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In Vitro Antibacterial activity of *Hygrocybe parvula* (Peck) Pegler

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Abstract

The antibacterial activities of petroleum ether, chloroform and methanol extract of mushroom *H. parvula* was analyzed *in vitro* by agar well diffusion method. The yields of extracts obtained from different solvents were petroleum ether (10.88) gm, chloroform (11.21gm) and methanol (51.16gm). The growth inhibitory effects of crude extracts were tested against three plants and six human pathogenic bacteria. The maximum antibacterial activity of petroleum ether extracts of *H. parvula* was found against *A. tumefaciens* (16mm) at 100% concentration and minimum against *X. campestris* (3mm) at lower concentration of chloroform and methanol extract in plant pathogenic bacteria. Whereas petroleum ether extracts were showed more inhibition zone against *P. aeuroginosa* (18mm), at 100% concentration in human pathogenic bacteria, the activity was less than the standard Tetracycline and Ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (12.5% -100%). The antibacterial activity of all the solvent extracts were calculated by measuring inhibition zone formed around the well.

Key-Words: *Hygrocybe parvula*, Antibacterial activity, Tetracycline, Agar well diffusion method

Introduction

Mushrooms are spore bearing fruiting bodies of fungi, typically produced above ground on soil or on food surface. They may be edible, poisonous or indigestible (Surekha et al., 2011). Mushrooms are invaluable sources of useful therapeutic agents (Reshetnikov et al., 2001) in addition to their increasing use as functional foods for the prevention of diseases such as diabetes mellitus, hypertension and cancer (Mau et al., 2002; Perera et al., 2011). This is due to the presence of useful nutrients and secondary metabolites (Manzi et al., 2001). A nutraceuticals can be defined as a substance that may be considered a food or part of a food that provides medical or health benefits like the prevention and treatment of disease. Mushrooms have become attractive as a functional food and as a source for the development of drugs and nutraceuticals (Lakhanpal and Rana, 2005) responsible with their antioxidant, antitumor (Jones and Janardhanan, 2000) and antimicrobial properties.

Mushrooms have been shown to produce several biologically active compounds that are usually associated with cell wall, and these have been suggested to contribute to enhancement of immunity and tumor-retarding effects.

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Among the local communities, mushrooms may represent potential sources of antibacterial drugs, since in the early days, screening for antibiotics started with mushrooms and proved to be successful (Opige et al., 2006). In recent decades, various extracts of mushrooms and plants have been of great interest as sources of natural products (Aziz et al., 2007). Researchers have reported antimicrobial activity of several mushrooms (and Fung, 2004; Gao et al., 2005; Hirasawa et al., 1999). The chloroform and ethyl acetate extracts of the dried mushrooms have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Karaman et al., 2003). In recent years, the most human pathogenic microorganisms have developed multiple drug resistance mechanism, due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various sources of novel antimicrobial chemotherapeutic agents (Pellegrini et al., 1999). Furthermore, in previous studies various biologic activities such as antioxidant, antibacterial, antifungal (Turkoglu et al., 2007).

In the continuous search for new antimicrobial structures, mushrooms are of interest to investigators. Sixty antimicrobial compounds have been isolated from mushrooms; however, only the compounds from

microscopic fungi have been present in the market as antibiotics until now (Wong et al., 2009).

Material and Methods

Fungal material

The *Hygrocybe parvula* were collected from semi evergreen forest region (13˚51'56.30"N, 75˚03'12.50"E) which is located in Haniya, Hosanagar taluk, Shimoga district, Karnataka, India, during the month of June to August 2012. The *H. parvula* of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and air dried in an oven at 40˚ C for 48 h. dried mushroom samples were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics with the help of standard literatures like Purkayastha and Chandra (1985), Singer (1986), Roy and De (1996), Das and Sharma (2005) and Pegler and Spooner (1981).The voucher specimen (KUABARN-63) has been deposited at the herbarium of mycology laboratory, Department of Applied Botany, Kuvempu University, Jnana Sahyadri, Shimoga district, Karnataka, India.

Chemicals

All the solvents *viz.*, petroleum ether, chloroform and methanol used in this study were purchased from HiMedia Laboratoris Pvt. Ltd, of analytical grade.

Preparation of crude extracts

The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 1500 ml pet ether following 1500 ml of chloroform and methanol with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent (Lin et al., 1999). The extracts were concentrated in a vacuum at 40°C using a rotary evaporator. For the entire analysis, compounds of extract were dissolved in dimethylsulfoxide (DMSO). The yield of extracts obtained from pet ether was 10.88 gm, followed by chloroform (11.21gm) and methanol (51.16gm). Each extract was transferred to glass vials and kept at 4°C before use.

Microbial strains

Nine different bacterial strains were used: *Escherichia coli* (MTCC-1698), *Klebsiella pneumoniae* (MTCC-7028), *Pseudomonas aeuroginosa* (MTCC-1934), *Salmonella typhi* (MTCC-733), *Xanthomonas campestris* (MTCC-2286), *Pseudomonas syringae* (MTCC-1604) and *Agrobacterium tumefaciens* (MTCC-431), gram negative. *Staphylococcus aureus* (MTCC-902) and *Streptomyces pneumoniae* (MTCC-4734), gram positive. They were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The viability of the organisms were maintained by regular transfer into freshly prepared nutrient agar (Himedia) and stored at 4˚C until used.

Antibacterial assay

Antibacterial activity was carried out by Agar well diffusion (Perilla et al., 2003). Mueller- Hinton broth was applied for growing and diluting the bacterial suspensions. Bacterial strains were grown to exponential phase in Mueller-Hinton at 37˚C for 18 hrs and adjusted to a final density of 10^8 CFU/ml by diluting fresh cultures and comparison with Mc Farland density, poured into petridishes and allowed then to cool under strict aseptic conditions. After the medium was solidified a well was made in petridishes with the help of a sterile metal borer (6mm). 50µl of each extracts were filled in each well by using adjustable volume digital Finn pipette. After that the plates were incubated at 37˚ C for 24 hrs. After proper incubation, antibacterial activity was determined by measuring the diameter of the zone of the inhibition around the well by using Hi-antibiotic zone scale-c and the activity was compared with standard antibiotic, Tetracycline and Ciprofloxacin (30µg/ml). Simultaneously, control (DMSO) was also maintained without extract. Three replicates were carried out for each extract against each of the test organism.

Results and Discussion

The antibacterial activities of petroleum ether, chloroform and methanol extract of mushroom *H. parvula* was analyzed *in vitro* by agar well diffusion method. The growth inhibitory effect of crude extracts of *H. parvula* were tested against three plants and six human pathogenic bacteria viz., *X. campestris, P. syringae, A. tumefaciens, K. pneumoniae, S. aureus, S. pneumoniae, S. typhi, P. aeuroginosa* and *E. coli*. The antibacterial activity of all the solvent extracts were calculated by measuring inhibition zone formed around the well in millimeter (mm).

The antibacterial activities of *H. parvula* against plant pathogenic bacteria were presented in Table-1. The three organic solvent extracts, showed good activity against all the tested bacteria. The maximum antibacterial activity of petroleum ether extracts of *H. parvula* was found against *A. tumefaciens* (16mm) at 100% concentration, followed by *P. syringae* (14mm) and *X. campestris* (12mm) and moderate against *P. syringae* (13mm) and *A. tumefaciens* (14mm) at 50% concentration, followed by *A. tumefaciens* (3mm), *P. syringae* (4mm) and *X. campestris* (3mm) at lower concentration of chloroform and methanol extract.

The antibacterial effects of different solvent extracts of *H. parvula* were tested against six human pathogenic bacteria and results were tabulated in Table 2. Among

the three organic solvent extracts, showed more effective inhibitory activity against all the tested bacteria. The petroleum ether extracts were showed more active antibacterial proficiency against *P. aeuroginosa* (18mm), *S. typhi* (15mm), *S. aureus* (14mm) and *E. coli* (13mm) at 100% concentration, moderate effect against *S. aeuroginosa* (16mm) followed by *S. typhi* (14mm), *E. coli* (10mm) and *S. pneumoniae* (10mm) at 50% concentration. The methanol and chloroform extract were highly active against *P. aeuroginosa* (18mm) at 100% concentration, followed by *S. typhi* (11mm) and *P. aeuroginosa* (12mm) at 50% concentration. However, the activity was less than the standard Tetracycline and Ciprofloxacin. The extract shows increasing inhibitory activity with increase in concentration (12.5% -100%).

Researchers already reported potent anticancer, hepatoprotective activities of other edible mushrooms (Acharya et al., 2012 and Chatterjee et al., 2007). The results of the present study strengthened the outcomes of earlier works done by others that showed mushrooms produced a great variety of antimicrobial agents. For instance, it is known that the extract from fruit bodies of several *Lactarius* sp. (Bergendorf and Sterner, 1988; Anke et al., 1989); *Fomitopsis* sp. (Keller et al., 1996); *Boletus* sp. (Lee et al., 1999); *Cortinarius* sp. (Nicholas et al., 2001); *Ganoderma lucidum, Navesporus floccosa* and *Phellinus rimosus* (Sheena et al., 2003); *Pleurotus tuber-regium* (Ezeronye et al., 2005); *Amanita caesarae, Armillaria mellea, Chroogomphus rutilus, Clavariadelphus truncates, Clitocybe geotropa, Ganoderma* sp., *Ganoderma carnosum, Hydnum repandum, Hygrophorus agathosmus, Lenzites betulina, Leucoagaricus pudicus, Paxillus involutus, Polyporus arcularius, Rhizopogon roseo, Sarcodon imbricatus, Suillus collitinus, Trametes versicolor, Tricholoma auratum, Tricholoma fracticum* (Yamac and Bilgili, 2006); *Lactarius deliciosus, Sarcodon imbricatus* and *Tricholoma portentosum* (Barros et al., 2006); *Russula delica* (Turkoglu et al., 2007); *Pleurotus eryngii* var. *ferulae (*Akyuz and Kirbag, 2009); *Infundibulicybe geotropa, Lactarius controversus, Lactarius delicious* and *Phellinus hartigii* (Altuner and Akata, 2010); *Lactarius indigo* (Ochoa-Zarzosa, 2011) and *Stereum ostrea* (Praveen et al., 2012) contain a wide range of antimicrobial activity. This difference in response of mushroom extracts to test organisms might be due to a number of factors, as studies suggest that the antimicrobial activities of all mushroom extracts are changeable (Iwalokun et al., 2007), depending upon the nature of environment and media in which it was grown. It also depends upon the genetic structure of mushroom species, physical and biochemical constituents, extraction solvents and test organisms. The sensitivity pattern of microorganisms also changes to chemotherapeutic agents depending on their strains, and susceptibility or resistance to antibiotic (Gao et al., 2005).

Conclusion

Petroleum ether extract was found to be effective against tested plant pathogenic bacteria compared to methanol and petroleum ether, whereas, Petroleum ether extract, was found to be effective against tested human pathogenic bacteria compared to chloroform and methanol. Whole world is frantically in search of new antibiotics because of an alarmingly increase in bacterial resistance to existing antibiotics due to their inappropriate and indiscriminate use. Further studies on isolation and characterization of the active compounds from agaricomycetes may provide a better source for developing new therapeutic and pharmacological agents. The effect of antibacterial potential was examined against gram positive bacteria and gram negative bacteria; petroleum ether extract of the *H. parvula* has showed consistently significant inhibitory activity on different bacterial species tested.

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Table 1: Antibacterial activity of different extracts of *Hygrocybe parvula* on plant pathogens

Name of the bacteria	Crude extract	Diameter of Zone of Inhibition(in mm)					
		Extract concentrations (µg/ml)				Standard	Control
		100%	50%	25%	12.5%	Tetracycline (30µg/ml)	DMSO (100%)
Xanthomonas campestris	Petroleum ether	12	10	9	7	40	-
	Chloroform	9	7	5	3		
	Methanol	10	8	6	4		
Pseudomonas syringae	Petroleum ether	14	13	9	6	38	-
	Chloroform	8	8	7	5		
	Methanol	8	7	5	4		
Agrobacterium tumefaciens	Petroleum ether	16	14	12	10	30	-
	Chloroform	10	8	7	6		
	Methanol	8	6	4	3		

--: no activity

Table 2: Antibacterial activity of different extracts of *Hygrocybe parvula* on human pathogens

Name of the bacteria	Crude extract	Diameter of Zone of Inhibition(in mm)					
		Extract concentrations (µg/ml)				Standard	Control
		100%	50%	25%	12.5%	Ciprofloxacin (30µg/ml)	DMSO (100%)
Escherichia coli	Petroleum ether	13	10	8	6	33	-
	Chloroform	8	6	4	4		
	Methanol	7	6	6	4		
Klebsiella pneumoniae	Petroleum ether	10	8	6	6	28	-
	Chloroform	10	9	8	7		
	Methanol	8	6	5	7		
Pseudomonas aeuroginosa	Petroleum ether	18	16	12	9	32	-
	Chloroform	12	10	8	6		
	Methanol	18	12	11	8		
Staphylococcus aureus	Petroleum ether	14	13	11	8	30	-
	Chloroform	8	7	9	6		
	Methanol	9	7	8	4		
Streptomyces pneumoniae	Petroleum ether	12	10	8	6	26	-
	Chloroform	10	8	6	4		
	Methanol	7	5	4	3		
Salmonella typhi	Petroleum ether	15	14	11	8	28	-
	Chloroform	11	9	7	5		
	Methanol	9	7	5	3		

--: no activity

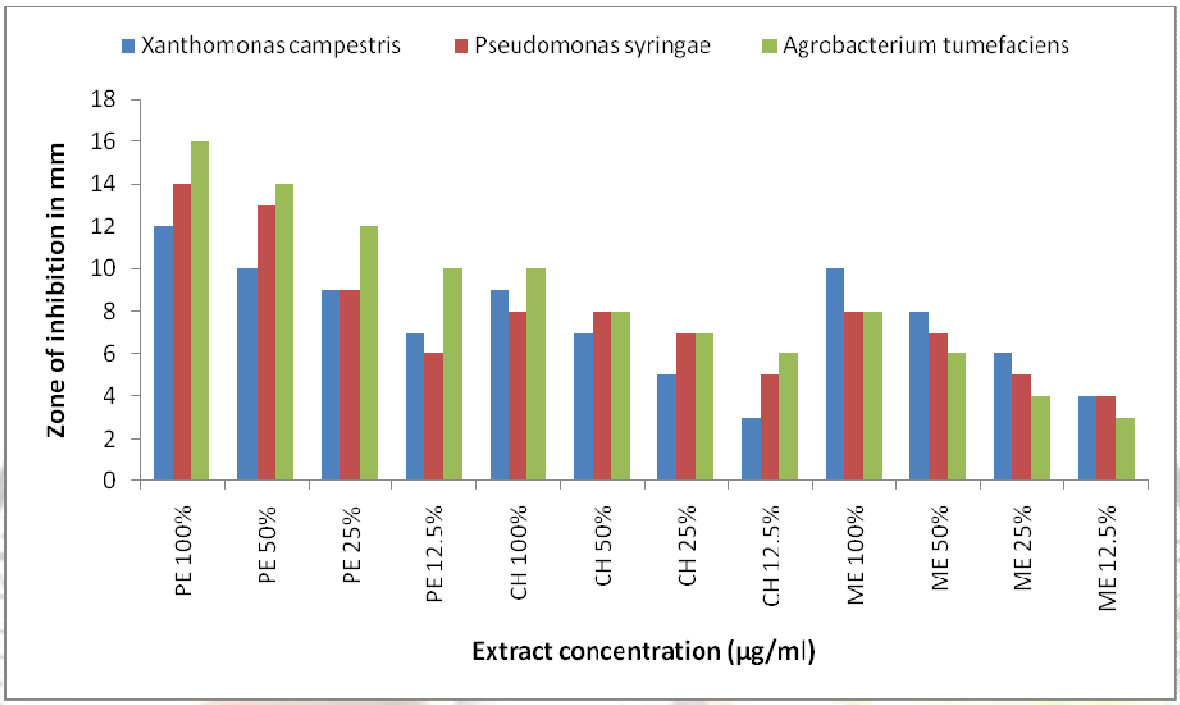

Fig. 1: Graphical representation of antibacterial activity of *Hygrocybe parvula* against plant pathogens

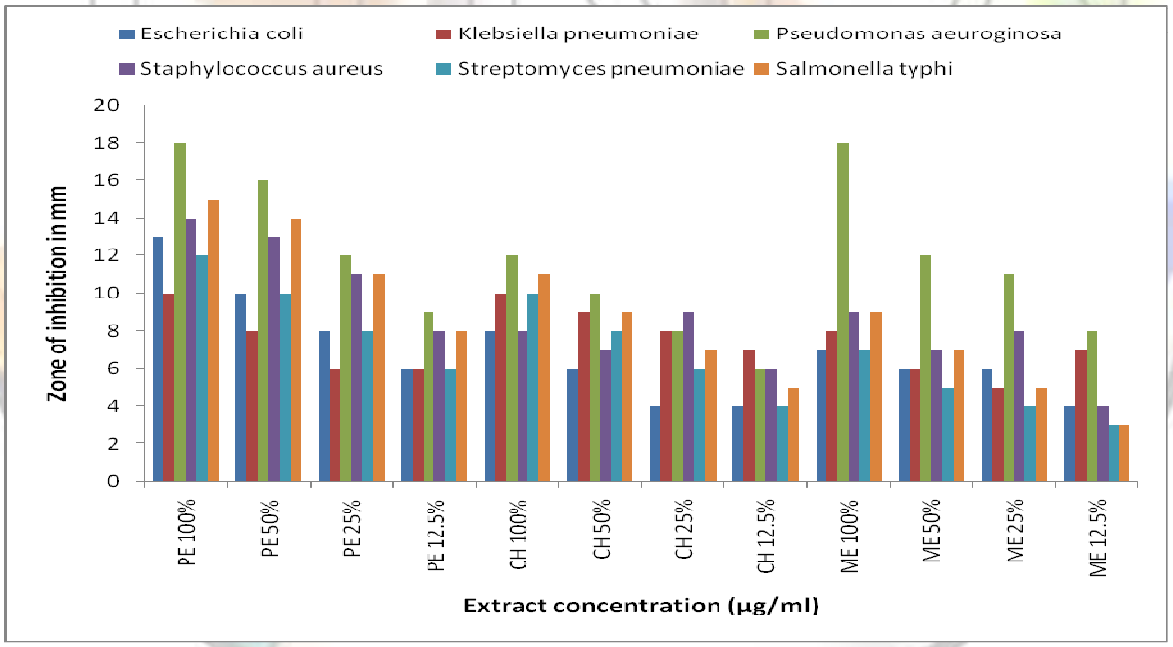

Fig. 2: Graphical representation of antibacterial activity of *Hygrocybe parvula* against human pathogens