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Phytochemical and Antifungal study of different solvent extracts of *Scleroderma bermudense* Corker. (Sclerodermataceae)

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ABSTRACT

The different solvent extracts of *Scleroderma bermudense* belongs to the family Sclerodermataceae 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 was subjected to phytochemical analysis for secondary metabolites and antifungal screening by agar well diffusion method against plant and 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*. Extracts were found to contain steroids, saponins, glycosides, flavonoids and phenols. The extract also showed significant antifungal activity against *C. albicans, C. indicum, C. merdarium, F. oxysporum, C. dematium, T. equinum, A. flavus C. capsici, F. solani, C. kruesi* and *C. lindemuthianum*, whereas least activity showed against *A. solani*, *M. gypseum* and does not showed inhibition zone against *A. alternate, A. tomentosa. T. kanei, C. zonatum, E. floccosum* and *T. rubrum.* However, the activity was less than the standard Clotrimazole, Fleuconazole, Mancozeb and Captan. 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.

Keywords: *Scleroderma bermudense*, Phytochemistry, Antifungal activity, Pathogens, Wild mushrooms, Western Ghats.

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INTRODUCTION

Both fruiting body and the mycelium of mushrooms contain compounds with wide ranging antimicrobial activity. Mushrooms need antibacterial and antifungal compounds to survive in their natural environment [1]. Hence, 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[2-4]. Phytopharmaceutical products have served as a major source of drugs for centuries and today about half of the pharmaceuticals in use are derived from natural products. The use of phytopharmaceutical substances, particularly isolated from plants, to control diseases is an old practice that has led to the discovery of more than half of all modern pharmaceuticals as complimentary or alternative medicine either to prevent or ameliorate many diseases has been noted in recent years[5]. World Health Organization (WHO) has estimated that more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Generally, mushrooms are used as food because of their good taste, appetizing aroma and nutrient contents[6, 7]. Humans and animals are prone to infection by several microorganisms, especially fungi[8, 9]. Besides, mushrooms have been used as traditional medicine for curing various types of diseases. Mushrooms such as *Agaricus bisporus, Lentinula edodes, Aricularia auricula* and *Pleurotus* sp. have antagonistic effects against bacteria, fungi and viruses[10].

The medicinal use of mushrooms has a very long tradition in the Asia and Africa, whereas their use in the Western countries has been increasing only moderately in recent decades[11-13]. In recent years, a number of pharmacological actions have been intensively investigated, including cytotoxic/ antitumor, immune suppressive, antipruritic, antierythema, and antifungal, antioxidant and free radical scavenging activities. Antibiotic resistance of human pathogenic bacteria has become a major worldwide public health concern [14, 15]; this is why the search for new substances with antimicrobial activity is a priority [16]. Antimicrobial activity has already been documented in extracts from the mycelium[17] and fruiting bodies[18] of different wild species from Basidiomycota. Extracts of various fungal fruiting bodies such as *Pleurotus ostreatus* (Jacq.) P. Kumm.[19], *Pholiota adiposa* (Fr.) P. Kumm. (Strophariaceae)[20], *Coprinus digitalis* (Batsch) Fr. (Agaricaceae) [21], *Podaxis pistillaris* (L.) Fr. (Agaricaceae) [22], *Lycoperdon pusillum* Batsch [now *Bovista pusilla* (Batsch) Pers.], and *Lycoperdon giganteum* Batsch [now *Calvatia gigantea* (Batsch) Lloyd] (Lycoperdaceae) [23] have shown activity against a range of different Gram+ve and Gram-ve bacteria and also fungi. 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. In the present study the crude extract and different solvent soluble fractions of the whole fruiting body of *Scleroderma bermudense* were used.

Figure: 1. Habitat of collected mushroom

MATERIALS AND METHODS:

Mushroom:

The *Scleroderma bermudense* (Figure 1) was 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 during the month of September and October 2012. The *S. bermudense* of mushroom was picked from the litter and decaying soil surface, with help of forceps and then they were cleaned and kept for shade drying. The shade dried mushroom materials were powdered mechanically for further use. Identification was done by comparing their morphological, anatomical and physiological characteristics and monographs with descriptions given in the manual[24] and also through the electronic data on identification keys of mushrooms[25]. The voucher specimen (KUABARNH-82) was 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 [26]. 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 dimethyl sulfoxide (DMSO). The yield of extracts obtained from pet ether was (10.6 gm), followed by chloroform (22.9 gm) and methanol (32.4 gm). Each extract was transferred to

glass vials and kept at 4°C before use.

Preliminary phytochemical screening:

Qualitative phytochemical analysis of *S. bermudense* extracts (petroleum ether, chloroform and methanol) were carried out using standard methods[27]. The extracts obtained in Soxhlet extractor were dissolved with their respective solvents**.**

Fungal strains:

Nineteen different fungal strains were used: *Alternaria alternate* (MTCC-7202), *Aspergillus flavus* (ATCC-9170), *Alternaria solani* (ATCC-26934), *Alternaria tomentosa* (ATCC-16404), *Colletotrichum capsici* (MTCC-2071), *Colletotrichum dematium* (ATCC-60192), *Colletotrichum lindemuthianum* (ATCC-90028), *Fusarium oxysporum* (MTCC-2485) and *Fusarium solani* (MTCC-2935) plant pathogens. *Candida albicans* (ATCC-10231), *Chrysosporium indicum* (MTCC-4266), *Cnadida krusei* (ATCC-6258), *Chrysosporium merdarium* (ATCC-900628), *Chrysosporium zonatum* (ATCC-845981), *Epidermophyton floccosum* (MTCC-613), *Trichophyton rubrum* (MTCC-1538), *Microsporum gypseum* (MTCC-2157), *Trichophyton equinum* (ATCC-6275) and *Trichophyton kanei* (MTCC-2091) human pathogens. They were purchased from the Institute of Microbial Technology (IMTECH), Chandigarh, India and 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.

Screening for antifungal activity:

The antifungal activity was tested by agar well diffusion method[28]. 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 *S. bermudense* 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 (Figure 2).

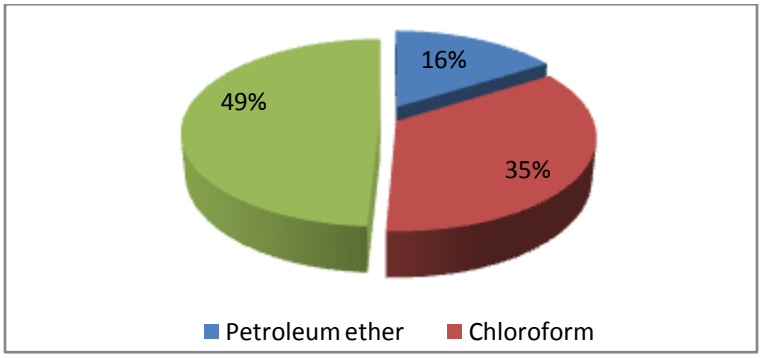

Figure: 2. Total yield of *Scleroderma bermudense* extracts obtained in different organic solvents (200 gm in 2000 ml)

Phytochemical investigation:

The qualitative phytochemical screening of *Scleroderma bermudense* has revealed the presence of various secondary metabolites of therapeutic importance namely steroids, saponins, phenols, glycosides, and flavonoids whereas absent of alkaloids, tannins and terpenoids (Table 1). The result shows that all extracts contain the highest secondary metabolites. Several of these constituents may possibly be responsible for the mushrooms antimicrobial activity. Flavonoids, for instance, have been of interest to the scientific community because of recent reports on their antiviral, antifungal, anti-inflammatory, and cytotoxic[29] and have also shown that antibacterial, anti-HIV property [30].

Table: 1. Phytochemical analysis of *Scleroderma bermudense*

Sl. No	Secondary metabolites	Name of the test		Petroleum ether extract	Chloroform extract	Methanol extract
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.

Table: 2. Antifungal activities of *Scleroderma bermudense* 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*	8	7	5.6	3.6	-	-	-	-	7.3	6.3	5	5
3	*A. solani*	7	5.3	-	-	-	-	-	-	8	-	-	-
4	*A. tomentosa*	-	-	-	-	-	-	-	-	-	-	-	-
5	*C. capsici*	7	6	4.6	4.	7.3	6	4.3	3.6	-	-	-	-
6	*C. dematium*	10	8.6	7.6	6.3	7.3	6.3	5.3	4.3	8	7	6	-
7	*C. lindemuthianum*	-	-	-	-	9	8	6.3	5.3	-	-	-	-
8	*F. oxysporum*	11	9.3	8	7	-	-	-	-	9	8	6.6	5.6
9	*F. solani*	6.6	5.6	4.6	3.6	-	-	-	-	7.6	6.3	5	-

Note: '–'- No activity.

Table: 3. Antifungal activities of *Scleroderma bermudense* 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*	-	7.6	-	-	-	11	-	-	-	-	-	-
2	*T. equinum*	9	8	7	5.6	12	10	8	7.6	11	9	8	6.3
3	*T. kanei*	-	-	-	-	-	-	-	-	-	-	-	-
4	*C. albicans*	13	12	11	9	14	12	11	9	10.3	8.6	7	6.3
5	*C. indicum*	13	12	10	8	12	11	9	7.3	13	12	9	7.6
6	*C. krusei*	-	-	-	-	12	11	9	8	12.3	10	-	-
7	*C. merdarium*	12	11	9	7	14	11	9.6	-	-	-	-	-
8	*C. zonatum*	-	-	-	-	-	-	-	-	-	-	-	-
9	*E. floccosum*	-	-	-	-	-	-	-	-	-	-	-	-
10	*T. rubrum*	-	-	-	-	-	-	-	-	-	-	-	-

Note: '–'- No activity.

Antifungal activity:

Antifungal screening of different solvent extracts of *Scleroderma bermudense* by agar well diffusion method against plant pathogenic fungi *viz.*, *A. alternate*, *A. flavus*, *A. solani*, *A. tomentosa*, *C. capsici*, *C. dematium*, *C. lindemuthianum*, *F. oxysporum* and *F.* solani (Table 2). 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* (Table 3).

Among the three solvent tested, petroleum ether extract recorded a significant activity compared to chloroform and methanol. In petroleum ether extract *F. oxysporum* (11 mm), *C. dematium* (10 mm), *A. flavus* (8 mm), *C. capsici* (7 mm) and *F. solani* (6.6 mm) recorded a complete inhibition and where as in chloroform extract shown significant activity against *C. lindemuthianum*, *C. dematium* and *C. capsici*, in case of methanol solvent extract, moderate inhibition zone against *F. oxysporum* and *A. flavus* at 12.5-100% concentration, also least record shown against *A. solani* at 50-100% concentration, but there is no activity against *A. alternate* in all the solvent extract at 12.5, 25, 50 and 100% concentration (Table 2).

From human pathogenic fungi the highest antifungal activity was shown against *C. albicans* (14 mm) followed by *C. merdarium* (14 mm), *C. indicum* (13 mm), at 100 % concentration in all the three solvents and the weakest activity against *T. equinum* (9 mm) at 100 % and *M. gypseum* (7.6 mm) at 50 % concentration in petroleum ether extract. Whereas no activity was observed in all the three solvent extracts at 12.5, 25, 50, and 100 % concentration tested against four fungi *T. kanei*, *C. zonatum*, *E. floccosum* and *T. rubrum* (Table 3). 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 4).

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[31, 32]. So, the antifungal activity showed by petroleum ether, chloroform and methanol extracts of *Scleroderma bermudense* may be due to presence of alkaloids, saponin, phenolic compounds and flavonoids.

This study indicated that there are differences in the antimicrobial effects of mushroom groups, due to phytochemical differences among species. They claimed that the sensitivity of microorganism to chemotherapeutic compounds change even against different strains. The results revealed that antifungal and phytochemical constituents of petroleum ether, chloroform and methanol extracts varied in usefulness which may be attributed, the author also notable the antibacterial activity[33]. 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[19], 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[34].

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[35].

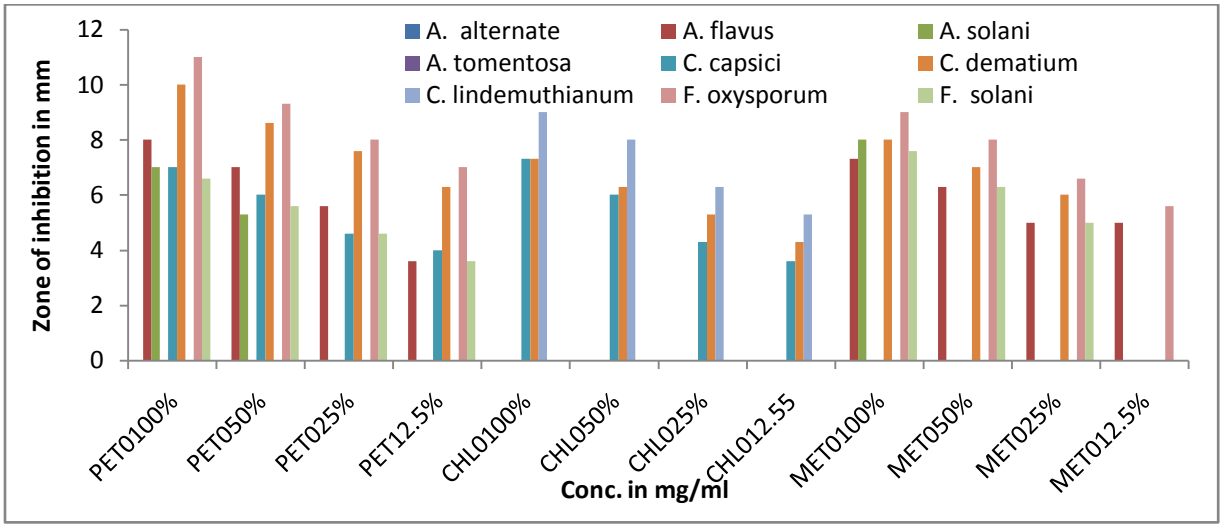

Figure: 3. Graphical representation of antifungal activity of *Scleroderma bermudense* against plant pathogenic fungi

C. lindemuthianum, F. oxisporum, F. solani

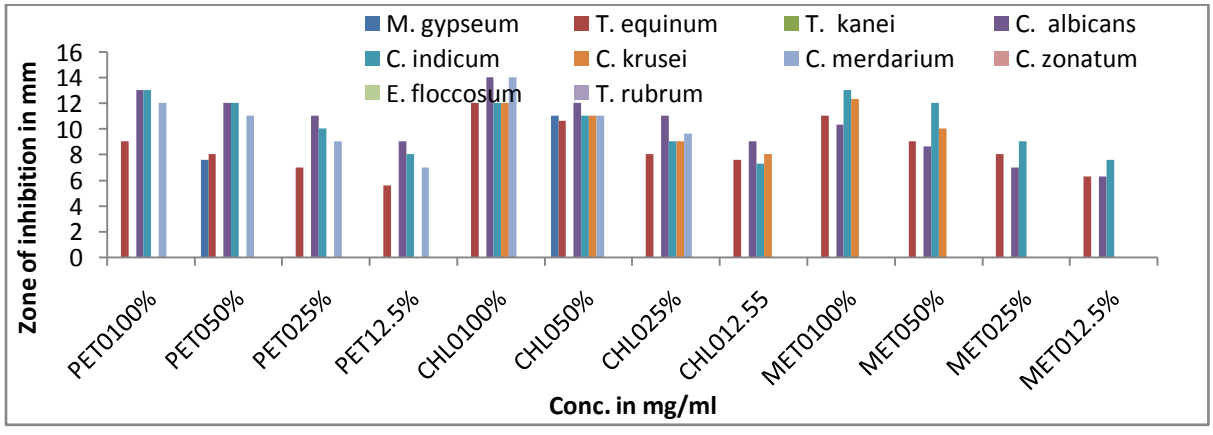

Figure: 4. Graphical representation of antifungal activity of *Scleroderma bermudense* against human pathogenic fungi

C. merdarium, C. zonatum, E. floccosum, T. rubrum

Table: 4. Antifungal activity of standard drug and control against plant and human pathogenic fungi

Sl. No	Test organism	Standard				Control
		Clotrimazole	Fleuconazole	Mancozeb	Captan	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.

CONCLUSION:

The present study of antifungal activity of the mushroom extract of the *Scleroderma bermudense* against plant and human pathogenic fungi are confirmation that the extracts are potential source of antibiotics with a wide spectrum of properties. In general, reference wells are more active than different solvent extracts of mushrooms but microorganisms get resistant to the antibiotics after sometime. Therefore, extracts of mushroom may be used as source of antimicrobial agents for safe and lacking side effects. Bioactive substances from this mushroom can therefore be employed in the formulation of antimicrobial agents for the treatment of various bacterial and fungal infections including gonorrhea, pneumonia, eye infections and mycotic infections. Isolation, identification and purification of these phytoconstituents and determination of their respective antimicrobial potencies and toxicological evaluation with the view to formulating novel chemotherapeutic agents should be the future direction for investigation.

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