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RESEARCH ARTICLE :

Antimicrobial activities of important mushrooms against *Alternaria solani*

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SUMMARY : Antimicrobial study was carried out by poisoned food technique against the pathogens by using the methanol, acetone and water extracts. The methanol, acetone and water extracts of mushroom species namely *G. lucidum*, *G. sapidum*, *P. florida*, *Russula* sp., *Boletus edulis* and combination of *Russula* sp. with *Curcuma caesia* showed well per cent growth inhibition against *Alternaria solani* except in *Agaricusbisporus* which showed zero per cent growth inhibition in all 5 per cent, 10 per cent, 20 per cent and 40 per cent concentrations, respectively. *G.lucidum*, *Boletus edulis* and combination of *Russula* sp. with *Curcuma caesia* are the most promising species as antimicrobial agents.

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KEY WORDS :
Antimicrobial activities, *Alternaria solani*, Mushroom extracts, Poisoned food technique, Fruiting bodies

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BACKGROUND AND OBJECTIVES

It has been known that macrofungi are used as a valuable food source and traditional medicines since Greek and Roman antiquity (Anke, 1989). It is believed that mushrooms need antibacterial and antifungal compounds to survive in their natural environment. Antimicrobial compounds could be isolated from many mushroom species and some proved to be of benefit for humans (Lindequist *et al.*, 2005). As an antifungal and antibacterial compound, Sparassol was isolated in the early 1920s from *Sparassiscrispa*. Since then, several antifungal and antibacterial compounds have been isolated from different macrofungi species. In recent decades, various extracts of mushrooms have been of great interest as sources of natural products (Turkoglu *et al.*, 2007). The effects of different mushroom extracts on pathogens and micro-organisms are studied by a very large number of researchers in different parts of the world (Jonathan and Fasidi, 2003; Rosa *et al.*, 2003 and Demirhan *et al.*, 2007).

The aim of the present work is investigate antimicrobial properties of important mushroom extracts with the help of methanol, acetone and water solvent system against *Alternaria solani*.

RESOURCES AND METHODS

Collection of mushroom fruit body:

Mushroom fruit bodies were collected

from forest area of Chhattisgarh, India, during the month of July-August 2015. Collected fruit bodies 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 (Purkayastha and Chandra, 1985; Singer, 1986; Roy and De, 1996 and Das and Sharma, 2005).

Preparation of crude extracts of mushroom fruit bodies:

The coarse powder material of mushroom fruit bodies was used for extraction with solvents like methanol, acetone and distilled water. 100 g of each powdered sample (mushrooms) was separately stirring with 100 ml of each solvent (methanol, acetone and distilled water) at the ratio of 1:1 separately in 250 ml conical flasks by keeping 24 hours at room temperature for initial warming followed by flask were kept at rotary shaker for 3 hours at 120 rpm at room temperature and filtering through Whatman no.1 filter paper in separate sterilized containers. All extracts were stored in a refrigerator in air tight containers and were analyzed for antifungal activity.

Test fungal pathogen:

Alternaria solani was isolated from infected plant sample.The infected specimens were collected from research farm of IGKV, Raipur. The collected diseased samples were washed thoroughly in running tap water for 30 minutes to remove soil particles, air-dried, and cut into 5 mm pieces. Roots, stems and leaves were surface sterilized in 0.1% $HgCl_2$ for 15 seconds and rinsed three times in sterilized distilled water and were on sterilized dry blotting sheet. The surface sterilized segment will be placed on petridishes (three to four segments per plate) containing potato dextrose agar medium. The plates were incubated for 4 to 5 days at 25 ± 1°C in the incubator. Pure cultures of the test fungi were obtained by hyphal tip method (Hyamumachi *et al*., 2005).

Evaluation of antimicrobial activities of mushroom extracts:

Different mushroom extracts (methanol, acetone and water) were tested by poisoned food technique (Vincent 1947). Desired quantity of mushroom extracts (5%, 10%, 20% and 40%) were aseptically mixed into double strength sterilized PDA medium. This medium was poured in sterilized petriplates and allowed to solidify. Medium without any extract served as control.After solidification a 5 mm diameter mycelial disc of test fungus was placed in the middle of plates. Each treatment was replicated for three times. Immediately after inoculation the plates were incubated in incubator at 25±1°C. Data on mycelia growth were recorded when control petriplate was fully covered.

OBSERVATIONS AND ANALYSIS

Data from the Table 1 depict that, 5 per cent

Table 1: Antimicrobial activities of mushroom extracts (5%) against *Alternaria solani* and its mycelial growth characteristics

Sr. No.	Mushroom sp.	Methanol 5%		Acetone 5%		Water 5%	
		% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics
1.	*G.lucidum*	78.14 (62.14)	Fluffy, blackish	0 (0)	Black, velvety	0 (0)	Black, velvety
2.	*G.sapidum*	67.03 (54.96)	Blackish, smooth	63.70 (52.95)	Cottony black	0 (0)	Black, velvety
3.	*P. florida*	45.18 (42.23)	Black velvety	53.70 (47.12)	Fluffy	64.07 (53.18)	Smooth
4.	*Russula* sp.	11.85 (20.12)	Blackish, smooth	0 (0)	Black, velvety	67.03 (54.57)	Irregular edge, velvety
5.	*Boletus edulis*	78.51 (62.39)	Fluffy	61.85 (51.86)	Black, cottony	0 (0)	Black, velvety
6.	*Russula*sp. + *Curcuma caesia*	65.18 (53.54)	Velvety, fluffy	62.22 (52.08)	Velvety black	43.70 (41.38)	Black fluffy
7.	*Agaricusbisporus*	0 (0)	Black, velvety	0 (0)	Black, velvety	0 (0)	Black, velvety
8.	Control	0 (0)	Black, velvety	0 (0)	Black, velvety	0 (0)	Black, velvety
	S.E.±	0.46		0.46		0.33	
	C.D. (P=0.05)	1.40		1.39		1.00	

All values are mean of three replications. Figures in the parentheses are arcsine transformed values

methanol extracts of mushrooms it was observed that *Boletus edulis* showed the maximum zone of inhibition (78.51%) with fluffy mycelial growth at par with *Ganodermalucidum* (78.14%) with fluffy-blackish mycelial growth. The least per cent growth inhibition was recorded in *Russula* sp. (11.85%) with blackish-smooth mycelial growth. In case of 5 per cent acetone extracts it was observed that *Ganoderma sapidum* showed maximum per cent growth inhibition (63.70%) with cottony-black mycelial growth while, *Ganoderma lucidum* and *Russula* sp. could not inhibit mycelial growth of *A. solani*. In case of 5% water extracts it was observed that *Russula* sp. showed maximum growth inhibition (67.03%) of *A. solani* with irregular margin mycelial growth while, *Ganoderma lucidum*, *Ganoderma sapidum*, *Boletus edulis* and *Agaricus bisporus* could not inhibit mycelial growth of *A. solani*.

Data from the Table 2 depict that, 10 per cent methanol extract of *Ganoderma lucidum* showed maximum zone of inhibition (84.07%) against *A. solani*

Table 2 : Antimicrobial activities of mushroom extracts (10%) against *Alternaria solani* and its mycelial growth characteristics

Sr. No.	Mushroom sp.	Methanol 10%		Acetone 10%		Water 10%	
		% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics
1.	*G.lucidum*	84.07 (68.48)	Irregular edge, blackish	0 (0)	Black, velvety	0 (0)	Black, velvety
2.	*G.sapidum*	71.85 (57.96)	Black, serrate edge	68.15 (55.65)	Fluffy	0 (0)	Black, velvety
3.	*P. florida*	50.37 (45.21)	Cottony, fluffy	57.40 (49.26)	Cottony, fluffy	67.40 (50.20)	Serrate edge, dull black
4.	*Russula* sp.	17.03 (24.36)	Velvety, dull black	0 (0)	Black, velvety	71.85 (57.96)	Serrate edge, blackish
5.	*Boletus edulis*	82.59 (65.35)	Cottony, irregular edge	66.29 (54.51)	Dull black with serrate edge	0 (0)	Black, velvety
6.	*Russula* sp. + *Curcumacaesia*	68.89 (56.11)	Serrate edge, dull black	68.52 (55.88)	Serrate edge, blackish	48.14 (43.94)	Dull black, serrate edge
7.	*Agaricusbisporus*	0 (0)	Black, velvety	0 (0)	Black, velvety	0 (0)	Black, velvety
8.	Control	0 (0)	Black, velvety	0 (0)	Black, velvety	0 (0)	Black, velvety
	S.E.±	0.53		0.43		0.41	
	C.D. (P=0.05)	1.61		1.31		1.25	

All values are mean of three replications. Figures in the parentheses are arcsine transformed values.

Table 3 :Antimicrobial activities of mushroom extracts (20%) against *Alternaria solani* and its mycelial growth characteristics

Sr. No.	Mushroom sp.	Methanol 20%		Acetone 20%		Water 20%	
		% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics
1.	*G.lucidum*	88.15 (69.85)	Fluffy	0 (0)	Dull black, cottony	0 (0)	Dull black, velvety
2.	*G.sapidum*	77.03 (61.35)	Fluffy type	73.70 (59.13)	Fluffy type	12.59 (20.47)	Fluffy type
3.	*P. florida*	55.18 (47.95)	Fluffy	62.59 (52.27)	Cottony	72.59 (58.41)	Fluffy type
4.	*Russula* sp.	22.59 (28.35)	Black velvety	22.59 (28.35)	Black, irregular edge	79.25 (62.89)	Serrate type
5.	*Boletus edulis*	85.92 (67.94)	Fluffy type	75.55 (60.42)	Cottony / Fluffy	11.85 (20.11)	Dull black colour
6.	*Russula* sp. + *Curcumacaesia*	80.36 (63.78)	Serrate type	73.70 (59.12)	Serrate type	53.70 (47.10)	Dull black, irregular edge
7.	*Agaricusbisporus*	0 (0)	Dull black, smooth	0 (0)	Dull black, smooth	0 (0)	Dull black, smooth
8.	Control	0 (0)	Black, smooth	0 (0)	Black, smooth	0 (0)	Black, smooth
	S.E.±	0.85		0.80		0.53	
	C.D. (P=0.05)	2.58		2.41		1.63	

All values are mean of three replications. Figures in the parentheses are arcsine transformed values.

with blackish-irregular marginmycelial growth followed by *Boletus edulis* (82.59%) with cottony, irregular marginmycelial growth. In case of 10 per cent acetone extracts it was observed that combination of *Russula* sp. with *Curcuma caesia* showed maximum growth inhibition (68.52%) of *A. solani* with blackish-serrate edgemycelial growth.In case of 10 per cent water extracts it was observed that *Russula* sp. showed maximum growth inhibition (71.85%) with blackish-serrate edgemycelial growth while, *Ganoderma lucidum, Ganoderma sapidum* and *Agaricus bisporus* could not inhibit mycelial growth of *A. solani*.

Data from the Table 3 depict that, 20 per cent methanol extracts of mushrooms it was observed that *Ganoderma lucidum* showed maximum per cent growth inhibition (88.15%) against *A. solani* with fluffy mycelial growth.The least per cent growth inhibition of *A. solani* was recorded in *Russula* sp. (22.59%) with black-velvety mycelial growth while, *Agaricus bisporus* could not inhibit mycelial growth of *A. solani*. In 20 per cent acetone extracts it was observed that *Boletus edulis* showed maximum growth inhibition (75.55%) of *A. solani* with fluffymycelial growth while, *Ganoderma lucidum* and *Agaricus bisporus* could not inhibit mycelial growth of *A. solani*. In 20 per cent water extracts it was observed that *Russula* sp. showed maximum growth inhibition (79.25%) with serrate edgemycelial growth. The least mycelial growth inhibition of *A. solani* was recorded in *Ganoderma sapidum* (12.59%) with fluffy mycelial growth while, *Ganoderma lucidum* and *Agaricus bisporus* could not inhibit mycelial growth of *A. solani*.

Data from the Table 4 depict that, 40 per cent methanol extracts of mushrooms it was observed that *Ganoderma lucidum, Ganoderma sapidum* and *Boletus edulis* showed complete inhibition (100%).In 40 per cent acetone extracts it was observed that *Boletus edulis* showed complete growth inhibition (100%) of *A. solani* followed by *Russula* sp. (90.00%) with irregular edge mycelial growth. All treatments were superior over control in arresting mycelial growth of *A. solani* except *Agaricus bisporus*, which could not inhibit mycelial growth of *A. solani*.In 40 per cent water extracts it was observed that *Russula* sp. showed maximum growth inhibition (82.96%) with serrate mycelial growth of *A. solani* followed by *Pleurotus florida* (78.14%) with serrate mycelial growth while, *Ganoderma lucidum* and *Agaricus bisporus*, which could not inhibit mycelial growth of *A. solani*.

Song and Ji (2006) previously reported the inhibitory effects of culture filtered and ultra-sonified mycelial extracts of 8 toxic mushroom strains (*Amanita virosa, Lepiotaclypeolaria, Lactariusvellereus, Amanita pachycolea, Amanita* sp., *Ramariaephemeroderma, Clitocybedealbata, Lepiotacristata*) on spore germination of *Alternaria alternata*. The culture filtered extracts inhibited (61.44 to 90 %.) *in vitro* spore germination of *Alternaria alternata*. Chu *et al*., 2005 reported antifungal peptide designated pleurostrin exhibited antifungal activity against *F. oxysporum*, *Alternaria alternata* and *Drashelaria* sp. A variety of antifungal proteins and peptides have been isolated and

Table 4: Antimicrobial activities of mushroom extracts (40%) against *Alternaria solani* and its mycelial growth characteristics

Sr. No.	Mushroom sp.	Methanol 40%		Acetone 40%		Water 40%	
		% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics	% Inhibition	Mycelial growth characteristics
1.	*G.lucidum*	100 (90.00)	Complete inhibition	76.66 (61.09)	Dull black, velvety	0 (0)	Dull black, velvety
2.	*G.sapidum*	100 (90.00)	Complete inhibition	79.26(62.88)	Fluffy type	23.70(29.11)	Cottony / Fluffy
3.	*P. florida*	65.55 (54.04)	Cottony	72.59(58.40)	Cottony	78.14(62.11)	Serrate type
4.	*Russula* sp.	77.03(61.34)	Serrate type	90.00(71.55)	Irregular type edge	82.96(65.61)	Serrate type
5.	*Boletus edulis*	100(90.00)	Complete inhibition	100(90.00)	Complete inhibition	27.40(31.54)	Dull black colour
6.	*Russula* sp. + *Curcumacaesia*	88.15(69.85)	Irregular edge	87.03(68.89)	Fluffy	60.74(51.18)	Dull black, irregular edge
7.	*Agaricusbisporus*	0(0)	Dull black, smooth	0(0)	Dull black, smooth	0 (0)	Dull black, smooth
8.	Control	0 (0)	Black, smooth	0(0)	Black, smooth	0 (0)	Black, smooth
	S.E.±	0.37		0.42		0.50	
	C.D. (P=0.05)	1.13		1.26		1.51	

All values are mean of three replications. Figures in the parentheses are arcsine transformed values.

purified from fruiting body of *Ganoderma* mushrooms.

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