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# Preliminary Phytochemical Screening of Selected Wood Degrading Fungi Collected from Chitteri Hills, Eastern Ghats of Tamil Nadu, India

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## A B S T R A C T

The present study shows that the role of wood degrading fungi which plays a vital role in maintaining the forest ecosystem. It acts as a decomposer and symbiotic relationship with other species. These findings showed that the selected fungi from the study area have great diversity and potential in the development of pharmaceutical and other curative products. The present study was intended to investigate the phytochemical screening of the different solvent extracts of selected wood degrading fungi. Preliminary phytochemical screening of selected extracts showed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins.

***Key words*:** Macrofungi, Phytochemical screening, Chitteri Hills, Pharmaceutical products

Macrofungi are renowned for its curative properties. These natural resources are gifted for the future and hence they are in need to be uncovered. This present study provides the information regarding macro fungi species with the high use values that could be employed in pharmacological research and further pharmaceutical approaches. In order to achieve adequate revenue for the tribal people who can start cultivating these macrofungi with the Government support. Fungi are identified worldwide in various forms like yeasts, molds and mushrooms, found in soil, on dead matter even some were identified as symbionts with other species [1]. The fungal species were primitive life forms and exhibits rich diversity hence studies on phytoconstituents of fungi were vital for future discoveries [2]. Identification of macrofungi found easier through grouping by habitat, spatial position and the appearance of the decayed wood found in the study area [3]. Macrofungi were identified from the study area by the presence and absence they were visible to the naked eye. The periodical investigation was carried out just after the rainfall. The recurrent surveys were performed in all sampling areas during January 2017 to December 2019. The intensive survey was done in the study area and found more than 105 species of macrofungi. All macrofungal species has some bioactive compounds which have pharmacological importance. The macrofungal specimens were collected from the decayed wood and three species were selected for phytochemical screening. This study reports the preliminary studies on bioactive compounds which may have enormous potential in pharmacological application to treat various diseases.

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## MATERIALS AND METHODS

Chitteri Hills (654.52 km²) are one of the fragments of Eastern Ghats of Tamil Nadu and the geographical limit of 78°15'–78°45' E longitude and 11°44'–12°08' N latitude. The withhold various vegetation types such as the evergreen, semi-evergreen, riparian, dry mixed deciduous, dry deciduous scrub and the southern thorn scrub forests. The least and highest temperature of the area is 19 °C (in winter) and 40 °C (in summer) correspondingly. The yearly rainfall differs from 620 to 900 mm and it received northeast and southwest monsoons mutually. The hills contain twisted ridges and narrow valleys running in the northeast and southwest directions, enclosing numerous constricted valleys (rivers), viz. Kallar, Varattar, Kambalai and Anaimaduvu. The study area is surging with an altitude varying from 240 to 1266 m. The study area covers Kambalai Beat, Arasanattham Beat, Tholthukki, Pallipatti North Beat, Irulappatti, Pallipatti Central Beat, Pallipatti Extension Beat, Nochikuttai Beat, Kalasapadi Beat, Sandhumalai East Beat, Sandhumalai West Beat, Kundal maduvu Beat, Chitteri Beat, Erumakadai, Kalnadu, Kalnadu Extension Beat, Suriyakadai Beat.

Seventeen random transects were selected each measure 100m length and 100m width were laid in the study area [4]. The subplots were also laid in each permanent plot for detailed investigation. The phenology of wood rot fungi differs

throughout the year. The study sites were randomly selected and latitude/ longitude were noted using GPS (Gramin ETrex 20).

*Macrofungal collection and preservation*

Macrofungi were collected carefully not disturbing any sporomas and carefully picked with the help of sterilized knife. Some sporomas were collected with the substratum and collected samples were scrutinized for free of insect and infection. The different morphological data and ecological data were noted in the fungal data book. They were photographed during the collection by using digital camera (Nikon D60 SLR Camera). The fungi were collected in tissue paper and stored in sterilized polythene bags which were labeled with unique collection number. The dehydrated fungi were sealed in covers and preserved by appropriate techniques. Delicate specimens were also preserved. The air – dried herbaria placed into the airtight container with silica gel [5]. The collected specimens were deposited in Department of Botany, School of Life Sciences, Periyar University, Salem.

*Morphological identification of fungi*

The specimens were identified based on their macro and micro morphological features [6]. Wood rot fungi were examined for the presence and absence of varying morphological features, mainly colour, shape, size, volva, annulus, gills, pileus, stipe, pores, peridium and veils. Spore prints were also taken to know the spore colour [7].

*Qualitative phytochemical analysis of the extract*

*Preparation of extract*

From a survey on fungi undertaken along the main trail from the hill bottom to the mountain top in early 2017 and late 2019 as part of the multidisciplinary study, more than 105 species of fungi were discovered. Among fungal specimens were collected three species were selected based on abundance namely *Ganoderma applanatum*, *Inonotus dryedeus*, and *Trametes hirsuta* (Fig 1) were selected for the studies. These fungal materials were air-dried for 14 days and ground with a commercial grinder. The powdered material (10 g) was extracted with 100 ml of selected organic solvents were extracted with Methanol and Petroleum ether using Soxhlet apparatus and filtered through Whatman No 1 filter paper for 24 hours at room temperature. The solvent was removed by filtration and then evaporated. The concentrated extracts of the taken samples were stored in small vials at 4°C and used for further analysis.

*Phytochemical screening (qualitative) of macrofungal species extracts*

A qualitative phytochemical analysis of the crude macrofungal extracts was carried out by following the standard protocols [8-16].

*Test for alkaloids*

Ethanolic extract of each Macro fungal species (0.5 g) were stirred with 5 ml of 1% aqueous hydrochloric acid (HCl) for two minutes on a steam water bath. After cooling the mixtures were filtered and few drops of Dragendorff's reagent were added. The change of the samples colour or turbidity was then recorded to draw inference.

*Test for flavonoids*

Diluted ammonia solution (5 ml) was added to portions of aqueous filtrate of each macro fungal extracts. This was then followed by the addition of a concentrated sulphuric acid. The solutions were observed for yellow coloration that disappears on standing to confirm the presence of flavonoids.

*Test for tannins*

Each sample (0.5 g) was dissolved in 5 ml of distilled water, followed by boiled gently and cooled. 1 ml of each solution was dispensed in a test tube and 3 drops of 0.1% ferric chloride solution were added. The change of colour observed for brownish green or blue black indicates the presence of tannins.

*Test for saponins*

To screen the presence of saponins the persistent frothing test was carried out. Distilled water (30 ml) was added to 1 g of each of the macro fungal extracts, vigorously shaken the mixture and heated on a steam water bath. The samples were then observed for the formation of froth to draw inference.

*Test for terpenoids*

For screening of terpenoids the Salkowski test was used. Macrofungal extracts (5 ml each) were mixed in 2 ml of chloroform and 3 ml concentrated sulphuric acid ($H_2SO_4$) were carefully added to form a layer. The change of colour observed for reddish brown coloration will confirms the presence of terpenoids.

*Test for steroids*

To screen the presence of steroids the macrofungal extract (0.5 g each) will be mixed with Acetic anhydride (2 ml) and filtered. Concentrated Sulphuric acid (2 ml) was added to the filtrate and observed for colour change from violet to blue or green, which indicates the presence of steroid.

*Carbohydrates - Benedict's test*

173 g of sodium citrate and 100 mg of sodium carbonate was dissolved in 500 ml of water. To this solution 17.3 g of copper sulphate dissolved in 100 ml of water was added. To 0.5 ml of the leaf extract, 5.0 ml of Benedict"s reagent was added and boiled for 5 minutes. Formation of a bluish green colour showed the presence of carbohydrates.

*Glycosides*

Five milliliters of 25% $H_2SO_4$ was transferred into a tube and 0.5 ml of the extract was added and boiled in water bath for 15 mins. Fehling's solution (5 ml) was then added to the boiled mixture. A reddish brown colour indicated the presence of steroidal ring of glycosides.

*Aminoacids and proteins-Biuret's test*

To 1.0 ml of leaf extract, 5-8 drops of 5% sodium hydroxide solution were added, followed by one or two drops of 1% copper sulphate. Formation of pink or purple colour confirmed the presence of amino acids and proteins.

*Phenols*

To little portions of the extract was 5 mL distilled water added, followed by the addition of 2 – 5 drops of neutral 5% ferric chloride solution. Appearance of dark green coloration showed the presence of phenol.

## RESULTS AND DISCUSSION

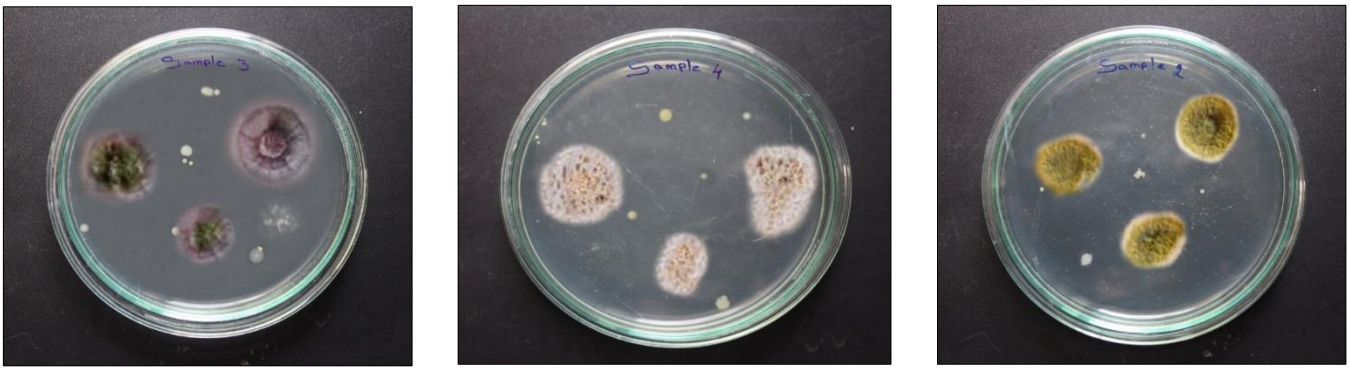
*Qualitative phytochemical screening*

The intensive survey was done in early 2017 and late 2019 from the study area. As an outcome of the present study, the selected study area shows marcofungal diversity hence more

than 105 species of fungi were discovered. Among fungal specimens were collected three species were selected based on abundance namely *Ganoderma applanatum*, *Inonotus dryedeus*, and *Trametes hirsuta* (Fig 1) were selected for the studies. The mycelial cultures were also done for further studies (Fig 2).

*Ganoderma applanatum* *Inonotus dryadeus* *Trametes hirsuta*

Fig 1 Selected wood degrading fungi from the study area




*Ganoderma applanatum* *Inonotus dryadeus* *Trametes hirsuta*

Fig 2 Mycelial textures of selected wood degrading fungi in PDA medium

Phytochemical screening result revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins in the selected macrofungal extracts in varying proportions. Methanol and Petroleum ether were selected as solvents for the presence of bioactive compounds. Methanol produced a higher yield than Petroleum ether when used for extraction. Results from the phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, aminoacids and proteins in methanol extract of *Ganoderma applanatum*. The Petroleum ether extract results the presence of alkaloids, phenols, flavonoids, tannins, carbohydrates, glycosides, aminoacids and proteins. Saponins, terpenoids, steroids were absent (Table 1).

Table 1 Qualitative analysis of phytoconstituents present in different solvent extracts of *Ganoderma applanatum*

| Phytochemicals | Extracts | |
|---|---|---|
| | Methanol | Petroleum ether |
| Alkaloids | ++ | + |
| Phenols | ++ | + |
| Flavonoids | +++ | + |
| Tannins | ++ | ++ |
| Saponins | + | - |
| Terpenoids | + | - |
| Steroids | + | - |
| Carbohydrates | ++ | ++ |
| Glycosides | ++ | ++ |
| Amino acids | +++ | ++ |
| Proteins | +++ | ++ |

Table 2 Qualitative analysis of phytoconstituents present in different solvent extracts of *Inonotus dryadeus*

| Phytochemicals | Extracts | |
|---|---|---|
| | Methanol | Petroleum ether |
| Alkaloids | +++ | ++ |
| Phenols | + | - |
| Flavonoids | + | - |
| Tannins | - | - |
| Saponins | - | - |
| Terpenoids | + | + |
| Steroids | + | + |
| Carbohydrates | ++ | ++ |
| Glycosides | ++ | ++ |
| Amino acids | ++ | ++ |
| Proteins | +++ | ++ |

The phytochemical analysis *Inonotus dryedeus* revealed the presence of alkaloids, phenols, flavonoids, terpenoids, steroids, carbohydrates, glycosides, aminoacids and proteins in methanol extract. Tanins and saponins were absent. The Petroleum ether extract results the presence of alkaloids, terpenoids, steroids, carbohydrates, glycosides, aminoacids and proteins. Phenols, flavonoids, tannins and saponins were absent (Table 2).

The phytochemical analysis revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, aminoacids and proteins in methanol extract of *Trametes hirsuta*. The Petroleum ether extract results the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, glycosides, aminoacids and proteins. The steroids, carbohydrates were absent (Table 3).

Table 3 Qualitative analysis of phytoconstituents present in different solvent extracts of *Trametes hirsuta*

| Phytochemicals | Extracts | |
|---|---|---|
| | Methanol | Petroleum ether |
| Alkaloids | ++ | ++ |
| Phenols | ++ | ++ |
| Flavonoids | ++ | + |
| Tannins | + | + |
| Saponins | + | + |
| Terpenoids | ++ | + |
| Steroids | + | - |
| Carbohydrates | + | - |
| Glycosides | + | ++ |
| Amino acids | +++ | ++ |
| Proteins | +++ | +++ |

+ → present in small concentration
++ → present in moderately high concentration
+++ → present in very high concentration
-- → absent

Ganoderma species was used as antitumor and antioxidant, and in some other medicinal therapy [17]. Fungi in general and mushrooms in particular are a good source for antimicrobial products [18]. Phytochemicals are abundant, locally renewable, user-friendly and environmentally safe, and attracts low capital [19-20]. Edible macro-fungi are widely consumed as food sources for their flavors and culinary features. In order to explore the potential of macro-fungi as a natural resource of bioactive compounds, the antioxidant properties and polysaccharide contents [21]. The first report about the antileishmanial activity showed by extracts from *Trametes versicolor*. The activity displayed by these extracts warrants the study of their composition as a potential source of new agents against Leishmania. A recent survey indicated that a large number of antitumor agents were produced by fungi [22].

Fungi from the division Basidiomycota have been of interest recently due to the numbers of biological active compounds that have been isolated from them [23]. Mushrooms were exhibiting the different bioactive compounds that are extractable and they have nutritional and medicinal features that may be used in the prevention and treatment of various diseases [24]. Wild mushrooms are commonly used in various pathologies. However, there are few studies concerning species characteristics from different geographical areas [25]. Hence the study was done from the selected study area to explore the various bioactive compounds from the macrofungal species.

The study reveals that, the crude methanolic and petroleum ether extract of *Ganoderma applanatum*, *Inonotus dryedeus*, and *Trametes hirsuta* revealed the presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, amino acids and proteins. The bioactive compounds present in these macrofungal species could be an accessible resource of innate antioxidant and antibiotics.

## CONCLUSION

The selected fungi *Ganoderma applanatum*, *Inonotus dryedeus*, and *Trametes hirsuta* have great potential to be developed into pharmacological products. The presence of alkaloids, phenols, flavonoids, tannins, saponins, terpenoids, steroids, carbohydrates, glycosides, aminoacids and proteins shows the presence of curative efficiency of the selected wood degrading fungal species. This macrofungal extracts could serve as potential sources of antioxidant and antimicrobial agents. The further research work can be done on anticancer study and also used for drug discovery. The study area endowed with rich resemblance of wood rot fungi and these kinds of forest areas to be protected and conserved for the abundance of natural resources.

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## LITERATURE CITED

1. Liew GM, Khong HY, Kutoi CJ. 2015. Phytochemical screening, antimicrobial and antioxidant activities of selected fungi from Mount Singai, Sarawak, Malaysia. *International Journal of Research Studies in Biosciences* 3(1): 191-197.
2. Dasgupta A, Rai M, Acharya K. 2015. Phytochemical analysis and in vitro antioxidant activity of a wild edible mushroom *Entoloma lividoalbum*. *Asian Journal of Pharmaceutical and Clinical Research* 8(5): 171-174.
3. Tainter FH, Baker FA. 1996. *Principles of Forest Pathology*. New York: John Wiley and Sons. pp 805.
4. Mohanan C. 2011. *Macrofungi of Kerala*. KFRI Handbook No. 27. Kerala Forest Research Institute, Peechi, Kerala, India. pp 597.
5. Drabkova LZ. 2014. DNA extraction from herbarium specimens. *In*: Molecular Plant Taxonomy Humana Press, Totowa, NJ. pp 69-84.
6. Yamashita S, Hattori T, Abe H. 2010. Host preference and species richness of wood inhabiting aphyllophoraceous fungi in a cool temperate area of Japan. *Mycologia* 102: 11-19. http://dx.doi.org/10.3852/09-008.
7. Magurran A. 2004. *Measuring Biological Diversity*. Blackwell Publishing, Oxford, UK. pp 260.
8 Odebiyi OO, Sofowora EA. 1978. Phytochemical screening of Nigerian medicinal plants. *Lloydia* 41: 234-246.
9 Harborne JB. 2005. *Phytochemical Methods*. A guide to modern techniques of plant analysis. Springer Pvt. Ltd., New Delhi.
10 Awala SI, Oyetayo VO. 2015. The phytochemical and antimicrobial properties of the extracts obtained from *Trametes elegans* collected from Osengere in Ibadan, Nigeria. *Jordan Jr. Biol. Sciences* 289-299.
11. Savithramma N, Linga Rao M, Suhrulatha D. 2011. Screening of medicinal plants for secondary metabolites. *Middle-East Journal of Scientific Research* 8(3): 579-584.
12. Onyeike EN, Osuji JO. 2003. *Research Techniques in Biological and Chemical Sciences*. Springfield Publishers Ltd., Owerri, Nigeria. pp 403.
13. Peach D, Tracey MV. 1955. *Modern Methods of Plant Analysis*. 4th Edition, Springer Berlin, Verlag. pp 373-374.
14. Raaman N. 2006. *Phytochemicals Techniques*. New India Publishing Agency, New Delhi. pp 19-25.
15. Trease GE, Evans WC. 1989. *Pharmacognosy*. ELBS/Bailliere Tindall, London. pp 345-346.

16. Lukas H, Matus K, Jana H, Martin R, Jana P. 2016. Antimicrobial activity of crude extracts from some medicinal mushrooms. *Jr. Microb. Biotech Food Sci. M* 5: 60-63.
17. Ulirike L, Timo HJ, Julich WG. 2005. The pharmacological potentials of mushrooms. *eCAM* 2: 285-299.
18. Janes D, Kreft S, Jurc M, Seme K, Strukelj B. 2007. Antibacterial activity in higher fungi: Mushrooms and endophytic fungi from Slovenia. *Pharma Biol*. 45: 700-706.
19. Anjorin TS, Salako EA. 2009. The status of pesticidal plants and materials identification in Nigeria. *Nigerian Journal of Plant Protection* 23: 25-32.
20. Rotimi MO, Moens M. 2003. The use of leaf extracts of some herbs in the control of *Meloidogyne incognita*. *Proceedings of Nigerian Society for Plant Protection* 21: 34-39.
21. Ya-Jun Guo, Gui-Fang Deng, Xiang-Rong Xu, Shan Wu,a Sha Li, En-Qin Xia, Fang Li, Feng Chen, Wen-Hua Linga, Hua-Bin L. 2012. Antioxidant capacities, phenolic compounds and polysaccharide contents of 49 edible macro-fungi. *Food Funct*. 3: 1195-1205.
22. Hata T. 1997. *Recent Advances in Medical and Veterinary Mycology*. University Park Press, Baltimore, MD. pp 299.
23. Hatvani N. 2001. Antibacterial effect of the culture fluid of Lentinus edodes mycelium grown in submerged liquid culture. *Int. Jr. Antimicrob. Agents* 17(1): 71-74.
24. Borchers AT, Keen CL, Gershwin ME. 2004. Mushrooms, tumors, and immunity: An update. *Exp. Biol. Med*. (Maywood). 229(5): 393-406.
25. Vamanu E, Voica A. 2017. Total phenolic analysis, antimicrobial and antioxidant activity of some mushroom tinctures from medicinal and edible species, by in vitro and in vivo tests. *Scientific Bulletin. Series F. Biotechnologies* 21: 318-324.