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Biochemical and antimicrobial analysis of *Phellinus phomaceus* from Melghat forest of Amravati MS

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Abstract

The present paper includes Hymenochaetaceous macrofungi Phellinus phomaceus from melghat forest Maharashtra, India. Author collected many interesting and rare macrofungi out of which Phellinus phomaceus selected here for further studies. Morphotaxonomy of the macrofungi studied. Extraction of primary and secondary metabolites for the biochemical analysis carried out and antimicrobial potential also investigated. The mycelium, gills and stipe extracted and used for antimicrobial test carried out by Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus. The study results that Phellinus phomaceus showed antimicrobial activities.

Keywords: Hymenochaetaceous Macrofungi, *Phellinus phomaceus*, biochemical analysis, Antimicrobial potential.

Introduction

Hymenochaetaceae belongs to family of basidiomycotina which contains polyporoid, hydnoid and steroid genera[1]. Hymenochaetaceae of the class Basidiomycetes is only one family[2]. The important characteristics of the family are characteristics of xanthochroic basidiocarps. All the members of family members shared common ancestry[3]. Poroid genera classified in nine families[4]. Species of Hymenochaetales are mainly saprobiotic on litter[5,6] and are considered as the major wood decomposers. Mushrooms are medicinal[12,13]. Every part of mushroom useful for industrial product[14]. Antimicrobial properties[15,16]. *Phellinus* used to cure various diseases[17-21,23,24].

Materials and methods

Melghat forest located in the Amaravati district of Maharashtra, A brief information regarding growing habit, habitat, locality, altitude, collection number, date of collection, forest type, especially the plants growing around recorded each time on the note paper which is further tagged to the packet in which the collection is wrapped. Most care was taken to avoid mixing of different collection to different places[23]. After noting down the taxonomically importance macroscopic characters on the field key on the above stated line, the collections are preserved for undertaking microscopy. The amount materials dried between 40-50˚C in oven[23].

Extraction: Extraction of primary and secondary metabolites carried out by standard methods[25]. In the present investigations; the mushroom specimens were grinded to a fine powder. Mushroom powder was taken for Soxhlet extraction. The extracts were kept at 35^0C for further analysis. Standard methods followed for further investigations.

Microorganisms tested: In vitro antimicrobial susceptibility test was performed using a set of microbes such as Gram negative, Gram positive bacteria and filamentous fungi which included both human clinical pathogen and laboratory control strains. The panel consisted of E. coli (MTCC 443), P. aeruginosa, (MTCC 779), S, aureus, (MTCC 187), Klebsilla. Antibacterial activity was studied.

Antibacterial Assay: The antibacterial assay was carried out by Disc diffusion method each 0.5mg extract was diluted. Ten microlitre extract was put in paper disc and zone of inhibition measured and compared with standard one and MIC observed.

Antifungal activity: The method of was adopted to evaluate the effect of sample on the growth of fungus. 20ml of sterilized and cooled (40^0C) growth media (PDA) with desired concentration of antibiotic were poured into pre-sterilized Petri plate. Requisite amount of different concentrations of extracts were added into the plates. The assay plates rotated clockwise and anticlockwise to ensure an even distribution of the extract into the medium. In control plates the medium was subjected with respective solvents. After the solidification of agar medium, a disc (5mm diameter) of test fungal strain from 7 days old culture was placed aseptically in the centre of each plate.

Results and discussion

Phellinus pomaceus (Figure-1)**: Morphology**: Basidiocarps poroid - reflexed pilei ungulate solitary, or embricate upto 3.5cm; upper surface at first light grayish, Brown, Smooth, tomentose becoming blackened rimose and glaburous margin light brown, rounded pore surface dark yellowish to reddish-brown the pores circular 7-9per mm with thick entire

dissepiments, context yellowish raddish brown shining zonate woody upto 1cm thick tube layer concolorous, distinctly stratified, tubes dull, becoming whitish within. Contextual hypae mostly brownish in KOH, thick - walled, with rare to frequent branching, sample - septate 2.5-5.5µm in clam, some hyline, thin-walled, sample septate, 2-3µm. in diam; tramal hypae usually similar some thin-walled, pale brownish to hyaline, smooth, negative in melzer reagent 4-5×3-4.5µm.

Taxonomy: Hyphal system dimitic Genrative hyphae up to 3.8 µm wide, branched, simple septate, subhylaline to pale yellow, thin-walled. Skeletal hypae up to 5.8µm wide, rarely branched, aseptate, brownish, thick-walled. Setal hyphae Hymenial setae 25×31×8-9.2µm, ventricose, acuminate, dark brown, thick- walled. Basidia broadly clavate, 4-sterigmate, 10-13×5-5.6µm simple septate at the base. Basidispore ovoid to broadly ellipsoid, hyaline, smooth, thick walled negative in melzer reagent.

Host: Living type of Development: Parasite type of plant: Gliricidia sepium soil Condition: Moist Soil Type: clay and silt.

Locality: Chikhaldara in Melghat region Latitude: $20^0$32' and 21^o46' north Longitude: 76^o37' and 78^o27' east Type of Forest: chikhaldara Temperature: Max. 22^0C and Min. 13^0C Humidity: 20^0 to 30^0C. Date of collection: 29 September 2018.

Qualitative phytochemical analysis: Qualitative phytochemical analysis are given in Table-1 and 2.

Table-1: Primary metabolits test *Phellinus pomoceus*.

Test	Ethanol	Acetone	Ethyl acetate	Distilled water
Fat	+	+	+	+
Protein	++	++	++	++
Non reducing sugar	—	—	—	—

Note present + and absent -.

Table-2: Phytochemical analysis test of *phellinus pomaceus*.

Test	Ethanol	Acetone	Ethyl acetate	Distilled water
Tannin	_	_	_	_
Alkaloid	+++	++	++	++
Anthroquinone	_	_	_	_
Saponin	_	_	_	_
Terpenoid	_	+	+	_
Flavonoid	_	_	+	++
Glycoside	_	_	_	_
C. glycoside	_	_	_	_
Lecoanthocynin	_	_	_	_
Steroid	_	+	+	_
Triterpenoid	_	+	_	_

Note: present + and absent -.

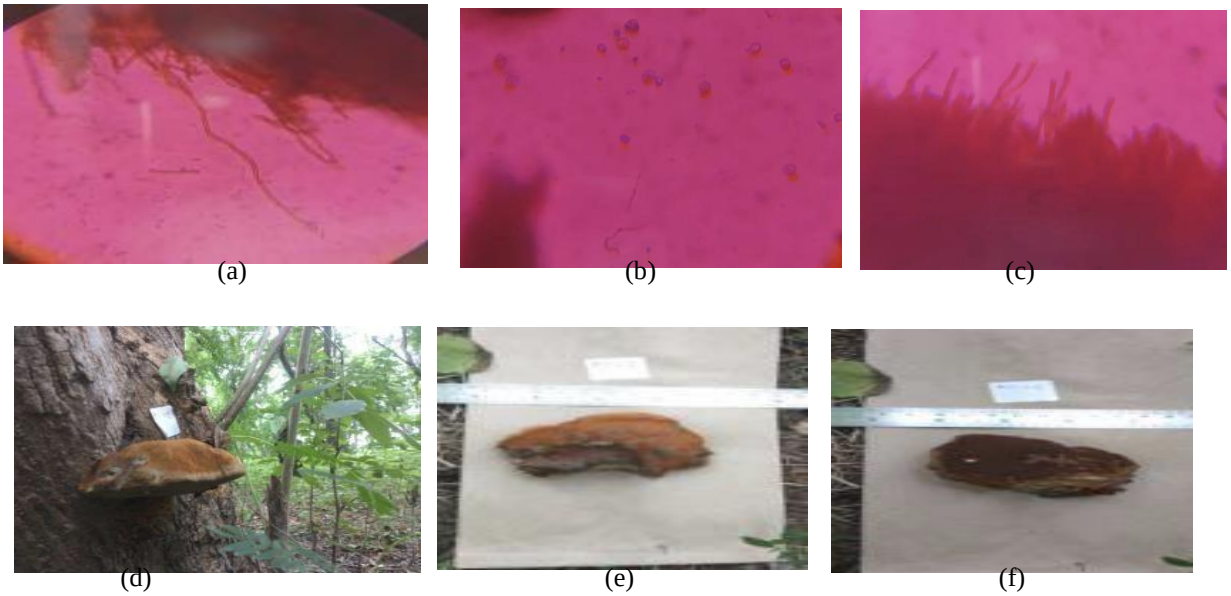

Figure-2: Collection examined: *Phellinus pomaceus*: a. Basidiocarp Showing Abhyaminial Surface b. Basidiocarp Showing Hymenial Surface c. Basiodiospore d. Context e. Spore f. Hyphae.

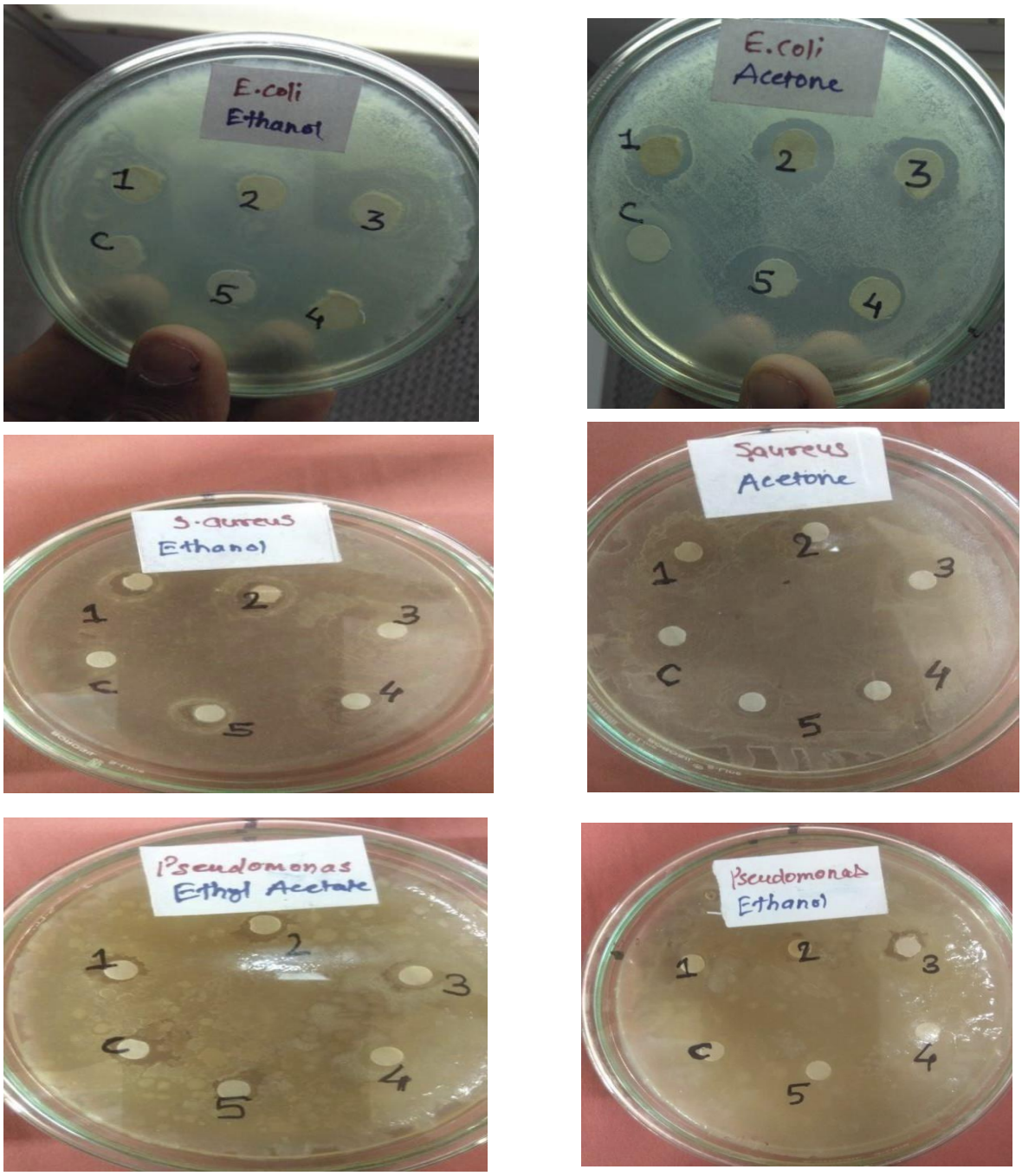

Figure-3a-f: Antibacterial activity of *Phellinus pomaceus*

Antimicrobial analysis of *Phellinus pomoceus*: Antimicrobial activity of Ethanol, Ethyle acatate, Acetone and distilled water extract of *Phellinus pomoceus* were investigated using disc diffusion.

Antimicrobial analysis of Ethanol extract; (Table-3).: By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e. in *E. coli* 20mm, *S. aureus* 12mm, Pseudomonas12mm, *Klebsilla* 18mm Respectively.

Antimicrobial analysis of acetone extract; (Table-3): By using disc diffusion method, P. pomoceus showed maximum zone of inhibition i.e. in *E. coli* 20mm, *S. aureus* 18mm, Pseudomonas 12mm, *Klebsilla* 21mm Respectively.

Antimicrobial analysis of Ethyle acetate extract; (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E. coli* 20mm, *S. aureus* 10mm, Pseudomonas 12mm, Klebsilla 12mm Respectively.

Antimicrobial analysis of distilled water extract; (Table-3): By using disc diffusion method, P. pomoceus showed maximum zone of inhibition i.e. in *E. coli* 13mm, *S. aureus* 10mm, *Pseudomonas* 10mm, *Klebsilla* 10mm Respectively.

Table-3: Zone of inhibition in mm.

Name of bacteria	Ethanol	Acetone	Ethyle acetate	Distilled water
E .coli	20mm	20mm	20mm	13mm
S .aureus	12mm	18mm	10mm	8mm
Pseudomonas	12mm	12mm	12mm	10mm
Klebsilla	18mm	21mm	12mm	10mm

Antimicrobial analysis of Ethanol extract; (Table-3): By using disc diffusion method, P. pomoceus showed maximum zone of inhibition i.e in E.coli 12mm, S. aureus 14mm, Pseudomonas10mm, Klebsilla 12mm Respectively.

Antimicrobial analysis of Acetone extract; (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E.coli* 18mm, *S. aureus* 17mm, *Pseudomonas* 8mm, *Klebsilla* 18mm Respectively.

Antimicrobial analysis of Ethyle acetate extract; (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E.coli* 18mm, *S. aureus* 12mm, *Pseudomonas* 12mm, *Klebsilla* 18mm Respectively.

Antimicrobial analysis of distilled water extract; (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E.coli* 12mm, *S. aureus* 10mm, *Pseudomonas* 8mm, *Klebsilla* 10mm Respectively.

Antimicrobial analysis of Acetone extract; (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E.coli* 20mm, *S. aureus* 12mm, *Pseudomonas* 8mm, *Klebsilla* 15mm Respectively.

Antimicrobial analysis of Ethyle acetate extract; (Table-3): By using disc diffusion method, P. pomoceus showed maximum zone of inhibition i.e in E.coli 18mm, S. aureus 12mm, Pseudomonas 13mm, Klebsilla 20mm Respectively.

Antimicrobial analysis of distilled water extract: (Table-3): By using disc diffusion method, *P. pomoceus* showed maximum zone of inhibition i.e in *E. coli* 18mm, *S. aureus* 17mm, *Pseudomonas* 14mm, *Klebsilla* 15mm Respectively.

FTIR: The FTIR spectrum was used to identify the functional group of the active component present in extract based on the peak values in the region of IR radiation .the result of FTIR analysis confirmed the presence of O-H Stretching, C-H stretching and O=C=O stretching functional groups showed and aromatic C-H stretch carboxylic OH and O-H and N-H stretching show. FTIR spectroscopy is provided to be a reliable and sensitive method for detection of bio molecular composition.

The result of antimicrobial activity on the convenes of present investigation that during a course of activity the ethanol acetone, ethyl acetate and Distilled water extracts found more interactive than acetone extract in material .however the acetone extract found maximum interactive in the *Phellinus pomaceus* and other extract the like ethanol ,ethyl acetate, distilled water showed profound activity and result that is 21mm maximum and 10 mm zone of inhibition were recorded in both the sample.

Conclusion

In the present investigations *Phellinus linteus* synthesized useful metabolites isolation of different active compounds. A protein synthesized by the mushroom is significant. The finding of present work reveals that *Phellinus linteus* (Berk. and M.A. Curtis) proves its powerful antimicrobial activity. Flavonoids and alkaloids extracted from species. *Pseudomonas* and *Klebsilla* are significantly inhibited by chloroform and methanol extracts.

Table-3: FTIR spectral peak value and functional group obtained for the sample *Phellinus pomoceus.*

No.	Peak	Intensity	Corr. Inte	Base (H)	Base (L)	Area	Corr. Are
1	393.5	3.3627	0.0617	412.78	378.06	51.0245	0.1451
2	520.8	3.4527	0.0194	532.38	493.8	56.2763	0.034
3	574.81	3.4388	0.0473	628.82	536.23	135.2106	0.2967
4	659.68	3.4715	0.147	752.27	632.68	171.8004	0.8989
5	771.56	3.9174	0.0548	864.15	756.13	150.5834	0.1951
6	891.15	4.1299	0.0425	918.16	868	69.2939	0.1047

7	1068.61	2.8902	0.721	1138.05	922.01	318.8709	10.7428
8	1153.48	3.3183	0.0819	1188.2	1141.91	67.9378	0.2533
9	1207.49	3.5547	0.0116	1215.21	1192.06	33.5183	0.0218
10	1311.65	3.343	0.0902	1338.66	1219.06	175.1295	0.7094
11	1373.38	3.2628	0.1054	1400.38	1342.51	85.5988	0.4064
12	1423.53	3.2869	0.0728	1438.96	1404.24	51.3577	0.1768
13	1519.97	3.25	0.0529	1523.83	1489.11	50.829	0.2164
14	1550.83	3.0073	0.2067	1585.55	1523.83	93.0394	0.8594
15	1643.42	2.9327	0.1901	1666.57	1589.41	117.2095	1.1956
16	1917.33	5.7451	0.0209	1936.61	1905.75	38.2611	0.0204
17	1952.05	5.7633	0.0081	1959.76	1940.47	23.899	0.0071
18	1994.48	5.7506	0.0097	2006.05	1975.19	38.2633	0.0127
19	2067.78	5.6983	0.0157	2083.21	2009.91	91.0884	0.0678
20	2137.22	5.6943	0.0116	2175.8	2110.22	81.5936	0.0331
21	2333.97	4.3082	0.0403	2337.83	2179.66	200.0331	0.0221
22	2360.97	3.976	0.7595	2391.83	2341.68	67.9029	1.7292
23	2924.21	3.4941	0.5399	2993.65	2395.69	807.7592	6.9776
24	3391	2.7195	2.064	3711.2	2997.51	1058.882	105.5526
25	3738.21	5.4164	0.3094	3780.64	3715.06	81.9036	0.7029
26	3811.5	5.8316	0.0492	3815.36	3784.5	37.8747	0.0775
27	3896.38	5.84	0.0687	3919.52	3884.8	42.7252	0.0851

Sr. No	Sample	Peak	Functional Group
1	*Phellinus pomoceus*	3738.21	O-H Stretching
		2924.21	C-H Stretching
		2360.21 and 2333.97	O=C=O Stretching

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