

In vitro Evaluation of Sesquiterpenoid Extracts of *Phellinus* Samples from Western Ghats of Maharashtra for Antimicrobial Activity

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Summary

Phellinus Quel. is a folk medicinal, wood inhabiting fungus special, recently been studied for its pharmacological activities. In the current study, sesquiterpenes extracted from six species of *Phellinus* viz. *P. fastuosus*, *P. merrillii*, *P. aureobrunneus*, *P. crocatus*, *P. lloydii* and *P. sublinteus* were tested against twelve virulent strains of bacteria and fungi. A significant broad spectrum anti-bacterial and anti-fungal activity of sesquiterpenes isolated from *Phellinus* spp. was revealed through the study.

Key Words: *Phellinus*, Sesquiterpenoids, Anti-bacterial

Introduction

Phellinus species (family: Hymenochaetaceae) receiving special attention due to their potent pharmacological activities including immunostimulation, anti-tumor, anti-oxidant, and antihepatotoxicity activity. *Phellinus* are routinely used as a folk medicine to cure teeth, tongue and throat related ailments to stop excessive salivation in case of children against diarrhea [1,2,3].

In recent years, multiple drug resistant in human pathogenic microorganism is rampant, due to indiscriminate use of antimicrobial drugs. More than sixty antimicrobial compounds have been isolated from mushroom [4].

One species, *Phellinus linteus* extensively used for medicinal properties like anticancer, antitumor, antidiabetic, antibacterial, Hepatoprotective etc. [5,6]. *In vitro* susceptibility testing of *Phellinus merrillii* and *Phellinus swieteiniiae* against *Acinetobacter baumannii* from Maharashtra was done [7].

Phellinus contains many bioactive compounds like triterpenoids, sesquiterpenoids, Polysaccharides, alkaloids, sterols etc. having different type of activities. *Phellinus* is extensively used in Traditional Chinese medicine to treat stomachache, arthritis and cancer of stomach, esophagus and lung [8]. However, not much scientific work has been done on the bioactivity of the fungus especially with reference to the antimicrobial activity of this fungus.

Therefore present investigation aims to study the antimicrobial activity of different *Phellinus* species. In the present study screening of different species of *Phellinus* like *Phellinus adamantinus*, *P. aureobrunneus*, *P. crocatus*, *P. fastuosus*, *P. lloydii*, *P. merrillii*, *P. sublinteus*, against human pathogenic organisms for antimicrobial assay was done.

Material and Methods

Mushrooms

Phellinus samples were collected from Western Ghats of India which were authenticated at Mycology Research Laboratory, Department of Botany, University of Pune. A specimen of this sample has been deposited at the Mycological Herbarium, Forest Research Institute, Dehradun, India.

Extraction of Sesquiterpenoids

Phellinus samples powder (100gm) was suspended in 2 ltr. chloroform ($\times 2$) and left overnight with initial warming. The filtrated solutions were combined and evaporated under vacuum (Medica Instrument MFG.Co., India). The residue thus collected was dissolved in 500 ml of aqueous lead acetate (4% w/v) and 500 ml (95% ethanol) in (1:1 v/v) proportion. The resultant solution was evaporated to dryness under vacuum. The residue (3.1 g) was dissolve in methanol and used for assay [9].

Assay for Antibacterial activity

Bacterial inoculums were prepared from the primary culture by comparing with the turbidity standard. The plates were inoculated with inoculum suspension by using sterile cotton swab. Wells were made with the help of cork boarer (6 mm), and samples extracts were added to the wells by micropipette (80 μ l) in each well also with positive control (solvent in which the component is dissolved). The plates were kept in the incubator at 35-37°C for 18-24 hours, the activity was calculated by measuring the diameter of zone of inhibition [10].

Micro-organisms Used

Table:-Source and Code of Micro-organisms used-

Cultures	Strain	Sources
<i>Acinetobacter calcoaceticus</i> (Ac.)	NCIB 2886	NCL
<i>Bacillus subtilis</i> (Bs.)	NCIM 2010	NCL
<i>Candida albicans</i> (Ca.)	MTCC 1637	IMTECH
<i>Candida albicans</i> (Ca.)	MTCC 3017	IMTECH
<i>Candida albicans</i> (Ca.)	ATCC 2091	NCL
<i>Escherichia coli</i> (Ec.)	MTCC 724	IMTECH
<i>Escherichia coli</i> (Ec.)	MTCC 739	IMTECH
<i>Escherichia coli</i> (Ec.)	ATCC 2046	NCL
<i>Klebsiella pneumonia</i> (Kp.)	MTCC 432	IMTECH
<i>Proteus mirabilis</i> (Pm.)	MTCC 1429	IMTECH
<i>Pseudomonas aeruginosa</i> (Pa.)	ATCC 2036	NCL
<i>Staphylococcus aureus</i> (Sa.)	HAL 2079	NCL

IMTECH = Institute of Microbial Technology Chandigarh India.

NCL= National Chemical Laboratory, Pune India.

Assay of Minimum Inhibitory Concentration (MIC)

The MIC of test samples were carried out by broth dilution method. After the dilution was done, 10µl/ml of the suspension of microbial test was added to each tube, blanks and positive controls were included in the prepared suspension. The test tubes were incubated in incubator at 37°C for 18-24 hours. The test tubes were then observed for growth of microorganisms [11,12].

Results

In the present study the bioactive compounds from the *Phellinus* species were extracted for sesquiterpenes. These compounds were tested for their antimicrobial activity using well assay method against the human pathogenic microorganisms (Table-2&3).

Table 2: Activity of the sesquiterpenoid extracts different *Phellinus* samples against different microorganisms:

Sample	Zone of Inhibition in response to various extracts (mm)											
	A.c	B.s	E.c 724	E.c 739	E.c 2046	K.p	P.a	P.m	S.a	C.a 1637	C.a 2091	C.a 3017
<i>Phellinus fastuosus</i>	26	21	23	20	26.67	28	19	36	23	35	25	32
<i>Phellinus merrillii</i>	25	23	21	19	24	26	20	34	24	33	23.33	30
<i>Phellinus aureobruneus</i>	21	19	18	23	19	19	18	29	20	25	20	18
<i>Phellinus crocatus</i>	16	18	19	20	19	19	17	29	25	20	25	23
<i>Phellinus lloydii</i>	18	25	17	23	18	15	17	21	21	31	27	25
<i>Phellinus sublinteus</i>	15	16	15	16	16	15	15	27	15	22	21	20

Table 3: MIC of Sesquiterpenoid extract of *Phellinus* on different microorganisms

Sample	Zone of Inhibition in response to various extracts (mm)											
	A.c	B.s	E.c 724	E.c 739	E.c 2046	K.p	P.a	P.m	S.a	C.a 1637	C.a 2091	C.a 3017
<i>Phellinus fastuosus</i>	32	32	32	32	32	16	32	16	32	16	32	32
<i>Phellinus merrillii</i>	32	32	32	32	32	16	32	16	32	16	32	32
<i>Phellinus aureobruneus</i>	32	32	32	32	32	16	32	16	32	16	32	32
<i>Phellinus crocatus</i>	32	32	32	32	32	16	32	16	32	16	32	32
<i>Phellinus lloydii</i>	32	32	32	32	32	16	32	16	32	16	32	32
<i>Phellinus sublinteus</i>	32	32	32	32	32	16	32	16	32	16	32	32

During the present investigation, it was observed that sesquiterpenoid extracts of the *Phellinus* spp i.e. *Phellinus aureobruneus*, *P. crocatus*, *P. fastuosus*, *P. lloydii*, *P. merrillii*, *P. sublinteus* exhibited strong activity (15-36mm) against all tested gram negative and gram positive microorganisms (Table 2).

All the bacterial strains studied were found to be sensitive to the sesquiterpenoid extracts of all *Phellinus* spp.

Species of *Phellinus* showed strong activity against the *Candida albicans* strains. *Phellinus fastuosus* and *Phellinus merrillii* showed maximum activity against all the bacteria with

inhibition zone in the range 19-36mm, when compared with other *Phellinus* spp.

The values of minimum inhibitory concentration (MIC) of all the microorganisms were recorded in between 16-32 µl/ml (Table 3). Sesquiterpenoid extracts of all the *Phellinus* spp. exhibited strong activity against *Proteus mirabilis* when both zone of inhibition and MIC values were considered (Table 2 and 3) suggesting that it was more sensitive organism amongst the studied organism.

Similarly, comparing the MIC values of the sesquiterpenoid extracts of all the *Phellinus* spp. showed significant activity against the *Klebsiella pneumonia*, *Candida*

albicans and *Proteus mirabilis* (Table 3). *Pseudomonas aeruginosa* was found to be most resistant organism amongst the studied.

Conclusion

The finding of MIC values indicates that the sesquiterpenoid extracts had -statistic effect at lower concentration, suggesting a low dose of *Phellinus* is required to inhibit all the bacterial strains. The sesquiterpenes from *Phellinus* exhibits broad spectrum anti bacterial activity and anti fungal activity. In this event sesquiterpenoids from the mushroom *Phellinus* has antibacterial activity and it has potential biopharmaceutical prospectus in future.

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