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Broad spectrum antimicrobial activity of ectomycorrhizal specie; *Russula delcica*

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Abstract: Ectomycorrhizal species produces antibiotic substances found to be inhibitory for various pathogenic microorganisms. The scientific community has reported antimicrobial activity of several mushrooms. *Russula delica* Fr. is a well known macro fungi which is used as a food in many parts of world. Antimicrobial activity of *R.delica* was tested against disease causing fungal and bacterial pathogens using disc-diffusion method. Three antibiotics; amoxicillin, phnexin and hexamox were used as positive control and DMSO (Dimethyl sulfoxide) was used as negative control. Ethanol extract of *R. delica* showed significant antimicrobial activity against one fungal and three bacterial pathogens. Positive fractions were further tested for minimum inhibitory concentration (MIC). Study showed that ethanol extract of ectomycorrhiza can be used as better alternative therapeutic agent than commercially used synthetic drugs which also have many side effects.

Keywords: **Antimicrobial, Ectomycorrhiza, Minimum inhibitory concentration, *Russula delica***

I. INTRODUCTION

The ectomycorrhizal symbiosis represents one of the most prominent and ecologically crucial mutualistic associations in terrestrial habitats. It involves several species of fungi grouped within the phyla Bassidobycota, Ascomycota, Zygomycota and hundreds of mostly woody plant species worldwide. Ectomycorrhizas occur in most of the temperate and boreal ecosystem and in large forested area of tropical and subtropical regions (Smith and Read, 1997; Caimey and Chambers, 1999; Verbeken and Buyck, 2001; Comandini et al., 2006; Wang and Qiu.2006). Mycorrhizal plants are often more resistant to diseases, such as those caused by microbial soil borne pathogens. This may be due to the improved water and mineral uptake. The resistance may also be due to the mechanical barrier afforded by mycorrhizal fungal mantle. However, species of certain ectomycorrhizal fungal genera such as *Lactarius, Hygrophorus* and *Cortinarius* produce antibiotic substances. Some of these antibiotics are found to be antifungal on *Rhizoctonia solani, Pythium debaryanum* and *Fusarium oxysporium* (Liu *et al*. 2007).

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Natural products with antimicrobial activity are used to aid the endogenous protective system, increasing interest in the antimicrobial role of nutraceutic products. Mushrooms have also become attractive as a functional food and as source for the development of drugs and nutraceuticals (Barros et al., 2007). Medicinal mushrooms have an established history of use in traditional oriental therapies. The scientific community has found various therapeutic activities such as anti-inflammatory, immunosuppressor, anticarcinogenic and antibiotic and researchers have showed antioxidant and antimicrobial activities of several mushrooms (Dulger *et.al.* 2002, Gao *et.al.* 2005, Karaman *et.al.* 2003, Mercan *et.al.* 2006 and Turkoglu *et.al.* 2007). Chloroform and ethyl acetate extracts of dried mushrooms have antimicrobial effect against some bacterial and fungal species. MDR (Multiple drug resistance) in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. According to some toxicologists and nutritionists, the side effects of some commercially used antimicrobial drugs have already been documented like allergy. All these situations forced scientists for searching new antimicrobial chemotherapeutic agents. The chloroform and ethyl acetate extracts of the dried mushroom have been reported to have antibacterial activity against *Streptococcus mutans* and *Prevotella intermedia* (Hirasawa *et.al.*1999). Fruiting bodies and the mycelium of both the species contain compounds with wide-ranging antimicrobial activity (Jong *et.al.*1993).

To the best of our knowledge, not much work has been reported on antimicrobial activities of the extract of *Russula delica* and in the light of facts mentioned above this study was planned with the objective to evaluate the antimicrobial potential of *Russula delica*.

II. MATERIAL AND METHODS

Source of plant material

Fruiting bodies of ectomycorrhizal species *Russula delica* was obtained from forest of Nainital region and identified on the bases of various phenotypic characters.

Determination of antimicrobial activity

For the evaluation of broad spectrum antimicrobial activity of *R.delica,* ethanol extract of *fruiting body* was tested against the four fungal (*Rhizactonia solani, Tricoderma atroviride, Alternaria solani* and *Fusarium oxysporium*) and four bacterial pathogens (*E. coli, Erwinia herbicola, Staphlococcus aureus* and *Agrobacterium rhizogens*) which are known to cause various diseases in plants and animals using Disc-diffusion method. Three most commonly used antibiotics (Amoxicillin, Phnexin and Hexamox) were used as positive control and DMSO (Dimethyl sulfoxide) was used as negative control. All the fractions which showed significant antimicrobial (antifungal and/or antibacterial) activity were further tested to evaluate minimum inhibitory concentration (MIC).

Preparation of fungal extract

Fresh mushrooms (450g) were air dried in hot air oven at 40^0c before the analysis. Dried mushroom samples (50g) were extracted by stirring with 500 ml of ethanol at 30^0c at 150 rpm for 24 hrs and filtered through Whatman no. 4 filter paper (Turkoglu *et. al.* 2007). The residue was then extracted with additional 500 ml of ethanol as described above. The combined ethanol extract were then rotary evaporated at 40^0c to dryness and dissolved in ethanol to a concentration of 10 mg/ml and stored at 40^0c for further use.

Culture and maintenance of pathogenic microorganisms

Different media were used to grow pathogenic fungal and bacterial species. All the pathogenic microorganisms tested for the antimicrobial activities were spread on their respective solidified agar media in petri plates and incubated for two days at appropriate temperature (table 1).

Table 1: Media and appropriate growth temperatures used for different pathogens

S.NO.	Microorganism	Media used	Growth temperature
1.	*E. herbicola*	L.B. medium	37^0C
2.	*E. coli*	Nurient agar medium	37^0C
3.	*S. aureus*	Nurient agar medium	37^0C
4.	*A. rhizogenes*	Yeast Extract Mannitol Agar	25^0C
5.	*R. solani*	Czapek-Dox Agar medium	25^0C
6.	*T. atroviride*	Czapek-Dox Agar medium	25^0C
7.	*A. solani*	Czapek-Dox Agar medium	25^0C
8.	*F. oxysporium*	Czapek-Dox Agar medium	25^0C

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Application of discs

Whatman filter paper no.4 discs of approximately 5 mm diameter were placed in petridish and autoclaved. These discs were impregnated with ectomycorrhizal extract, 3 antibiotics and DMSO separately. Once plates were inoculated with pathogenic microorganisms, five filter paper discs; one impregnated with ethanol extract of *R. delica,* another impregnated with DMSO (negative control) and three impregnated with standard antibiotics; Amoxicillin (10 mg/ml), Phnexin (10 mg/ml) and Hexamox (10 mg/ml) (positive control) were applied under aseptic conditions. The plates were inverted and placed in incubator set at appropriate temperature for the growth of the inoculated pathogens just after the discs were applied (table 1).

Reading of plates

After 24 to 48 hrs of incubation, each plate was examined. The diameter of complete inhibition zone was measured, including the diameter of disc using a ruler. The zone margin was taken as the area showing no obvious, visible growth of any tiny colonies.

MIC evaluation

Fourfold serial dilution of ethanol extract of *R. delica* was prepared by diluting 10% DMSO to achieve a decreasing concentration range from 2000 µg/ml to 500 µg/ml. Autoclaved filter paper discs were impregnated with the ethanol extract of *R. delica* of different concentration and then applied in the petriplates inoculated with pathogenic microorganisms for all the positive fractions. After that plates were incubated at appropriate temperature for the growth of the inoculated pathogenic microorganisms for 24 to 48 hrs (table 1). The least concentration of each fugal extract showing the inhibition zone was taken as MIC.

III. RESULTS AND DISCUSSION

Antimicrobial screening

Antimicrobial screening reveals that the ethanol extract of *R. delica* have a broad antimicrobial spectrum as found effective against one fungal and three bacterial pathogens (table 2). When antimicrobial activities of ethanol extract of *R.delica* was compared with those of commonly used antibiotics against pathogenic microorganisms it was found that in some cases, fungal extracts were even more active than the commercially used antibiotics as shown in table 2. Antimicrobial activity of ethanol extract of *R. delica* against various pathogenic microbes has been reported (Turkoglu *et.al.* 2007 and Jain & Pande 2013). *R. delica* is easily available at high altitude of eastern zone of India. The extracts of ectomycorrhizal fungus can be used as better alternative therapeutic agent than commercially used synthetic drugs which also have many side effects.

Table 2. Antimicrobial activity of *R. delica* against different pathogens.

S.NO.	Microorganisms	*R.delica*	Antibiotics		
			A#	P#	H#
1.	*E. herbicola*	+	-	-	-
2.	*E. coli*	-	+	+	+
3.	*S. aureus*	+	+	-	-
4.	*A. rhizogenes*	+	+	+	+
5.	*R. solani*	-	-	-	-
6.	*T. atroviride*	-	-	-	-
7.	*A. solani*	-	-	-	-
8.	*F. oxysporium*	+	+	-	-

(A- Amoxicillin, P- Phnexin, H- Hexamox, + - Effective, **-** - Not effective)

Most interesting result was obtained in case of bacterial pathogen *E. herbicola* as it was found to be resistance against all the three antibiotics and no inhibition zone was obtained but extract of *R. delica* was found inhibitory as an inhibition zone of 7mm was observed. Extract of *R. delica* also showed inhibition against *S. aureus, A. rhizogense*, *F. oxysporium* with inhibition zones of 9.5, 6 mm and 5.5 mm respectively. Out of three antibiotics used only Amoxicillin was found effective against all three pathogens. *R. solani, T. atroviride* and *A. solani* were found to be resistant against *R. delica* and all the three antibiotics as no inhibition zones were observed (table 3).

Table 3. Inhibition Zone (mm) formed by ethanol extract of *R. delica* and three antibiotics.

S.NO.	Microorganisms	*R.delica*	Antibiotics		
			A#	P#	H#
1.	*E. herbicola*	7	-	-	-
2.	*E. coli*	-	7	5.8	6
3.	*S. aureus*	9.5	8	-	-
4.	*A. rhizogenes*	6	11	6	5.5
5.	*R. solani*	-	-	-	-
6.	*T. atroviride*	-	-	-	-
7.	*A. solani*	-	-	-	-
8.	*F. oxysporium*	5.5	6	-	-

(A- Amoxicillin, P- Phnexin, H- Hexamox, + - Effective, **-** - Not effective)

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MIC evaluation

All the fractions of ethanol extract of *R. delica* found significantly active in disc diffusion analysis at 2000 µg/ml concentration were further analyzed to determine MIC. It was observed that to inhibit the growth of *A. rhizogens* and *F. oxysporium* a minimum concentration of 1.0 mg/ml of *R. delica* extract was required whereas in case of *E. herbicola* minimum 1.5 mg/ml of extract was required for inhibition. In case of *S. aureus* only 0.5 mg/ml of *R. delica* extract was sufficient for inhibition (table 4).

Table 4. Minimum concentration required to inhibit the growth of test pathogenic microorganism.

Test microorganism	MIC (mg/ml) of *R. delica*
F. oxysporium	1.0
E. herbicola	1.5
S. aureus	0.5
A. rhizogenes	1.0

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