Hiralal Sonawaneet al. / Journal of Pharmacy Research 2014,8(7),1008-1013

Research Article
ISSN: 0974-6943

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Antimicrobial activity of *Phellinus* and *Ganoderma* samples against human pathogenic organisms

Hiralal Sonawane[1*], Bapat Gauri[2], Bhosle Shekhar[3], Ghole V. S[4]
[1]Anantrao Pawar College, Pirangut, Tal: Mulshi, Dist: Pune 412115,Maharastra, India.
[2]Department of Botany, University of Pune, Ganeshkhind, Pune-411 007,Maharastra, India.
[3]Science and Technology Park, University of Pune campus, Pune-411 007,Maharastra, India.
[4]National Institute of Virology, Pashan Pune,Maharastra, India.

Received on:16-05-2014; Revised on: 22-06-2014; Accepted on:23-07-2014

ABSTRACT

Medicinal mushroom contains the bioactive compound like polysaccharides, terpens, proteins having bioactivity. *Phellinus and Ganoderma* has been used effectively in various diseases including improving blood circulation, enhancing detoxication and hepatoprotection, cobmbating allergy, arthritis, cancer, diarrhea, diabetes etc. *Phellinus* which are used by traditional healers in their treatment of infection, with the hope of isolating and identifying the compound responsible for its activity. Several extracts using various solvents have been evaluated for the antimicrobial activity. The extract of *Phellinus* contains different bioactive compounds like sesquiterpens, triterpensetc that may act on *Acinetobacter.* The extract of *Phellinus* was found to be stable in alkaline and acidic conditions. Thus it can be used as an oral antibiotic and it will be potent antibiotic drugs.

KEYWORDS: *Acinetobacter*, Antibiotics, bioactive compounds, *Ganoderma*, *Phellinus*

INTRODUCTION:

The medicinal mushrooms includes both edible and non-edible species such as *Ganoderma lucidum, Lentinus edodes, Phellinus linteus, Poriacocos, Auricularia auricular, Hericium erinaceus, Flammulina velutipus, Pleurotus ostreatus, Trametes versicolor, Tremella fuciformis* and *Cordyceps sinensis*[1,2].

ß-glucan polysaccharides isolated from mushroom is well known for immuno-modulatory properties, however the researchers and health care professionals are now aware that many secondary metabolites, extra cellular secretions of mycelia have antibacterial, antiviral properties. Also the exudates from mushroom mycelia are active against *Plasmodium falciparum* (protozoa that cause malaria) and other micro-organisms[3].

In a recent *in vitro* study, extracts of 204 mushroom species (polypores and gilled mushroom) were tested and 75% of polypores and 45% of agarics showed antimicrobial activity, inhibiting growth of wide variety of micro-organisms. Species of *Ganoderma* was effectively active against *Bacillus subtilis* and less active against Gram negative *E. coli* and *Pseudomonas aeruginosa*. Polyacetylenes also occur frequently in mushrooms, which are responsible for anti-bacterial activities[4].

Phenolic compounds have attracted much attention recently because *In vitro* and *In vivo* studies suggest that they have a variety of beneficial biological properties, which may play an important role in the maintenance of human health [5].

Phellinus is another potent mushroom which has not been extensively studied for medicinal uses in comparison with other. *Phellinus* mushroom is used for the treatment of palsy, gonorrhoea and abdominal pain and effective for helping urination disorders, stomach problems, hematuria, grips, lymphatic tumor and diarrhea [6]. Several fungi belonging to genera *Phellinus* have been used as traditional medicines for treatment of gastrointestinal cancer, liver or heart diseases, and stomach ailments without adverse effects[7]. *Phellinus* mushroom contains many bioactive compounds like triterpenoids, sesquiterpenoids, Polysaccharides, alkaloids, sterols etc. having different type of activities[10].

Phellinus usually used in traditional oriental medicine and has been

***Corresponding author.**
Hiralal Sonawane
Anantrao Pawar College, Pirangut,
Tal: Mulshi, Dist: Pune 412115,
Maharastra, India.

reported to have many pharmaceutical attributes, including anti-mutagenicity and anti-cytotoxicity, anti-cancer as well as immune enhancer [11,12,13]. *Phellinus* spp. Showed significant activity against the *Klebsiella pneumonia*, *Candida albicans* and *Proteus mirabilis*[14].

General mode of action of the antimicrobial compounds

Based on the effectiveness of the drugs these are primarily classified into two categories as a narrow spectrum drugs which are effective only against a very limited variety of pathogen while others are broad spectrum drugs which attack different kind of pathogen. Drugs may be further classified based on their action against a particular group of microorganisms as antimicrobial, antifungal, antiprotozoal and antiviral. Some antimicrobial drugs can be effective against more than one group e.g sulfonamids are active against bacteria and some protozoa. Furthermore the mode of action is to interfere with microbial metabolism without producing a like effect in a host cell[10].

***Human pathogenic microorganisms*:**

The most bacteria that live on the skin or in the gut of human and are usually harmless but can cause disease under some certain condition. Some species of bacteria are known as pathogen but affect less while some are highly pathogenic and are lethal. Some bacteria had caused terrible epidemics in part and today are much less dangerous; the reason could be the development of vaccines of resistance in humans[15].

***Mushroom as antimicrobial agent*:**

Several pathogenic microorganisms gradually developed resistance to the available antibiotic infections by multidrug resistant. Isolates of *Candida* species, *Staphylococcus aureus*, *Streptococcus* species, *Enterococcus* species and *Escherichia coli*, became more and more resistant. This stimulated the search for new antibiotics with novel mechanisms of action

Mushroom which are used by traditional healers in their treatment of infection, with the hope of isolating and identifying the compound responsible for its activity. Several extracts using various solvents have been surveyed for the antimicrobial activity[8,9].

MATERIAL AND METHODS

***Preparation of Extracts of Phellinus and Ganoderma samples*[2]**

Mix basidiocarp powder in solvents (acetone, methanol and ethyl acetate) respectively. Incubate in water bath at 60°C for 4-6 hours. Filter and concentrated it. Solvent is added to dissolve the extract. Extracted samples are used for further experiments.

Broth Tube Dilution Method

A set of tube containing broth medium was prepared. To the first tube, known amount of *Phellinus* sample extract was added and the solution was mixed thoroughly. This was followed by serial dilution. Incubate at 37°C for 24 hours.

Antimicrobial Assay by Disc Diffusion Method

Acinetobacter strains were revived and suspension of each strain was spread on Nutrient agar. Sterile Whatmann filter paper discs were placed on these plates. 40 µl of the extracted samples were loaded on the respective discs and incubated at 37°C for 24 hours.

Plasmid Extraction (Alkali Lysis Method)

a) Treated samples

Acinetobacter strains with extract of *Phellinus* in Luria broth were incubated at 37°C for 24 hrs.

b) Untreated samples

Acinetobacter strains in Luria broth were incubated at 37°C for 24 hrs.

Centrifuged culture and resuspend in alkaline lysis solution I. Add alkaline lysis solution II. Mix the contents and incubate it on ice-bath for 10 minutes. Add Alkaline Lysis solution III. Centrifuged and add equal volume of Phenol:Chloroform (25:24) to supernatant. Transfer the aqueous upper layer to a fresh tube by a centrifugation at 4°C. Add sodium acetate into supernatant. Dissolve the DNA pellet in 50 µl of TE buffer (P^H=8). Load the sample on the 1% agarose gel.

Determination of the shelf life of the Phellinus extract:

The effect of incubation time on antimicrobial activity of *Phellinus* extract was determined by storing the extract at room temperature as well as 4°C in the refrigerator for a period of 0 time, 2-6 months. After the specified incubation period, the antimicrobial activity of the extract was tested by disc diffusion against *Acinetobacter* strains. The results were compared with inhibition zone produced by fresh *Phellinus* extract[16].

***Effect of pH on activity of Phellinus extract*[17]**

Antimicrobial activity of *Phellinus* against *Acinetobacter* was evaluated against different values, by disc diffusion method on Nutrient agar. The pH of the extract was changed using the following buffers acetate buffer (pH 3,4,5), phosphate buffer (pH 6,7,8), Tris HCL buffer (9) and carbonate-bicarbonate buffer (pH 10,11). An aliquot (50 µl) of extract was added separately to 50 µl of each buffer. After 1hr incuba-

tion at 37°C, 10 µl of each solution was used to impregnate separately the sterile discs on nutrient agar seeded with *Acinetobacter* strains. The discs impregnated with 10 µl of each buffer and incubated it.

RESULTS AND DISCUSSION

Plasmid isolation

In the case of untreated samples where the organisms alone were incubated respectively, showed a good banding pattern at their respective lanes. While in case of treated samples where the organisms were incubated with the extracts respectively, showed presence of no bands.Thus it can be concluded that the *Phellinus* extract might be acting at the genomic level of the bacteria, contributing to its inhibition (Fig 1).

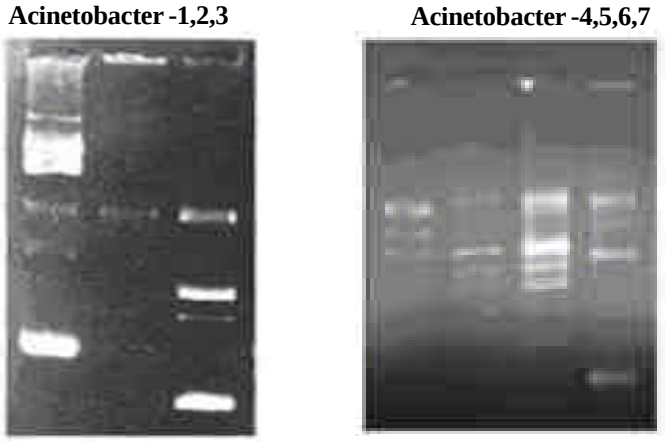
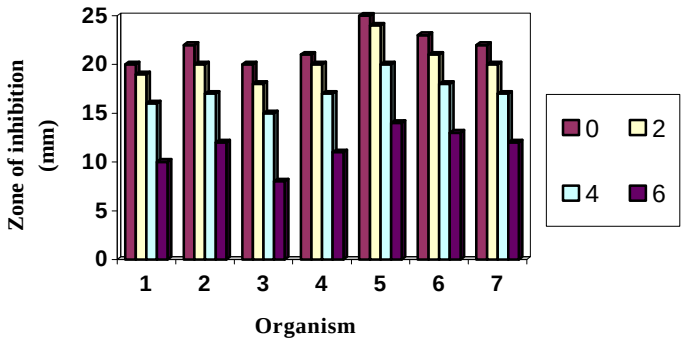


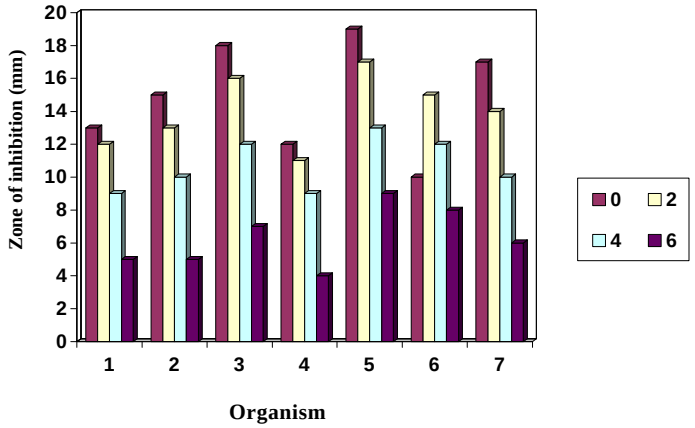
Fig 1: Plasmid isolation of untreated samples

Shelf life of the Phellinus extract

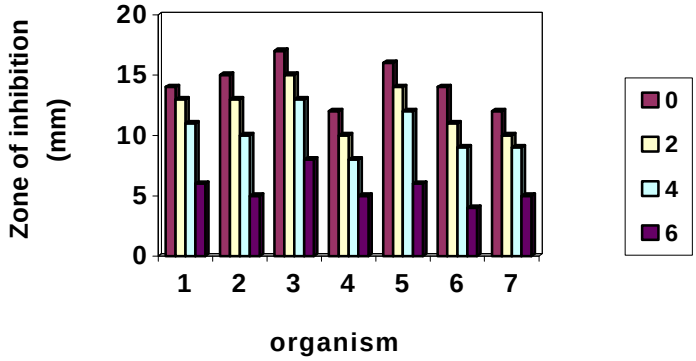
It was observed, however that the extract retains activity upon staring at 4°C upto six months. But the extract kept at room temperature decreases the activity after about four months. It is therefore seemed that the extract needs to be kept at 4°Cif it has to be administered as an antibiotic for effective use (Graph 2,3,4).



Graph 2:- Shelf life of *Phellinus* species extracted by using acetone extract.



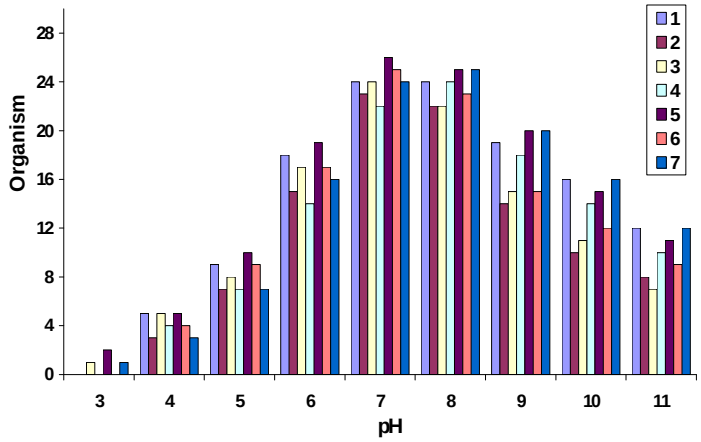
Graph 3:- Shelf life of Phellinus species extracted by using methanol extract.



Graph 4:- Shelf life of *Phellinus* species extracted by using ethyl acetate extract.

Stability of antimicrobial activity of Phellinus extract at different pH

The *Phellinus* extract showed good activity at different pH values ranged from 4-11. Thus the *Phellinus* extract was found to be stable at alkaline and acidic conditions. Hence it can be used as an oral antibiotic (Graph 5).



Graph 5:- Stability of antimicrobial activity of *Phellinus* extract at different pH against *Acinetobacter*

Antimicrobial activity Ganoderma against different bacterial strains:

Ganoderma is a medicinal mushroom used for antimicrobial activity against *Escherichia coli* 2064, *Candida albicans* 3017, *Bacillus subtilis, Staphylococcus aureus, Escherichia coli* 724, *Candida albicans*1637, *Acinetobacter strains.* All these organisms showed good inhibitory effects at the concentration 20 µl. But the *Acinetobacter* strains were resistant to the extract (Table 1 & 2).

Table 1: Antimicrobial activity *Ganoderma* sample 1 against different bacterial strains

Solvent used→ Organism↓	Acetone	Methanol	Ethyl Acetate
	Zone of inhibition (mm)		
Escherichia coli 2064	12	10	06
Candida albicans 3017	***12***	***08***	***08***
Bacillus subtilis	11	12	10
Staphylococcus aureus	13	10	10
Escherichia coli 724	09	11	07
Candida albicans 1637	11	10	09
Acinetobacter strains	-	-	-

Table 2: Antimicrobial activity *Ganoderma* sample 2 against different bacterial strains

Solvent used→ Organism↓	Acetone	Methanol	Ethyl Acetate
	Zone of inhibition (mm)		
Escherichia coli 2064	10	10	10
Candida albicans 3017	12	09	09
Bacillus subtilis	12	10	07
Staphylococcus aureus	15	09	10
Escherichia coli 724	14	11	07
Candida albicans 1637	13	10	08
*Acinetobacter*strains	-	-	-

Total yield of Phellinus species extracted in different solvents:

The respective extracts in ethyl acetate, methanol and acetone solvents were screened against the seven strains of *Acinetobacter* for the activity. The acetone and ethyl acetate have same polarity but they differ in their activity. The Methanol extract have less polarity as compared to acetone and ethyl acetate, hence it shows comparatively good activity as compared to ethyl acetate. This indicated that the active compound has high polarity and present in acetone extract (Table 3).

Table 3:-Total yield of *Phellinus* extract made at different solvent.

Solvent	Polarity	Yield
Acetone	5.1	2.6%
Methanol	4.4	0.86%
Ethyl Acetate	5.1	5.3%

Broth tube dilution method

After the respective incubation of all the seven strains of *Acinetobacter* with the *Phellinus* extracts, there was no growth or turbidity observed. It concludes inhibitory action of *Phellinus* against *Acinetobacter* strains (Table 4).

Table 4: Result for broth tube dilution method.

Solvent used→ Organism↓	Acetone	Methanol	Ethyl Acetate
1	-	-	-
2	-	-	-
3	-	-	-
4	-	-	-
5	-	-	-
6	-	-	-
7	-	-	-
Positive control	+	+	+
Negative control	-	-	-

(-):-No Turbidity, (+) :-Turbidity

Table 5: Antimicrobial sensitivity test of clinical isolates of *Acinetobacter* strains by Disc Diffusion Method.

Organism→ Solvent used↓	1	2	3	4	5	6	7
	Zone of inhibition (mm)						
Acetone	2	2.2	1.5	1.9	2.5	2.3	2.2
Methanol	1.3	1.5	1.8	1.2	2.0	1.6	1.7
Ethyl Acetate	1.4	1.5	1.7	1.2	2.0	1.4	1.2

Antimicrobial susceptibility testing:

The *Acinetobacter* strains showed significant inhibitory results with respect to acetone extract as compared methanol and ethyl acetate extracts. *Acinetobacter* strains were tested against Erythromycin and Ampicillin. It was observed that all strains were resistant to both the antibiotics (Fig 2).

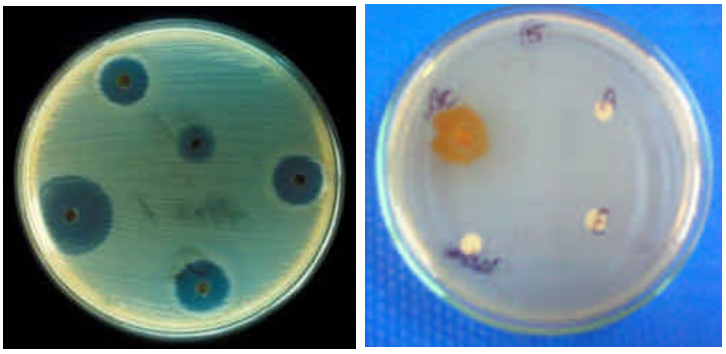


Fig: 2
A] Antimicrobial susceptibility testing of *Phellinus* against *Acinetobacter* strains.

B] Antimicrobial susceptibility testing of *Phellinus* against Erythromycin and Ampicillin.

Plasmid isolation:

Organisms were incubated respectively, showed a good banding pattern at their respective lanes. While in treated samples the organisms were incubated with the extracts respectively, showed presence of no

bands. Thus it can be concluded that the *Phellinus* extract might be acting at the genomic level of the bacteria, contributing to its inhibition (Fig 1).

Shelf life of the Phellinus extract

It was observed, however that the extract retains activity upon staring at 4°C up to six months. But the extract kept at room temperature decreases the activity after four months. It is therefore concluded that the extract needs to be kept at 4°C and shows potent activity for administered as an antibiotic for effective use (Graph 2,3,4).

Stability of antimicrobial activity of Phellinus extract at different pH:

The *Phellinus* extract showed good activity at different pH values ranged from 4 to 11. Thus the *Phellinus* extract was found to be stable at alkaline and acidic conditions. Hence it can be used as an oral antibiotic if its other characteristics are suitable as recommended for antibiotics (Graph 5).

CONCLUSION

The active compounds of acetone and methanol extracts have the same polarity but they showed different activity. The antimicrobial assay showed zone of inhibition against different strains of *Acinetobacter* and acetone extract has potent activity. Therefore, the extract of *Phellinus* may contain different bioactive compounds like sesquiterpens, triterpens etc that may act on *Acinetobacter.* The extract retains activity upon staring at 4°C up to six months but the extract kept at room temperature looses the activity after two months. The extract of *Phellinus* was found to be stable in alkaline and acidic conditions. Thus it can be used as an oral antibiotic.

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Source of support: Nil, Conflict of interest: None Declared