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Research article

Evaluation of antimicrobial activity of *Phellinus linteus* (Berk. & M.A. Curtis.) with their wild collections from Western Ghats of India

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Abstract: A survey was conducted in Sagar taluk Shivamogaa district, in the month of June to August 2015 to collect the *Phellinus linteus* from the wood logs of Jack fruit. The samples were dried and powdered, subjected to extraction using petroleum ether, chloroform and methanol successively by soxhlet method. Extracts were tested for secondary metabolites and showed the presence of alkaloids, phenols, sterols and flavonoids. The sporocarp extract was screened for their antimicrobial activity against different human pathogens like *Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus, Candida albicans, Chrysosporium keratinophilum, Pencillium chrysogenum, Aspergillus niger* and plant pathogens like *Xanthomonas campestris* and *Agrobacterium tumefacien*s using agar well diffusion method. The methanol extracts showed maximum activity followed by chloroform, petroleum ether. The inhibition effects on fungi were low compared to bacteria. GCMS analysis showed the presence of ergosterol. The study reveals that *Phellinus linteus* showed good antimicrobial activity against *Candida albicans* and *Staphylococcus aureus*.

Keywords: *Phellinus linteus* - Sporocarp - Extract - Pathogens - GCMS - Antimicrobial.

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INTRODUCTION

Macrofungi are well known for their valuable food and traditional medicines around the world since times. Mushrooms are used as antitumor, antiviral, anti-allergants (Wisitrassameewong *et al.* 2012) and anti-inflammatory (Mourao *et al.* 2011). Elements from sporocarp and mycelia both contain antimicrobial compounds which can be isolated for the welfare of human beings (Yamac & Bilgili 2006). Antimicrobial includes antibacterial, antifungal and antiviral (Guler *et al.* 2009, David *et al.* 2012). *Phellinus* Quél. is a potent mushroom as *Ganoderma*. The secondary metabolites of Phellinus were used to cure gonorrhoea, abdominal pain, stomach ailments and diarrhoea (Sonawane *et al.* 2012). Few species of *Phellinus* are tested and proved to be good antimicrobial nature. *Phellinus baumii* Pilát showed good hypoglycaemic effects against ob/ob mice (Cho *et al.* 2007). *Phellinus* are wood inhibiting fungi and may be used as antioxidant and anticancerous (Khatun 2012). *Phellinus hartigii* (Allesch. & Schnabl) Pat., *P. swieteniae* (Murrill) S. Herrera & Bondartseva, *P. merrillii* (Murrill) Ryvarden are antimicrobial nature (Altuner & Akata 2010, Belsare *et al.* 2010). *P. igniarius* (L.) Quél. proved good antiviral agent against influenza virus (Song *et al.* 2014). *P. durissimus* (Lloyd) A. Roy and *P. linteus* (Berk. & M.A. Curtis) shows antioxidant activity (Liang *et al.* 2009, Lahiri *et al.* 2010). Out of these all, *Phellinus linteus* is a medicinal mushroom with a history of about 2000 years of being used for the treatment of haemorrhage, haemostasis and menstruation in China and Korea (Chen *et al.* 2016). Thus the present work is an attempt to find out the antimicrobial activity of *Phellinus linteus* (Berk. & M.A Curtis.) which found in Sagara on the jack fruit (*Artocarpus heterophyllus* Lam.) tree which prove good antibiotic for the welfare of the human beings.

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MATERIALS AND METHODS

Sample collection and preparation

The fruiting bodies of *Phellinus linteus* (Berk. & M.A Curtis.) are collected from the Sagara taluk, Shivamogga district, during June to August 2015. The major field characters of *Phellinus linteus* were recorded. The sporocarps were brought to the laboratory and morphological characters were recorded. The samples were oven dried at 45–50°C. The fruiting bodies were ground to a coarse powder using a mixer. Three hundred grams of the same material was subjected to Soxhlet extraction for 24 hrs each, using 1000 mL of three solvents, petroleum ether, chloroform and methanol respectively. Secondary metabolites were extracted from sporocarp of *Phellinus linteus* extracts were dried to powder and kept at 4°C (Dulger *et al.* 2004, Sridhar *et al.* 2011). These three extracts were screened against different pathogenic fungal and bacterial species by the well diffusion method. The test organisms were collected from the Microbial Type Culture Collection (MTCC), The Institute of Microbial Technology. Chandigarh, India.

Bacteria used to check antibacterial activities

Xanthomonas campestris [MTCC-2286], *Pseudomonas syringae* [MTCC-1604], *Agrobacterium tumefaciens* [MTCC-431], *Klebsiella pneumonia* [MTCC-7028], *Escherihia coli* [MTCC-1559], *Salmonella typhi* [MTCC-734], *Pseudomonas aeruginosa* [MTCC-1934], *Staphylococcus aureus* [MTCC-4734] and *Streptomyces pneumoneae* [MTCC-4734].

Fungi used to check antifungal activities

Candida albicans [MTCC-1637], *Chrysosporium merdarium* [MTCC-4608], *Trichophyton rubrum* [MTCC-3272], *Chrysosporium keratinophilum* [MTCC-1367], *Fusarium solani* [MTCC- 1040], *Penicillium chrysogenum* [MTCC-947], *Aspergillus flavus* [MTCC-1783] and *Aspergillus niger* [MTCC-514].

Preparation of extract

100% = 400 mg in 4 ml of DMSO, 50% = 200 mg in 4 ml of DMSO, 25% = 100 mg in 4 ml of DMSO.

Agar Well diffusion method

The antibacterial and antifungal activity of the mushroom extracts were tested using Agar well diffusion method (Sridhar *et al.* 2011). The culture plates were prepared by inoculating with different bacteria and fungi. Wells were made with 6 mm cork borer. The wells were loaded with extracts which were dissolved in dimethyl sulfoxide (DMSO) of different concentration (100%, 50% and 25%) using micro pipette. Ciprofloxacin for bacteria, Terbinafine for fungi were used as standard and DMSO was used as control for test microorganisms. The plates were incubated at 27±2 ºC for 24 hrs for bacterial activity and 48 hrs for fungal activity (Das *et al.* 2010). The zone formation was observed in plates around the wells and calculated by measuring the diameter of the inhibition zone around. The readings were taken in 4 replicates and the average values were tabulated.

Physicochemical analysis

a. Determination of Foreign Matter: One gram of sample was weighed and foreign matter was carefully separated. The matter differing in colour and texture were considered as foreign. The separated matter was weighed and subtracted from one gram and percentage was calculated.

b. Determination of Moisture Content: One gram of powder was weighed and dried at 80°C for 24 hrs in hot air oven. After 24 hrs, the powder was weighed again and the difference in the weight was determined. The percentage of moisture was calculated.

c. Determination of pH: The 5% (w/v) (5 g in 100 ml of water) powder was kept on shaker for 5 h with 140 rpm and filtered. The filtrate was analysed for the pH using pH meter (Elico, India) (Iqbal *et al.* 2010).

d. Determination of Water Soluble Extractive: Five Grams of powder was weighed and added into a 100 ml conical flask, 25 ml of distilled water is added into to it and kept on a rotator shaker (140 rpm) for 24 hrs. After 24 hrs it was filtered and dried in hot air oven at 80ºC for 24 hrs and weighed again. The difference in weight was determined and percentage of water soluble extractive were calculated (Gupta 2003).

e. Determination of Alcohol Soluble Extractive: Five grams of powdered material is taken in a 100 ml conical flask, 25 ml of absolute alcohol is added to it and kept on rotator shaker at 140 rpm for 24 hrs. After 24 hrs it was filtered and dried in hot air oven set at 80ºC for 24 hrs and weighed again. The difference in weight was determined and percentage of Alcohol soluble extractive were calculated (Gupta 2003).

f. Determination of Total Ash Content: The clean and dried silica crucible was weighed, 10 g of powder was taken and kept in muffle furnace and heated up to 300ºC for 3–4 hrs until the whole powder turns into ash. The crucible was cooled and weighed again. The difference in the weight was calculated. (Gupta 2003, Indrayan *et al.* 2005).

g. Determination of Water Soluble Ash: 1 g of powder was weighed and 10 ml of distilled water is added into it. The mixture was kept on a shaker with 140 rpm for 8h and filtered through ash less filter paper. The ash remained in the paper was kept in a crucible (silica) and burnt ash again in a muffle furnace for 3–4 hrs. The weight of ash obtained was noted and percent of water soluble ash was determined (Ahmad & Sharma 2001).

h. Determination of Acid Insoluble Ash: 1gm of ash was weighed and 10 ml of distilled water is added into it. The mixture was kept on a shaker with 140rpm for 8 h and faltered through ash less filter paper. The ash remained in the paper was kept in a crucible (Silica) and burnt to ash again in a muffle furnace for 3–4 hrs. The weight of ash obtained was noted and percentage of acid insoluble ash was determined. (Ahmad & Sharma 2001).

RESULTS AND DISCUSSION

Table 1. Result of physicochemical analysis of extract.

Physicochemical Parameters	%
Foreign Matters	0.80
Moisture Content	7.30
Water Soluble Extractive	8.88
Alcohol Soluble Extractive	3.20
PH	6.64
Determination Of Ash	21.60
Water Soluble Ash	85.00
Acid Insoluble Ash	21.00

Physicochemical analysis showed 0.8% of foreign matter, 7.3% of moisture content 8.88% of water soluble extractive, 3.2% of alcohol soluble extractive; pH is 6.4, 21.6% of determination of ash, 85% of water soluble ash and 21% of acid insoluble ash (Table 1). The extracts were tested for presence of secondary metabolite. Petroleum ether showed the presence of alkaloids and tannins. Chloroform extracts showed positive to alkaloids, tannins, steroids, glycosides and phenols. Methanol showed the presence of flavonoids, glycosides triterpenoids and phenols (Table 2).

Table 2. Phytochemicals test of *Phellinus linteus* (Berk. & M.A Curtis.).

Tests	Pet, ether	Chloroform	Methanol
Alkoloids	+	+	+
Saponins	–	–	–
Tannins	+	+	+
Flavonoids	–	–	+
Steroids	–	+	+
Glycosides	–	+	+
Triterpenoids	–	–	+
Phenols	–	+	+

The extracts showed varied antimicrobial result when tested against pathogens tables 2. The petroleum ether extract inhibited *Salmonella typhi* and *Staphylococcus aureus* (12 mm) at maximum. *Agrobacterium tumefaciens, Klebsiella pneumonia, Pseudomonas aeruginosa* were (10 mm) moderately inhibited. *Xanthomonas campestris, Pseudomonas syringae, Escherichia coli, Streptomyces pneumoneae* were (8–0) least inhibited by petroleum ether extracts. The fungal pathogens like *Trichophyton rubrum, Fusarium solani, Aspergillus flavus* and *Aspergillus niger* were completely resistant showing no inhibition whereas *Chrysosporium keratinophilum* was inhibited maximum that of other test organism. *Candida albicans* and *Chrysosporium merdarium* were inhibited moderately.

Chloroform extracts inhibited test organisms better than petroleum ether. *K. pneumonia* and *S. aureus* were inhibited *maximum* by showing 16 and 17 mm zone. The chloroform extract inhibited *P. syringae, E. coli, P. aeruginosa, S. pneumoneae, A. tumefaciens* and *S. typhi* moderately by showing 13–14 mm inhibition zone. Whereas *X. campestris* were least inhibited with 8 mm (Table 3, Fig. 1). *F. solani* (22 mm) had the maximum

effect of chloroform extract followed by *A. niger* and *C. keratinophilum* (20 mm). *C. merdarium*, *T. rubrum* and *P. chrysogenum* were moderately affected with 18–19 mm inhibition zone. *A. flavus* and *C. albicans* were least inhibited by chloroform extract (Table 4, Fig. 2).

Table 3. Antibacterial activity of *Phellinus linteus* (Berk. & M.A Curtis.) at different concentration and different solvent.

S.N.	Name of pathogen	Zone of inhibition in mm									Standard
		Petroleum ether			Chloroform			Methanol			
		100 %	50%	25%	100 %	50%	25%	100 %	50%	25%	
1	Xc	08±0	0	0	08±0.5	06±1.0	0	20±1.0	15±2.0	09±1.5	19±1.0
2	Ps	08±0	0	0	14±0.6	10±2.0	7±1.0	24±1.5	16±1.0	10±1.5	25±1.0
3	At	10±0	0	0	13±0.5	10±1.6	8±1.0	30±0.5	24±1.0	15±1.0	36±1.0
4	Kp	10±0	0	0	16±1.0	10±0.7	6±0.8	25±2.0	20±1.5	15±1.0	28±2.0
5	Ec	09±0	0	0	15±1.0	08±2.0	0	24±1.5	20±1.5	18±1.0	24±2.0
6	St	12±0	8±1	0	13±0.5	10±1.4	6±1.0	24±2.3	20±1.3	18±2.0	26±1.5
7	Pa	10±0	0	0	14±1.0	10±1.0	8±1.0	28±1.2	20±1.5	10±2.0	28±1.0
8	Sa	12±0	8±1	0	17±0.8	13±0.7	9±1.5	28±2.0	19±1.5	10±2.0	30±1.2
9	Sp	08±0	0	0	14±0.8	10±0.5	6±1.0	22±1.2	15±1.3	9±1.4	25±1.0

Note: Mean of 3 replicates for each concentration; XC= *Xanthomonas campestris*, Ps= *Pseudomonas syringae*, At= *Agrobacterium tumefaciens*, Kp= *Klebsiella pneumonia*, Ec= *Escherihia coli*, St= *Salmonella typhi*, Pa= *Pseudomonas aeruginosa*, Sa= *Staphylococcus aureus*, Sp= *Streptomyces pneumoneae*.

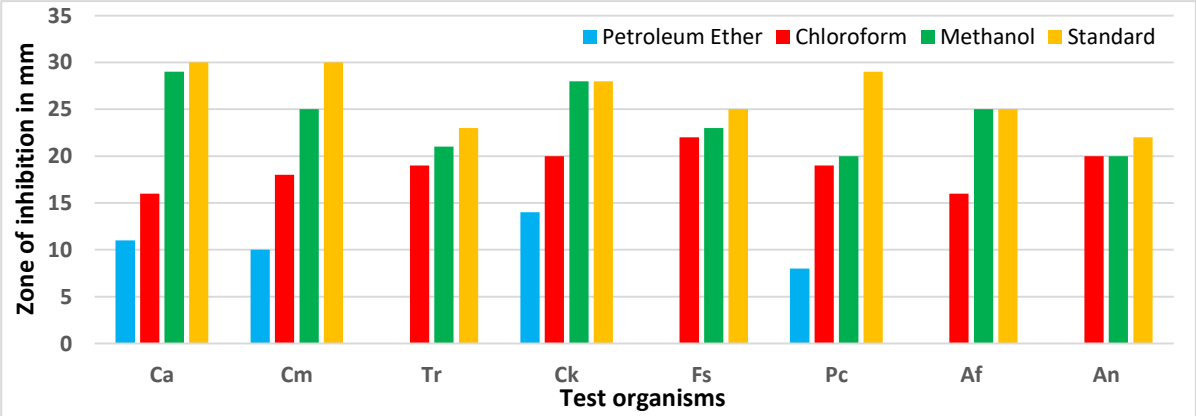

Figure 1. Antifungal activity of *Phellinus linteus* (Berk. & M.A Curtis.) at 100% concentration of different solvent extract. [Ca= *Candida albicans*, Cm= *Chrysosporium merdarium*, Tr= *Trichophyton rubrum*, *Ck= Chrysosporium keratinophilum*, Fs= *Fusarium solani*, Pc= *Penicillium chrysogenum*, Af= *Aspergillus flavus*, An=*Aspergillus niger*]

Table 4. Antifungal activity of *Phellinus linteus* (Berk. & M.A Curtis.) at different concentration and different solvent.

S.N.	Name of pathogens	Zone of inhibition in mm									Standard
		Petroleum ether			Chloroform			Methanol			
		100%	50%	25%	100%	50%	25%	100%	50%	25%	
1	Ca	11±1.6	10±2.0	08±1.0	16±0.0	12±0.5	10±0.6	29±1.5	22±2.0	19±1.5	30±2.3
2	Cm	10±0.0	08±1.0	0	18±1.0	10±0.5	08±0.5	25±1.5	19±1.5	15±1.0	30±1.0
3	Tr	0	0	0	19±1.2	16±1.0	14±0.5	21±1.0	19±1.2	14±0.4	23±1.2
4	Ck	14±1.0	08±0.5	0	20±1.3	13±0.5	06±0.0	28±2.0	25±2.2	20±1.7	28±0.5
5	Fs	0	0	0	22±1.8	19±1.8	15±1.0	23±1.2	20±1.7	15±1.0	25±1.4
6	Pc	08±2.0	06±0.5	0	19±0.4	14±0.5	09±0.7	20±1.0	15±1.0	11±0.2	29±1.2
7	Af	0	0	0	16±1.0	14±1.5	12±0.6	25±0.5	20±2.0	15±1.0	25±1.0
8	An	0	0	0	20±2.2	14±0.5	08±0.5	20±0.5	15±0.8	10±0.4	22±1.1

Note: Mean of 3 replicates for each concentration; Ca= *Candida albicans*, Cm= *Chrysosporium merdarium*, Tr= *Trichophyton rubrum*, Ck= *Chrysosporium keratinophilum*, Fs= *Fusarium solani*, Pc= *Penicillium chrysogenum*, Af= *Aspergillus flavus*, An= *Aspergillus niger*.

The GCMS analysis of methanol extract showed the presence of ergosterol. This agrees with the result of (Reis *et al.* 2014) which is anti-tumor component (Chen *et al.* 2016). *A. tumefaciens S. aureus* and *P. aeruginosa* showed more susceptibility to methanol extract followed by *K. pneumonia*, *S. typhi*, *P. syringae*, whereas *X. campestris* was resistant to methanol extract. *C. keratinophilum* and *C. albicans* were maximum inhibited by methanol extracts, almost equal to inhibition zone of standard drug. *C. merdarium*, *F. solani*, *A.*

flavus showed less susceptibility than the *C. keratinophilum* and *C. albicans* but *T. rubrum*, *P. chrysogenum* and *A. niger* were least inhibited by methanol extract compared to another test organism (Table 3 and 4). The relative decrease in the inhibition with the concentration of the extract shows that amount of bioactive components in extracts play an important role in inhibition of pathogens. The number of organisms inhibited decrease with the decrease in concentration of extracts. The observed result of the effectiveness of extracts, methanol proved best and petroleum ether showed less inhibition effect which is favourable with the findings of (Ehssan & Saadabi 2012). *S. aureus and E. coli* are inhibited maximum by methanol extract this agrees with the result of (Bala *et al.* 2011). Sonawane *et al.* (2012) reported methanol showed better activity than ethyl acetate, in present work methanol showed good result than chloroform. Antifungal activity of extracts was lower compared to bacteria this agrees with the result of Jonathan & Fasidi (2003, 2005). Oyetayo (2009) studied the effect of ethanol extracts of *Termitomyces* sp. and *Lentinus* sp. against *C. albicans* and *S. aureus*, ethanol extract showed good inhibition against *C. albicans* but *S. aureus* was inhibited hardly by 8 mm inhibition zone. Methanol extract showed good inhibition zone for *E. coli*, *S. typhi*, *P. aeruginosa* and *S. aureus*, this agrees with the result of (Balakumar 2010). When (Iftekhar *et al.* 2011) worked on the antibacterial activity of *Ganoderma lucidum*, *Auricularia auricula* and *Pleurotus florida* against *S. aureus* and *E. coli* none of the three mushroom inhibited *E. coli* but in present study *E. coli* is inhibited in all the concentration by all solvent extracts.

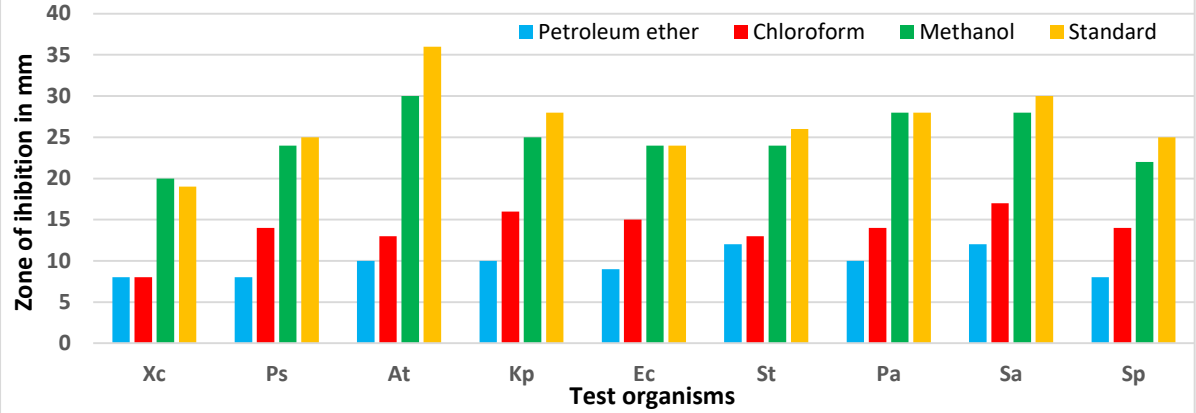

Figure 1. Antibacterial activity of *Phellinus linteus* (Berk. & M.A Curtis.) at 100% concentration of different solvent extract. [Xc=*Xanthomonas campestris*, Ps=*Pseudomonas syringae*, At=*Agrobacterium tumefaciens*, Kp= *Klebsiella pneumonia*, Ec=*Escherihia coli*, St=*Salmonella typhi*, Pa=*Pseudomonas aeruginosa*, Sa= *Staphylococcus aureus*, Sp=*Streptomyces pneumoneae*]

CONCLUSION

Resistance to a wide variety of antibiotics by pathogens and multiple drug resistant organisms has become a serious threat to growing population, there is a need to find new source of antibiotics which are capable of combating several disease causing pathogens. Mushrooms are such a group of organisms which are rich in useful metabolites so further identification and isolation of different active compounds from varieties of mushroom and commercializing their products is necessary. The finding of present work reveals that *Phellinus linteus* (Berk. & M.A. Curtis) proves its powerful antimicrobial activity. *Candida albicans* and *Staphylococcus aureus* are significantly inhibited by chloroform and methanol extracts. Further work is required to culture mycelia and extract metabolites from it so that there is no need to wait for the appropriate season for harvesting fruiting bodies, for the extraction of secondary metabolites and other bioprospecting activities.

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