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PHYTOCHEMICAL AND ANTIFUNGAL ACTIVITY OF *HYGROCYBE PARVULA* (PECK) PEGLER

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ABSTRACT

The phytochemical and antifungal activity of Hygrocybe parvula (Peck) Pegler, was examined using Petroleum ether, Chloroform and Methanol, as solvents and tested against nine plant and ten human pathogenic fungi viz., A. alternate, A. flavus, A. solani, A. tomentosa, C. capsici, C. dematium, C. lindemuthianum, F. oxysporum, F. solani, M. gypseum, T. equinum, T. kanei, C. albicans, C. indicum, C. krusei, C. merdarium, C. zonatum, E. floccosum and T. rubrum., using the agar well-diffusion method. Extracts were found to contain Alkaloid, steroids, saponins, glycosides, flavonoids and phenols. The maximum antifungal activity of methanol extracts of H. parvula was found against A. tomentosas (10 mm) and C. dematium (8 mm) at 100% concentration, but there is no inhibitory activity against A. alternate and F. oxysporum in plant pathogenic fungi. The petroleum ether extracts were showed more active antifungal activity against M. gypseum (13 mm), C. albicans (12 mm), C. zonatum (12 mm) and T. rubrum (10.33mm), in the same time, there is no activity against T. equinum and C. merdarium. The extract shows increasing inhibitory activity with increase in concentration (12.5%-100%).While comparing the solvent studied, petroleum ether and methanol extracts showed highest response in resisting microbial growth than chloroform. However, the activity was less than the standard Clotrimazole, Fleuconazole, Mancozeb and Captan.

KEY WORDS

Antifungal activity, Hygrocybe parvula, Phytochemistry, Pathogens, Wild mushrooms, Western Ghats.

INTRODUCTION

Mushrooms are generally treated as macro fungi which are known to produce many kind of bioactive compounds generally linked with mycelial cell wall, that help in enhancing the immune capacity to fight against many pathogenic microorganisms [1]. Macro fungi have long been used as a valuable food source and as traditional medicines around the world since ancient times, especially in Japan and China. A number of medicinal mushrooms, such as *Aleurodiscus, Coprinus, Clitocybe, Daedalea, Marasmius, Merulius, Pleurotus, Polyporus, Poria, Psathyrella* and *Tricholoma* spp, are rich sources of B-glucan, proteoglucan, lectin, phenolic compounds, flavonoids, polysaccharides, triterpenoids, diatery fibre, lentinan, schizophyllan, lovostatin, pleuran, steroids, glycopeptides, terpenes, saponins, xanthones, coumarines, alkaloid, purin, purimidin kinon, fensil, propanoid, kalvasin, volvotoksin, flammutoksin, porisin, AHCC, maitake D- fraction,

ribonucleas, eryngeolysin and also have been used extensively in traditional medicine for curing various types of diseases such as antimicrobial, antiviral, anticancer, antitumor, anti-inflammatory, cardiovascular disease, immuno modulating, central activities etc. [2-9].

Both fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. They are rich sources of natural antibiotics, where the cell wall glucans are well known for their immuno modulatory properties and many of the externalized secondary metabolites combat bacteria, fungi and viruses [10]. The effects of different mushroom extracts on pathogens and microorganisms are studied by a very large number of researchers in different parts of the world [11-19]. Antibiotic resistance has become a global concern [20]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multi drug resistant pathogens [21]. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microorganisms has led to the screening of several medicinal plants for their potential antimicrobial activity [22-23].

The wild mushrooms naturally having lot of medicinal values due to their secondary metabolites. Among them, the wild mushrooms growing in and around us having good medicinal properties. Therefore, by considering this fact, the proposed work is planned to undertake for the study phytochemical and antifungal activity of different solvent extracts of *H. parvula.*

MATERIALS AND METHODS

Collection of mushroom 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 [24-28]. 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 and reagents:

All chemicals and reagents used in the present study were purchased from reliable firms like HiMedia Laboratories Pvt. Ltd and were of analytical grade.

Preparation of mushroom extracts:

The dried and powdered by grinder (Bajaj Electrical Limited-Twister Mixer) mushroom material (200 g) was extracted successively with 2000 ml pet ether following chloroform and methanol with a Soxhlet extractor for 48 h at temperature not exceeding the boiling point of the solvent [29]. The extract was filtered with Whatman filter paper no.1 and the filter was 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 11.21 gm and methanol 51.16 gm (**Table 1**). Each extract was transferred to glass vials and kept at 4°C before use.

Phytochemical screening:

Phytochemical screening was carried out according to the standard procedures described by [31-32] in order to identify the constituents present in acetone extract of *H. parvula* whole fruiting body.

Culture and maintenance of microorganisms:

Pure cultures of all experimental fungi were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh.

American Type Culture Collection (ATCC). The viability of the organisms was maintained by regular transfer into freshly prepared on Potato dextrose agar (PDA) at 28˚C and stored at 4˚C until used. For the present study pure fungal cultures were taken (**Table 2**).

Preparation and its Sterilization:

For the agar well-diffusion method [32-33] antimicrobial susceptibility was tested on solid (Agar-agar) media in petriplates. For the fungal assay, PDA (39 gm/L), were used for developing surface colony growth. The suspension culture, for fungal cell growth was done by preparing 2.4% (w/v) PDB (Potato dextrose broth) was taken for evaluation. All the media prepared were then sterilized by autoclaving the media at (121˚C) for 20 minutes.

Agar Well-diffusion Method:

The antifungal activity was tested by agar well diffusion method [34]. The fungal spore suspension was prepared by the addition of a loopful of fungal spores in a 5 ml of sterile distilled water and 1 ml Tween 20. Then fungal spore suspension was spread evenly on the petriplate containing 20 ml of solidified potato dextrose agar. Four wells were punched at the corner by using sterile cork borer of 6 mm diameter. The different solvent extracts of *H. parvula* were loaded to the four wells by using 100µl micropipette in 4 different concentrations i.e., 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml respectively. Clotrimazole, Fleuconazole, Mancozeb and Captan are used as a positive control and DMSO is used as a negative control. All the plates were incubated at 23±2˚C fungal growth was determined by measuring the diameter of zone of inhibition after 5 days of incubation. The test was done in triplicates to arrive concordant result.

RESULTS AND DISCUSSION

The yield of the crude extract obtained in the mushroom samples shows that maximum yield (**Table 1**).

Table: 1. Total yield of the fruiting body of mushrooms extracts obtained in different organic solvents (200 gm in 2000 ml)

Mushroom species	Organic solvents	Yield of extract in gms	% of yield
Hygrocybe parvula	Petroleum ether	10.88	5.44
	Chloroform	11.21	5.61
	Methanol	51.16	25.58

Phytochemical investigation:

The qualitative phytochemical screening of *Hygrocybe parvula* has revealed the presence of various secondary metabolites of therapeutic importance namely alkaloids, saponins, phenols, glycosides, terpenoids and flavonoids whereas absent of steroids and tannins (**Table 3**).

Table: 2. Fungal cultures

Sl. No	Name of the Fungal	Type of pathogen	Numbers	
			MTCC	ATCC
1	*Alternaria alternate*	Plant	7202	
2	*Aspergillus flavus*	Plant		9170
3	*Alternaria solani*	Plant		26934
4	*Alternaria tomentosa*	Plant		16404
5	*Colletotrichum capsici*	Plant	2071	
6	*Colletotrichum dematium*	Plant		60192
7	*Colletotrichum lindemuthianum*	Plant		90028
8	*Fusarium oxysporum*	Plant	2485	
9	*Fusarium solani*	Plant	2935	
10	*Candida albicans*	Human		10231

11	*Chrysosporium indicum*	Human	4266	
12	*Candida krusei*	Human		6258
13	*Chrysosporium merdarium*	Human		900628
14	*Chrysosporium zonatum*	Human		845981
15	*Epidermophyton floccosum*	Human	613	
16	*Trichophyton rubrum*	Human	1538	
17	*Microsporum gypseum*	Human	2157	
18	*Trichophyton equinum*	Human		6275
19	*Trichophyton kanei*	Human	2091	

Table: 3. Phytochemical analysis of *Hygrocybe parvula*

Sl. No	Secondary metabolites		Name of the test	PE	CE	ME
1	Alkaloids	a	Mayer's test	-	-	+
		b	Wagner's test	-	-	+
2	Steroids	a	Salkowaski' s test	-	-	-
3	Saponins	a	Foam test	+	+	+
4	Tannins	a	Ferric chloride test	-	-	-
		b	Gelatin test	-	-	-
5	Glycosides	a	Keller-Killiani's test	+	-	-
		b	Legal's test	+	+	+
6	Triterpenoides	a	Salkowaski's test	-	-	-
7	Flavonoides	a	Ferric chloride test	-	-	+
		b	Shinoda test	-	-	+
		c	Alkaline reagent test	-	-	+
		d	Lead acetate solution test	-	-	-
8	Phenols	a	Test solution + 0.5 ml of ferric chloride solution	-	-	+
		b	Test solution + few drops of 5% glacial acetic acid & 5% sodium nitrate	-	+	-

Note: '+' is Present, '-'is Absent, PE-Petroleum ether extract, CE-Chloroform extract, ME-Methanol extract.

Phytoconstituents such as alkaloid, sesquiterpine, phenolic compounds and glycosides have been reported to inhibit bacterial growth and to be protective to plants against bacterial and fungal infections [35]. So, the antifungal activity showed by petroleum ether, chloroform and methanol extracts of *Hygrocybe parvula* may be due to presence of glycosides, saponin, phenolic compounds and flavonoids.

Antifungal activity:

In the present investigation, the inhibitory effects of different solvent extracts (*viz.,* Petroleum ether, Chloroform and Methanol) from *H. parvula* were evaluated against plant fungicidal strains *viz.*, *A. alternate, A. flavus, A. solani, A. tomentosa, C. capsici, C. dematium, C. lindemuthianum, F. oxysporum* and *F. solani*. Human pathogenic fungi *viz., M. gypseum, T. equinum, T. kanei, C. albicans, C. indicum, C. krusei, C. merdarium, C. zonatum, E. floccosum* and *T. rubrum*. The antifungal activity was determined using the agar well-diffusion method summarized in (**Table 4**) and (**Table 5**). The antibacterial activity of all the solvent extracts were calculated by measuring inhibition zone formed around the well in millimeter (mm).

The three organic solvent extracts, showed moderate activity against all the tested fungi. The maximum antifungal activity of methanol extracts of *H. parvula* was found against *A. tomentosas* (10 mm) and *C. dematium* (8 mm) at 100% concentration, whereas in chloroform *C. lindemuthianum* (9 mm) and moderate against *C. dematium* (9 mm) and *A. flavus* (7 mm) at 100% concentration, followed by *A. tomentosa* (8 mm), *C. dematium* (7 mm), *C. capsici* (6 mm) and *F. solani* (5 mm) at lower concentration of chloroform and methanol extract, but there is no inhibitory activity against *A. alternate* and *F. oxysporum* in plant pathogenic fungi (**Figure 1**).

Among the three organic solvent extracts, showed more effective inhibitory activity against all the tested human fungal pathogens. The petroleum ether extracts were showed more active antifungal activity against *M. gypseum* (13 mm), *C. albicans* (12 mm), *C. zonatum* (12 mm) and *T. rubrum* (10.33mm), moderate effect against *C. indicum* (9 mm) and *T. kanei* (6 mm) at 100% concentration. The methanol and chloroform extract were highly active against *T. rubrum* (12.66 mm), *C. zonatum* (11.33 mm) and *E. floccosum* (10.33 mm) at 100% concentration. Lowest zone of inhibition shown against *T. kanei* (4 mm), followed by *C. krusei* (3 mm) and *M. gypseum* (3 mm) at 12.5-50% concentration, in the same time, there is no activity against *T. equinum* and *C. merdarium* (**Figure 2**). The extract shows increasing inhibitory activity with increase in concentration (12.5% -100%).

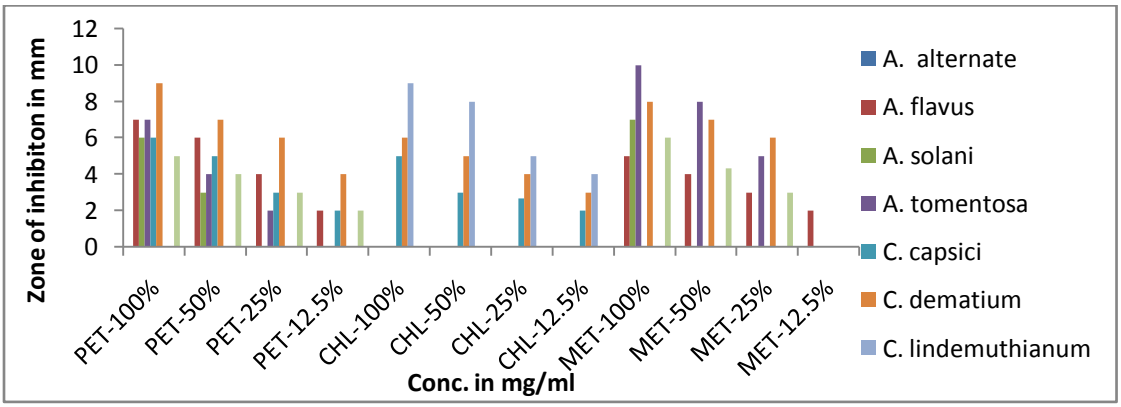

Figure: 1. Mean zones of inhibition of different solvent extract of *H. parvula* against plant pathogenic fungi

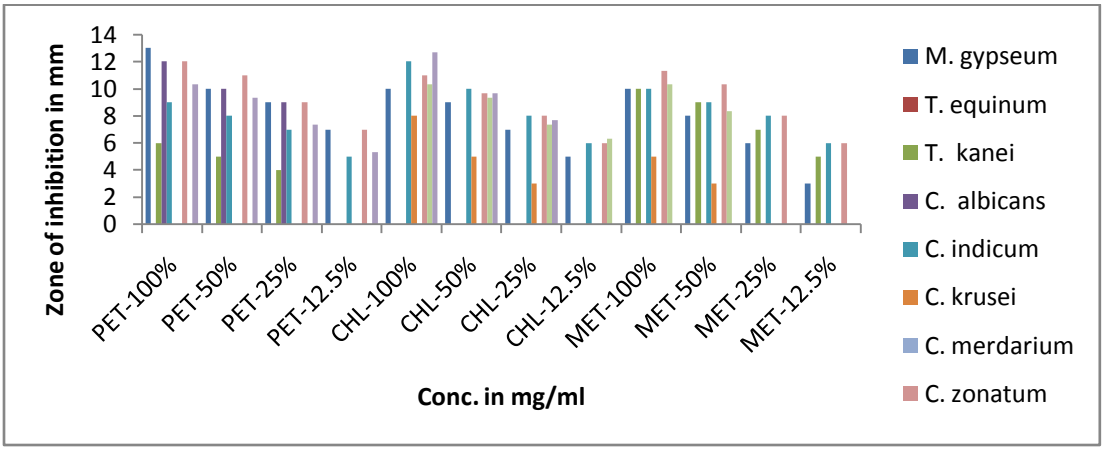

Figure: 2. Mean zones of inhibition of different solvent extract of *H. parvula* against human pathogenic fungi

Table: 4. Antifungal activities of *Hygrocybe parvula* extract against plant pathogenic fungi by agar well diffusion method

Sl. No	Organisms	Diameter of zone of inhibition (in mm)											
		Petroleum ether extract (Conc.mg/ml)				Chloroform extract (Conc.mg/ml)				Methanol extract (Conc.mg/ml)			
		100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %
1	*A. alternate*	-	-	-	-	-	-	-	-	-	-	-	-
2	*A. flavus*	7	6	4	2	-	-	-	-	5	4	3	2
3	*A. solani*	6	3	-	-	-	-	-	-	7-	-	-	-
4	*A. tomentosa*	7	4	2	-	-	-	-	-	10	8	5	-
5	*C. capsici*	6	5	3	2	5	3	2.66	2	-	-	-	-
6	*C. dematium*	9	7	6	4	6	5	4	3	8	7	6	-
7	*C. lindemuthianum*	-	-	-	-	9	8	5	4	-	-	-	-
8	*F. oxysporum*	-	-	-	-	-	-	-	-	-	-	-	-
9	*F. solani*	5	4	3	2	-	-	-	-	6	4.33	3	-

Note: '–'- No activity.

Table: 5. Antifungal activities of *Hygrocybe parvula* extract against human pathogenic fungi by agar well diffusion method

Sl. No	Organisms	Diameter of zone of inhibition (in mm)											
		Petroleum ether extract (Conc.mg/ml)				Chloroform extract (Conc.mg/ml)				Methanol extract (Conc.mg/ml)			
		100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %	100 %	50 %	25 %	12.5 %
1	*M. gypseum*	13	10	9	7	10	9	7	5	10	8	6	3
2	*T. equinum*	-	-	-	-	-	-	-	-	-	-	-	-
3	*T. kanei*	6	5	4	-	-	-	-	-	10	9	7	5
4	*C. albicans*	12	10	9	-	-	-	-	-	-	-	-	-
5	*C. indicum*	9	8	7	5	12	10	8	6	10	9	8	6
6	*C. krusei*	-	-	-	-	8	5	3	-	5	3	-	-
7	*C. merdarium*	-	-	-	-	-	-	-	-	-	-	-	-
8	*C. zonatum*	12	11	9	7	11	9.66	8	6	11.33	10.33	8	6
9	*E. floccosum*	-	-	-	-	10.33	9.33	7.33	6.33	10.33	8.33	-	-
10	*T. rubrum*	10.33	9.33	7.33	5.33	12.66	9.66	7.66	-	-	-	-	-

Note: '–'- No activity.

Table: 6. Antifungal activity of standard drug and control against plant and human pathogenic fungi

Sl. No	Test organism	Standard Clotrimazole	Fleuconazole	Mancozeb	Captan	Control DMSO
Plant pathogenic fungi						
1	*A. alternate*	x	x	25	20	-
2	*A. flavus*	x	x	23	25	-
3	*A. solani*	x	x	25.3	26	-
4	*A. tomentosa*	x	x	25	22	-
5	*C. capsici*	x	x	30	28	-
6	*C. dematium*	x	x	28	27	-
7	*C. lindemuthianum*	x	x	30	29	-
8	*F. oxysporum*	x	x	23	21	-
9	*F. solani*	x	x	25	21	-
Human pathogenic fungi						
1	*M. gypseum*	32	30	x	x	-
2	*T. equinum*	30	31	x	x	-
3	*T. kanei*	32	34	x	x	-
4	*C. albicans*	28	32	x	x	-
5	*C. indicum*	24	28	x	x	-
6	*C. krusei*	27	26	x	x	-
7	*C. merdarium*	26	30	x	x	-
8	*C. zonatum*	24	20	x	x	-
9	*E. floccosum*	21.3	24	x	x	-
10	*T. rubrum*	21.6	23.3	x	x	-

Note: 'x'-Not applicable, '–'- No activity.

The antifungal activity of different solvent extracts of mushroom is changeable and has a lower antifungal activity as to comparison of antibiotics *viz.,* Clotrimazole, Fleuconazole, Mancozeb and Captan (**Table 6**). The search for antimicrobials from natural sources has received much attention, and efforts have been put in to identify compounds that can act as suitable antimicrobial agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines for controlling the growth of microorganisms [36]. These compounds have a significant therapeutic application against human pathogens, including bacteria, fungi, or virus. Numerous studies have been conducted with the extracts of various plants, screening of antimicrobial activity, as well as for the discovery of new antimicrobial compounds [37].

In the present investigation, different extracts of *H. parvula* were evaluated for exploration of their antifungal activities against multi-drug resistant, clinically isolated microorganisms. Although the mechanism of action of these plant constituents is not yet fully known, it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided a more powerful antimicrobial activity, as compared to the aqueous extracts [38]. The extracts of various mushrooms inhibited the growth of some microorganisms at different ratios. Different mushroom species posses different constituents and in different concentration, which account for the differential antimicrobial effect, as suggested. The broad spectrum of antimicrobial activity may be attributed to the presence of bioactive metabolites of various chemical types in mushrooms compounds [39].

CONCLUSION

The public is becoming increasingly aware of problems with the over prescription and misuse of

traditional antibiotics. Worldwide spending on finding new anti-infective agents is increasing. The use of plant extracts as well as other alternative forms of medicinal treatments is being investigated by researchers. From these reports it is focused that mushrooms are a vital sources of medicinal compounds that may be use to cure different disorders and prevent pathogenic microorganisms. The wild mushrooms naturally having lot of medicinal values due to their secondary metabolites. Among them, the wild mushrooms growing in and around us having good medicinal properties. Therefore, by considering this fact, the proposed work is planned to undertake for the study phytochemical and antifungal activity of different solvent extracts of *H. parvula*. Definitely, this type of research is going to provide a potential contribution to the scientific knowledge and having good social relevance and national importance.

The present investigation shows that *Hygrocybe parvula* contains the potential antifungal components that may be of great use to the development of pharmaceuticals in industries, as a therapy against various diseases. The petroleum ether, chloroform and methanol extracts of *Hygrocybe parvula* possess significant inhibitory effects against tested pathogens. The results of the study support the folklore claim, along with the development of new antimicrobial drugs from both the mushrooms.

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