



Original Research Paper

Assessment of different media for in vitro culture of three wood-rotting fungal species: A screening of secondary metabolite occurrence

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Abstract

Wood rotting fungi, also known as lignicolous fungi, play a crucial ecological role in nutrient cycling through the degradation of lignocellulosic biomass. This study focuses on the isolation and in-vitro culture optimization of three wood rotting fungi viz. *Microporus xanthopus* (Fr.) Kuntze, *Hexagonia tenuis* (Hook.) Fr., and *Trametes elegans* (Spreng.) Fr. were collected from the Madhe Ghat, Maharashtra, India. The objective was to establish a reliable tissue culture establishment protocol and determine the most suitable growth medium for each fungal species. The parts of fruiting body were surface-sterilized and inoculated onto Potato Dextrose Agar (PDA), Czapek Agar, and Pentachloronitrobenzene (PCNB) Agar. Growth was monitored daily for seven days. Results indicated species-specific media preferences. *M. xanthopus* exhibited the most luxurious growth on PCNB medium, with observable mycelial growth within 24 hours. *H. tenuis* also showed optimal growth on PCNB medium, albeit with a slower initial growth rate. *T. elegans* demonstrated the best growth on Czapek medium. The specimens were also tested for chemical compounds. The strains viz. *M. xanthopus*, *H. tenuis* and *T. elegans* showed that various metabolites including flavonoids and alkaloids were present. Metabolite tannins were detected in *T. elegans* only. Glycosides and saponins were not detected in tested specimens. These findings provide a foundational protocol for the in-vitro culture of these understudied macrofungi, facilitating future research into their enzymatic potential and value-added applications, thereby contributing to the valorization of fungal biodiversity from the Western Ghats.

Keywords: Wood rotting fungi, *Microporus*, *Hexagonia*, *Trametes*, Media optimization, Secondary metabolites.

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1. Introduction

Since ancient times, fungi have fascinated humanity, particularly macrofungi which produce prominent fruiting bodies. Among these, wood rotting fungi, also known as lignicolous fungi, are a diverse group adapted to colonize and decompose wood (Dix and Webster, 1995; Floudas et al., 2020; Fukasawa, 2021). While some species are parasitic and cause decay in beneficial timber trees, many are saprophytic, playing an indispensable role in forest ecosystems by recycling nutrients locked in coarse woody material

(Marcot, 2017; Sudharsan, 2023). Their ability to degrade lignified cell walls through the enzymatic action of lignocellulolytic enzymes gives them profound ecological and economic significance (Chukwuma, 2020; Okeke, 2024). Wood rotting fungi are broadly classified into white rot and brown rot based on the nature of decay. White rot fungi, primarily belonging to the Basidiomycetes and some Ascomycetes, degrade both cellulose and lignin, leaving a white, stringy residue (Schwarze et al., 2013; Daniel, 2016; Isola et al., 2024). They are considered vital components of the global carbon cycle. In contrast, brown rot fungi predominantly break down cellulose and hemicellulose, modifying the lignin and leaving a brown, crumbly residue that often cracks into cubical pieces, a phenomenon known as cubical fracture (Goodell, 2020; Goodell and Nielsen, 2023). Brown rot fungi (e.g. *Serpula lacrymans* (true dry rot)), are of significant economic importance due to their attack on timber in buildings (Schmidt and Huckfeldt, 2011; Martinsson and Danielski, 2025).

The Western Ghats of India, a biodiversity hotspot, harbor a rich diversity of these fungi (Manjunath et al., 2026). The Madhe Ghat region, near Torna Fort at an altitude of approximately 850 meters, provides an ideal humid environment for the growth of wood rotting fungi, especially during the monsoon season. Among the various genera found in such regions, *Microporus*, *Hexagonia*, and *Trametes* are common examples with notable biotechnological potential. *Microporus* species are known for their secondary metabolites with antibacterial, antioxidant, and potential pharmaceutical applications (Sholola et al., 2022; Bui et al., 2026; Gautam et al., 2026). *Hexagonia*, a white rot genus widely distributed in tropical regions like the scrub jungles of Southwest India, has demonstrated a high capacity for laccase production, making it a candidate for textile dye degradation and other industrial applications (Kandasamy et al., 2016; de Oliveira et al., 2026). *Trametes*, particularly *Trametes versicolor* (turkey tail), is a mushroom with widely known medicinal properties like immune-modulatory, antitumor, and

antimicrobial properties and its use has been recorded in ancient Chinese medicine (He et al., 2022; Lucius, 2026).

Despite their potential, the successful cultivation of these fungi in a laboratory setting is a prerequisite for their exploitation (Rämä and Quandt, 2021; Mohdly et al., 2026). The optimization of culture media including factors like carbon and nitrogen sources, pH, and temperature is critical for enhancing fungal growth and productivity (Suh and Shin, 2000; Bhattacharyya and Jha, 2011; Krupodorova et al., 2021). In India, while foundational work on Aphyllophorales has been conducted by pioneers like Bagchee and Bakshi (1954), Thind and Adalakha (1956), and Ranadive et al. (2011), there remains a need for region-specific studies on the in-vitro culture optimization of these valuable species (Ranadive, 2011).

This study aims to collect *M. xanthopus*, *H. tenuis*, and *T. elegans* from the Madhe Ghat region and establish an in-vitro culture protocol. The objective is to screen and identify the most suitable growth medium among Potato Dextrose Agar (PDA), Czapek Agar, and Pentachloronitrobenzene (PCNB) Agar for each species. In addition, screening of preliminary metabolites has also undertaken thereby providing a foundation for future research into their biotechnological applications, including enzyme production, bioremediation, and the development of pharmaceutical compounds.

2. Materials and Methods

2.1. Collection of specimens

Fruiting bodies of *M. xanthopus*, *H. tenuis*, and *T. elegans* were collected from the Madhe Ghat region (approximately 850 meters above sea level), a dense forest area behind Torna Fort, Maharashtra, India. Collections were made during the rainy season when humidity and temperature are optimal for fungal growth (Fig 1 A-F). Specimens were examined in the field using a 15× hand lens and placed in sterile zip-lock polythene bags. They were transported to the laboratory and processed immediately. The specimens were identified as *M. xanthopus*, *H. tenuis*, and *T. elegans* (Bhagat et al., 2026).



Fig 1. Hymenial and abhymenial surface of sporocarp of collected specimen: Abhymenial (A), hymenial (B) surface of *T. elegans*. Abhymenial (C), hymenial (D) surface of *M. xanthopus*. Abhymenial (E), hymenial (F) surface of *H. tenuis*. Scale bar = 1 cm.

2.2. Glassware, Equipment, and Chemicals

Standard glassware (Petri plates, conical flasks, beakers, measuring cylinders, forceps, needles) and equipment (autoclave, laminar air flow, weighing balance, microscope) were used. Chemicals included ferrous sulphate (FeSO_4), magnesium sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), potassium chloride (KCl), dipotassium hydrogen phosphate (K_2HPO_4), sodium nitrate (NaNO_3), sucrose, ascorbic acid, agar, Potato Dextrose Agar (HiMedia), Tween 80, ethanol, mercuric chloride (HgCl_2), calcium hypochlorite ($\text{Ca}(\text{OCl})_2$), and sodium hypochlorite (NaOCl).

2.3. Culture Media Preparation

In the present study, three different media were tested for the culture of wood rotting fungi. The media were prepared as below:

Czapek Agar (ATCC medium 3/2): Composed of 6.018 grams (gm) sucrose, 0.618 gm NaNO_3 , 0.218 gm K_2HPO_4 , 0.1 gm KCl, 0.1 gm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.002 gm FeSO_4 and 3.018 gm agar in 200 mL distilled water (DW) with 7.3 ± 0.2 pH adjusted.

Potato Dextrose Agar (PDA): Ready-made PDA (HiMedia) was prepared according to manufacturer's instructions with 3 g of agar in 200 mL distilled water.

PCNB Agar: Composed of 5 gm peptone, 0.25 gm $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.50 gm K_2HPO_4 , 4 gm agar in 200 mL DW with 6.8 ± 0.2 pH adjusted.

All the media were sterilized in an autoclave at 121°C and 15 psi and for 20 minutes (min). The laminar air flow (LAF) cabinet was cleaned with 70% ethanol, and the UV light was switched on for 20 min prior to use. Sterilized media were dispensed into sterile Petri plates.

2.4. Surface Sterilization and inoculation

A multi-step surface sterilization protocol was followed. Fruiting bodies were cut into 0.5-1 cm explants. The explants were washed thoroughly with tap water and then with Tween 80 detergent. The explants were immersed in 0.1% HgCl_2 solution for 2-3 min and then in 0.5% NaOCl solution for 15 min, followed by 0.5% $\text{Ca}(\text{OCl})_2$ solution for 15 min and 1% ascorbic acid solution for 15 min. Finally, washing 3-4 times with sterile DW was done. All steps were carried out inside the LAF cabinet.

2.5. Inoculation and Incubation

Two sterilized explants of each fungus (*M. xanthopus*, *H. tenuis*, and *T. elegans*) were aseptically placed onto the center of each of the three different media (Czapek, PDA, PCNB). The experiment was conducted in triplicate for each fungus-medium combination, resulting in a total of 27 Petri plates (3 fungi $\times$ 3 media $\times$ 3 replicates). Plates were sealed with paraffin film to prevent contamination and incubated in a dark culture room at room temperature (approximately $25\text{--}28^\circ\text{C}$). Observations for mycelial growth were recorded daily for 7 days, and photographs were taken.

2.6. Extracts Preparation

The fruiting body were taken and extracted by following the method described as Szwajkowska-Michalek et al. (2022). One gram sample from each fungus were collected and homogenized into 80% methanol. Samples were flooded with 50 ml of methanol, then put in an ultrasonic bath for 30 minutes in special flasks before precipitate collection in distillation flasks. Three times, the extraction procedure was carried out. Then, combined extracts were evaporated in an evaporator to dryness. Final volume was made by methanol and extracts were used for further analysis.

2.7. Preliminary metabolite screening

For its several phytochemical tests, the crude methanolic extract was further diluted at a concentration of 5 mg/mL. Qualitative analysis of terpenoids, glycosides, phenolic compounds,

flavonoids, alkaloids and saponins were carried using below given test/s (Adebayo et al., 2012; Dutta et al., 2023).

2.7.1. Tests for alkaloids

Dragendorff's test: 1 mL of Dragendorff's reagent was added to 2 mL of extract and formation of orange red precipitate was observed as positive test.

Mayer's test: To 1.0 mL of extract, few drops of Mayer's reagent were added. A yellowish or white precipitation indicates the presence of alkaloid.

Hager's test: Taken a 2.0 ml of extract and added few drops of Hager's reagent. A yellow precipitation formed means that alkaloids were present.

Wagner's Test: Added a few drops of the solution to 2–3 mL of filtrate. Shaken well and observed the brown or reddish-brown precipitation for the presence of alkaloid.

2.7.2. Flavonoids

Two grams of the extracts were dissolved in ten percent sodium hydroxide (NaOH) and hydrochloric acid (HCl). Yellow solution that became colorless when HCl was added indicated that flavonoids were present.

2.7.3. Tannins

After adding ferric chloride to the extract, and stirring the extracts with 10 milliliters of hot distilled water, the precipitate was checked for blue-black, blue-green, or green.

2.7.4. Glycosides

Lieberman's test: 2 mL of acetic anhydride were used to dissolve a small amount of the extracts, which were then chilled in ice. Then, sulfuric acid (conc.) was added with caution. The existence of a steroidal nucleus, or the algycone part of the cardiac glycoside, was indicated by a color shift from violet to blue to green.

2.7.5. Saponins

After five minutes of boiling 0.1 gm powdered extract in 10 ml DW, the mixture was decanted and filtered. After diluting one milliliter of filtrate with four milliliters of distilled water, the mixture was violently agitated and checked for persistent foam that persisted for at least fifteen minutes.

2.8. Statistical analysis

The experiment was performed in triplicate. The mean values $\pm$ standard deviation was used to report the results. The DMRT test was used for ANOVA analysis. Statistical significance was defined at $P < 0.05$.

3. Results

3.1. Culture and growth of wood rotting fungi

The results demonstrated that all three fungal species could be successfully established in culture using the described surface sterilization and explant technique. However, the rate and extent of mycelial growth varied significantly depending on the fungal species and the culture medium.

3.1.1. *Microporus xanthopus* (Fr.) Kuntze

M. xanthopus showed the fastest and most vigorous growth on PCNB medium. Mycelial growth was observable within 24 hours of inoculation (Fig 2). Growth on PDA was moderate, while growth on Czapek medium was relatively sparse. By day 7, the mycelium on PCNB had covered the maximum area of the plate, exhibiting a dense, white, and uniform growth (Fig 2 H; Table 1).

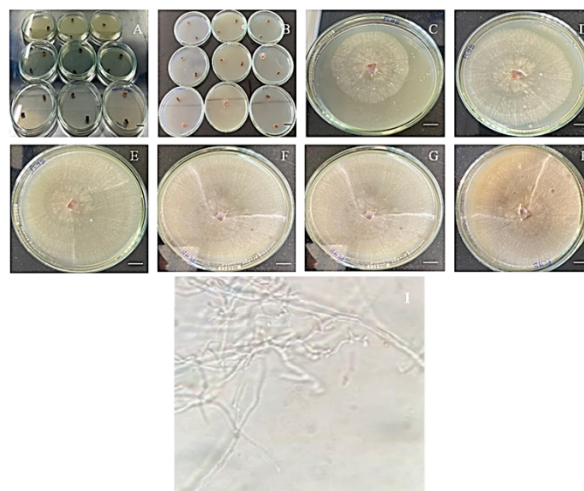


Fig 2. A- *M. xanthopus* inoculated on each medium; B- *M. xanthopus* inoculated on each medium; C to H - *M. xanthopus* inoculated on PCNB medium from Day 2 to 7; I - Hyphae; Scale bar- A to H – 1 cm, I– 10 μ m.

Table 1: Growth assessment of *M. xanthopus* on different media

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)
PDA	Slow (Day 2-3)	Moderate	Moderate
Czapek	Slow (Day 3)	Slow	Sparse
PCNB	Fast (Day 1)	Luxuriant	Maximum

3.1.2. *Hexagonia tenuis* (Hook.) Fr.

H. tenuis exhibited a slower initial growth compared to *M. xanthopus*. However, PCNB medium was again the most conducive for its growth. Mycelial emergence was observed after 2-3 days, and growth progressively increased, achieving substantial coverage by day 7 (Fig 3; Table 2). Growth on PDA and Czapek was notably slower and less dense.

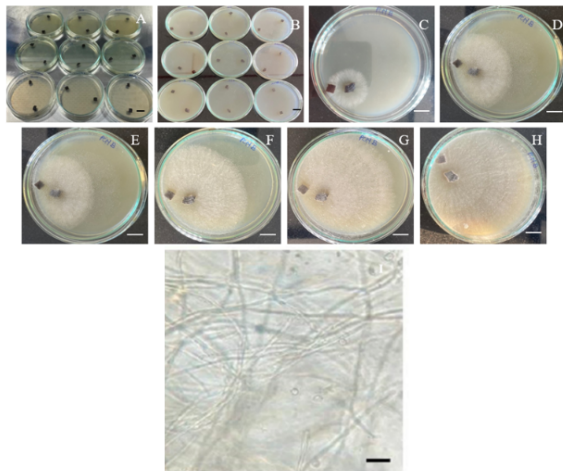


Fig 3. A – *H. tenuis* inoculated on each medium; B– *H. tenuis* inoculated on each medium; C to H. – *H. tenuis* inoculated on PCNB medium from Day 2 to 7; I- Hyphae and spores. Scale bar– A to H – 1 cm, I– 10 μ m.

Table 2: Growth assessment of *H. tenuis* on different media

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)
PDA	Slow (Day 3-4)	Slow	Low

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)
Czapek	Slow (Day 3-4)	Slow	Low
PCNB	Moderate (Day 2-3)	Moderate-Luxuriant	Maximum

3.1.3. *Trametes elegans* (Spreng.) Fr

T. elegans sp. displayed a distinct preference for Czapek medium. Growth initiation occurred within 2-3 days, and by day 6, the mycelium had grown well on the medium (Fig 4; Table 3). While growth was also observed on PDA and PCNB, it was not as robust. Some contamination was noted in one of the replicates for *T. elegans* on an unspecified medium, which was attributed to handling error or unfavorable environmental conditions.

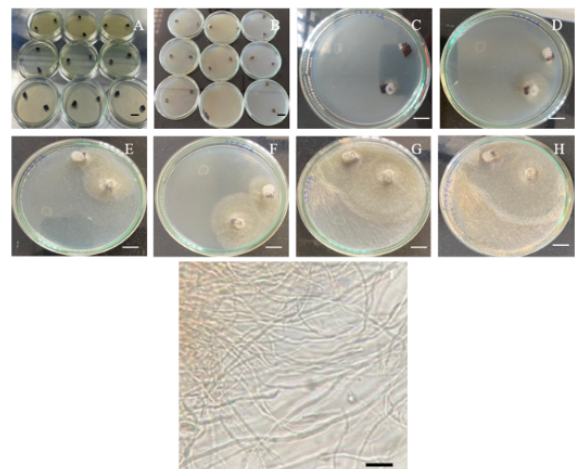


Fig 4. A – *T. elegans* inoculated on each medium; B – *T. elegans* inoculated on each medium; C to H – *T. elegans* spp. inoculated on Czapek medium from Day 2 to 7; I- hyphae. Scale bar- A to H- 1 cm, I – 10 μ m.

Table 3: Growth assessment of *T. elegans* on different media

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)
PDA	Moderate (Day 3)	Moderate	Moderate
Czapek	Fast (Day 2)	Luxuriant	Maximum

Medium	Growth Initiation	Growth Rate (Day 1-7)	Final Growth (Day 7)
PCNB	Slow (Day 3-4)	Slow	Sparse

3.2. Detection of secondary metabolites

Methanolic extracts showed that various metabolites were present (Fig 5; Table 4). It was observed that alkaloids and flavonoids were present all three strains viz. *M. xanthopus*, *H. tenuis*, and *T. elegans*. Whereas, tannins were detected only in *T. elegans*. The glycosides and saponins were not detected in any tested specimens.

Table 4. Qualitative phytochemical screening of wood rotting fungal extracts

Metabolite	Test	<i>M. xanthopus</i>	<i>H. tenuis</i>	<i>T. elegans</i>
Alkaloids	Mayer's test	Detected	Detected	Detected
	Hager's test	Detected	Detected	Detected
	Wagner's Test	Detected	Detected	Detected
Flavonoids	-	Detected	Detected	Detected
Tannins	-	ND	ND	Detected
Glycosides	Lieberman's test	ND	ND	ND
Saponins	-	ND	ND	ND

ND= Not detected

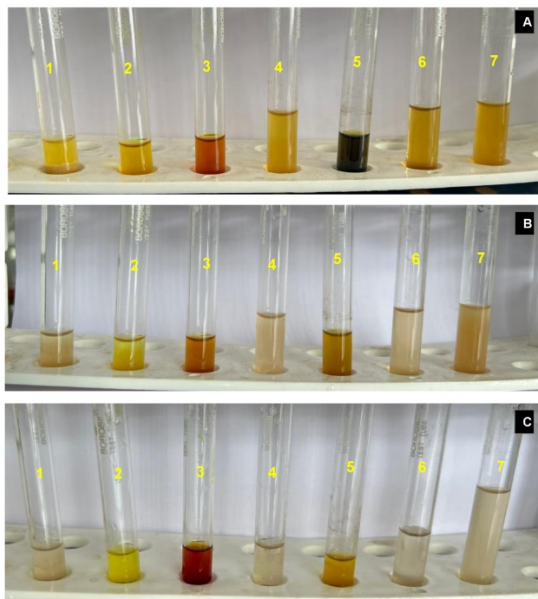


Fig 5. Preliminary secondary metabolites detection in (A) *T. elegans*, (B) *H. tenuis*, (C) *M. xanthopus*; 1-3= Test for alkaloids, 4= Test for Flavonoid, 5= Test for Tannins, 6= Test for Glycoside, 7= Test for Saponin

4. Discussion

4.1. Culture and growth of wood rotting fungi

The successful in-vitro cultivation of macrofungi is a critical first step for studying their biology and harnessing their biotechnological potential (Lodge et al., 2004; Qaraghuli et al., 2025). The tissue culture technique employed in this study proved effective for rescuing mycelial cultures from the collected fruiting bodies of *Microporus*, *Hexagonia*, and *Trametes*. The rigorous surface sterilization protocol, involving a sequential treatment with HgCl_2 , NaOCl , $\text{Ca}(\text{OCl})_2$, and ascorbic acid, was crucial in minimizing bacterial and fungal contamination, a common challenge in isolating wood-inhabiting fungi (Kirk et al., 2008; Nwankwegu et al., 2025). The choice of growth medium is paramount, as fungi have specific nutritional requirements (Borkert et al., 2025). General-purpose media like PDA are commonly used for fungal cultivation due to their rich nutrient content, which supports growth and sporulation for many species (Zhang et al., 2001; Mahadevakumar et al., 2025; Alhujaily, 2006). In this study, PDA supported the growth of all three species to some extent, confirming its utility as a basal medium. However, the species-specific preferences for other media highlight the importance of optimization.

M. xanthopus showed a strong preference for PCNB medium. The rapid growth initiation within 24 hours and subsequent luxuriant mycelial development suggest that the composition of PCNB, which includes peptone as a nitrogen source and a relatively simple carbohydrate source, is particularly well-suited for this genus. This finding aligns with research by Nguyen et al. (2019), who successfully optimized endoglucanase production from *M. xanthopus* using solid-state fermentation, indicating that *Microporus* species can thrive in nutrient conditions that promote enzyme secretion. The use of PCNB, a semi-selective medium often used for isolating *Fusarium* species, may have provided a competitive advantage by suppressing contaminants, thereby allowing *Microporus* to proliferate. Similarly, *H. tenuis* also exhibited optimal growth on PCNB medium. While its growth was slower than *Microporus*, the final mycelial biomass on PCNB was superior to that on PDA or Czapek. This result is interesting given that *Hexagonia* is known for its high laccase production in glucose yeast extract (GY) medium, as reported by Kandasamy et

al. (2016). The success of PCNB in this study suggests that this medium could be explored for optimizing laccase production from this isolate, complementing previous findings on carbon and nitrogen source optimization for *Hexagonia* growth (Nagadesi and Arya, 2013).

In contrast, *T. elegans* demonstrated a clear preference for Czapek agar, a defined medium with sucrose as a carbon source and sodium nitrate as a nitrogen source. This result is significant as it differs from common practices where *Trametes versicolor* is often cultivated on sawdust or other lignocellulosic substrates (Nguyen et al., 2012; Sen et al., 2025). The ability of this *Trametes* isolate to grow robustly on a defined synthetic medium like Czapek is valuable for physiological studies and for controlled production of bioactive polysaccharides and secondary metabolites, which are well-documented in this genus (Bains et al., 2021; He et al., 2022). The contamination observed in one plate underscores the need for stringent aseptic techniques, as the slow initial growth of some fungi can make them susceptible to overgrowth by contaminants (Deacon, 2005; Pasrija and Kumari, 2025).

From a broader perspective, this study contributes to the documentation of fungal biodiversity from the Western Ghats, a region that has been relatively underexplored for non-poroid resupinate Aphyllophorales (Ranadive et al., 2011; Patil et al., 2025). The ability to culture these fungi opens avenues for research into their economic importance, which spans from wood degradation to biotechnological applications (Covino et al., 2016; Dhiman et al., 2024). For instance, the enzymes from these white rot fungi, particularly laccases and peroxidases, have immense potential in bioremediation of environmental pollutants, textile dye decolorization, and biofuel production (Martinez-Inigo et al., 2000; Zheng et al., 2009; Deshmukh et al., 2016). Furthermore, the pharmaceutical potential of these fungi, especially *Trametes*, for their immunomodulating and anticancer properties, warrants further investigation (Standish et al., 2008; Luo et al., 2014; Wasser, 2017; He et al., 2025).

4.2. Detection of secondary metabolites

In the present study, alkaloids and flavonoids are detected in all tested specimens. The terpenes only detected in *Trametes*; while, glycosides and saponins were not detected in tested samples. The variation was observed in species to species. Similarly, in recent studies, it was observed that, the other chemical components were detected except alkaloids and tannins in *Aspergillus fumigatus* and *A. niger* (Ladoh Yemeda et al., 2015). In another study,

saponins, phenols, alkaloids and tannin was present in the ethyl acetate extract of *A. niger* and *A. fumigatus* extract showed terpenes presence (Yadav et al., 2014).

5. Conclusion

This study successfully established a protocol for the in-vitro culture of three wood rotting fungi viz *M. xanthopus*, *H. tenuis*, and *T. elegans* were collected from the Madhe Ghat region of Maharashtra, India. The results demonstrate that media optimization is crucial for the successful cultivation of these fungi. PCNB medium was identified as the most suitable for the growth of both *Microporus* sp. and *Hexagonia* sp., promoting rapid and luxuriant mycelial development. In contrast, Czapek medium was found to be optimal for *Trametes* sp. Three strains viz. *M. xanthopus*, *H. tenuis*, and *T. elegans* were rich in all tested phytochemicals. Whereas, tannins were detected only in *Trametes* sp., Glycosides and saponins were not detected in tested specimens. These findings provide a critical foundation for future research endeavours. The isolated cultures can now be utilized for exploring their enzymatic potential for bioremediation and industrial applications, as well as for investigating their bioactive secondary metabolites for pharmaceutical development.

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