibiotics and other bioactive substances[55,56]. Sułkowska-Ziaja *et al.*[57] stated that tests of bacterial and fungal susceptibility to a methanol extract of *I. hispidus* showed inhibiting the growth of *S. aureus*, *B. cereus*, and *E. faecalis*. As a result, researchers are always looking for new antimicrobial drugs from various biological sources[58]. This study demonstrated *I. vitis* potential as an antibacterial agent source. Extracts obtained from mushrooms have been shown by numerous authors to be very effective against gram-positive bacteria[59]. *Bacillus* subtilis, *V. cholerae*, and *P. acne* were all inhibited by an extract of *I. vitis* (Figure 6). The potential of *I. vitis* as an antibacterial agent was demonstrated by this investigation.

According to a World Health Organization assessment, 7.6 million persons worldwide lost their lives to cancer in 2008, out of an anticipated 12.7 million new cases of the disease[60]. By 2030, more than 21 million more instances of cancer and 13 million deaths are predicted, according to this report's estimates. It is commonly known that *I. obliquus* has anticancer properties. *I. obliquus* primarily stimulates the immune system, which is one way it can indirectly contribute to anticancer processes. Moreover, *I. obliquus* extracts antioxidative properties can stop the growth of cancer cells[61]. *I. vitis* extract performed well against the MCF-7cell line, demonstrating strong anticancer activity.

5. Conclusion

According to a number of studies, mushrooms are a great source of chemicals that have biological activity. By using phylogenetic and microscopic studies, *I. vitis* was identified. Based on conducted research, *I. vitis* extract plays a significant function in acting as an antioxidant and inhibiting the growth of specific bacteria. HR-LCMS analysis showed that, *I. vitis* has a many important secondary metabolites which increase its biological activities. The anticancer activity of the extract has also been demonstrated against the MCF-7Cell Line. This research indicates that *I. vitis* is an affluent resource of biologically dynamic substances; however, more research is needed to determine how these compounds affect different disorders.

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8. Authors Contribution

H.S. conceptualization, methodology, supervision, writing manuscript, and editing; Y.T. investigations, writing manuscript, and editing; D.S. data analysis, writing manuscript, and editing; G.G. writing manuscript, and editing; K.M. writing manuscript, and editing; S.A. conceptualization, methodology, supervision, and editing; B.B. conceptualization, methodology, supervision, and editing; S.G. conceptualization, methodology, supervision, and editing. All authors have read and agreed to the published version of the manuscript.

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