ANTIFUNGAL AND ANTIBACTERIAL ACTIVITY OF WILD EDIBLE MUSHROOM RAMARIA BOTRYTIS (FR.) RICKEN FROM NORTH WEST HIMALAYAS

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Abstract
The specimens of Ramaria botrytis were collected from nature during 2011-2012. Collections were made from different parts of Distt. Shimla (H. P.)[1], India. The antimicrobial activity, of Ramaria botrytis, at four concentrations (20, 40, 60 and 80%) of extracts prepared in different solvents (water, methanol and ethanol) were screened against five pathogens including three fungal pathogens (P. infestans, A. alternata and F. sambucinum) and two bacterial pathogens (E. coli and S. aureus). Maximum inhibition against all the five test pathogens including fungal as well as bacterial pathogens was observed in methanol extract at a concentration level of 80%, followed by ethanol and aqueous extracts at the same concentration. On comparing antifungal and antibacterial activity, it was observed that all extracts were having more antifungal property as compared to antibacterial property. In case of P. infestans, methanol and ethanol extracts in the concentration range of 40-80% completely checked the mycelial growth i.e. 100%. Whereas, growth inhibition of remaining four pathogens i.e. A. alternata, F. sambucinum E. coli and S. aureus increased with increase in concentration level of different solvent extracts. Except, aqueous extract at all concentrations remained totally ineffective against E.Coli.

Key Uses: antimicrobial, methanol, ethanol, extract, growth inhibition Ramaria botrytis.

I INTRODUCTION

Recently, in different parts of the world, attention has been paid towards exploitation of bio-products as novel chemo-therapeutants in plant protection because of non-phytotoxicity, sytemecity, easy biodegradability and stimulatory nature of host metabolism, plant products are considered valuable for controlling plant, animal and human diseases (Mishra and Dubey, 1994; Siva et al., 2008).

Although, fungicides and antibiotics have been very effective in controlling the fungal and the bacterial diseases, respectively but the use of those chemicals leads to health and environment hazards. Despite the use of half a million tones fungicides and pesticides annually, one third of all crop production is still lost. Continued use of fungicides is threatening the environment and health and is responsible for some major problems. Firstly, some fungi have acquired resistance against fungicides particularly the systemic fungicides; secondly, some fungicides are not biodegradable and tend to persist for years in the environment. This leads to third problem, the detrimental effects of chemicals on organisms other than target fungi (Brady, 1984; Agarwal et al., 2001). Similarly, society is facing serious public health dilemma over the emergence of infectious bacteria displaying resistance to many antibiotics (Kapil, 2005). Human infections, particularly those involving skin and mucosal surfaces constitutes serious problem, especially in tropical and subtropical countries (El-Mahmood and Amey, 2007). Methicillin Resistant Staphylococcus aureus (MRSA), Escherichia coli, Pseudomonas aeruginosa, Candida albicans were observed to be the most frequent skin pathogens (Obeidat, 2011). Therefore, human health and environmental safety are the two most important issues in the long term application of pesticides, fungicides and antibiotics (Lin, 1995).

Due to increasing awareness about the risk involved in use of chemicals much attention is being focused on alternative methods of pathogen control. Spiraling up the cost of synthetic fungicides, pesticides and antibiotics and pollution to soil, water, air by the accumulation of obnoxious chemical residues due to continuous use of these chemicals and development of resistant races to these chemicals are therefore now forcing the scientists to look for methods which are eco-friendly, safe, cost effective and specific for pathogen. The recurrent and indiscriminate use of fungicides have posed a serious threat to human health and existing human eco-geographical conditions as some of them have been proved to be either mutagenic, carcinogenic. Keeping in view the drawbacks of chemical management of animal and plant diseases and to increase world food production and feed the ever increasing population, agricultural production can be augmented with biological control instead of chemicals (Mukerji and Garg, 1993; Joseph et al., 2008).

Higher and lower plants contain a wide spectrum of secondary metabolites such as phenols, flavonoids, quinines, tannins, essential oils, alkaloids, saponins, sterols, polysaccharides particularly beta-glucan, chalcones, yellow polyphenol pigments composed of styryl pyrone. Such plant metabolites may be exploited for their different biological properties (Tripathi et al.,

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2004). Terresterial plants produce a wide spectrum of natural products viz. terpenoids, phenolic, alkaloids, tannins and quinines. Many of these are thought to be serving an ecological function for the plants from herbivores and pathogens (Islam and Akhtar, 2007). Both higher and lower plants generally produce many secondary metabolites which constitute an important source of micro-biocides, pesticides, fungicides and pharmaceutical drugs (Ibrahim, 1997; Mahesh and Satish, 2008).

The use of plants for curing various ailments is figured in ancient manuscripts such as the Rigveda and the Samhita etc. in early ages, man used raw drugs isolated or obtained from the plants leading to information about the interrelationship between primitive man and plants. Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper cost than modern medicines (Mann et al., 2008). Phyto-toxins which are safe and eco-friendly are considered a good alternative for the disease management (Kumar and Yadav, 2007).

Due to increasing awareness about micro-biocide and fungicides hazards, a need was felt to develop biological agents for the control of plant, animal and human diseases. In the field of biological control, mushrooms have attracted attention of scientists all over the world for a long time and yet studies in other areas of the world have shown that mushrooms contain many bioactive compounds with diverse biological activaties like higher plants.Since long, mushrooms have been cultivated world-wide for commercial purposes (Olila et al., 2008). Scientific research in this field indicates that metabolites of mushrooms are the potential source for the production of nutritiorial, neutraceutical and antimicrobial compounds (Veluchamy et al., 2012). Keeping into consideration the antimicrobial property of mushrooms extract, it is considered worthwhile to take up the studies with wild edible mushroom Ramaria botrytis against fungal pathogens viz, . Phytophthora infestans, Fusarium sambucinum, Alternaria alternata and bacterial strains viz. Staphylococcus aureus and Escherichia coli with the following objectives:

II MATERIALS AND METHODS

Test Pathogens

1 Procurement of test fungal and Bacterial pathogens

Fungal isolates of Phytophthora infestans, Alternaria alternata and Fusarium sambucinum were procured from the Department of Plant Pathology of Central Potato Research Institute Shimla. Pathogenic strains of Escherichia coli and Staphylococcus aureus were procured from Indira Gandhi Medical College, Department of Microbiology, Shimla.

Maintenance and preservation of pure culture

Pure cultures of all the fungal isolates of test pathogens were maintained on Potato Dextrose Agar (PDA) medium while, pure cultures of bacteria used as test pathogens were maintained on nutrient medium broth and were preserved in refrigerator at 4°C. Sub culturing was done at regular intervals in order to maintain the culture.
Each fungal /bacterial species of test pathogens was transferred from parent source to a fresh slant/fresh nutrient medium (broth) in order to maintain and preserve the parent culture, respectively.

Extraction procedure for preparation of methanol, ethanol and aqueous extracts from Ramaria botrytis mushroom.

A fine powder (20 meshes) was obtained using a mill (Restch ultra centrifugal mill and sieving machine). Dried mushroom powder sample (20 g) of Ramaria botrytis was extracted by stirring with 100ml of methanol (solvent) at 25°C at 150 rpm for 24h and filtering through Whatman No.4 filter paper. The residue was then extracted with two additional 100ml of methanol as described above. The combined methanol extracts were then rotary evaporated at 40°C to dryness, re-dissolved in methanol to a concentration of 50mg/ml (stock solution) and stored at 4°C in a refrigerator for further use. The whole procedure was repeated with ethanol and distilled water as solvents, to get ethanol and aqueous extracts, respectively. Preparation of extracts of mushrooms was based on procedures described by Barros et al., (2008) with some modifications.

Methodology for screening Ramaria botrytis for antifungal activity against fungal pathogens i.e. Alternaria alternata, Fusarium sambucinum and Phytophthora infestans.

Screening of methanol, ethanol and aqueous extracts of Ramaria botrytis against fungal plant pathogens viz .Phytophthora infestans, Alternaria alternata and Fusarium sambucinum was done using poisoned food technique (Grover and Moore, 1962; Perrucci et al., 1994; Mishra and Dubey, 1994).

Potato Dextrose Agar (PDA) medium (Potato: 200 gm, Dextrose: 20 gm, Agar-Agar: 15 gm, Distilled water: 1 lt.) was used for culturing Alternaria alternata and Fusarium sambucinum while Rye B medium was used for Phytophthora infestans.

The respective medium was autoclaved at 121.6°C for 30 minutes. After cooling the medium to 45°C, ten milligram of streptomycin was added to it and was mixed thoroughly to prevent bacterial contamination (Gupta and Banerjee, 1970; Srivastava, 2008).

In Poisoned food technique, each mushroom extract i.e. methanol, ethanol and aqueous extract was tested at 20%[2] (10ml/ml), 40%[3] (20mg/ml), 60%[4] (30 mg ml^{-1}) and 80%[5] (40mg/ ml) concentration, prepared separately by dissolving requisite amount in PDA medium in case of Alternaria alternata and Fusarium sambucinum and in Rye B medium for (Phytophthora infestans), cooled to 45°C in a beaker and then streptomycin was added to it and mixed thoroughly so as to prevent bacterial contamination. 10 ml of each concentration was poured into sterilized pertri plates (9.0 cm diameter). Disc (8mm diameter) of test fungal pathogen cut from the periphery of seven days old culture (A. alternata and F. sambucinum) and 12 days old culture (P. infestans) with the help of sterilized cork borer and was inoculated aseptically in each of the treatment.

Control sets: The medium of control sets, contained requisite amount of corresponding solvent (methanol, ethanol or distilled water) in place of corresponding extract. Three replicates were maintained in each case. The petri plates were incubated at 25 ± .5°C for seven days (A. alternata and F. sambucinum) and at 18°C for 12 days (P. infestans) in an incubation chamber. Diameters of fungal mycelial colonies of treatment and control sets were measured in mutually perpendicular direction on seventh day (A. alternata and F. sambucinum) and twelfth day (P. infestans). The percentage inhibition of radial growth of test fungus by different extracts was calculated following Pandey et al., (1982) method as:
% inhibition of fungal colony= dc-dt/dcx100
Where dc= average diameter of fungal colony in control sets.
dt= average diameter of fungal colony in treatment sets.
Percentage inhibition of growth of all the test fungi by different extracts of mushrooms samples using poisoned food technique was calculated on seventh day (A. alternata and F. sambucinum and twelfth day (P. infestans) and the results are represented in tables 1-10.

Methedolology for screening Ramaria botrytis for antibacterial activity against Staphylococcus aureus and Escherichia coli

Antibacterial activity of methanol, ethanol and aqueous extracts of Ramaria botrytis was determined by the agar well diffusion assay. All the microorganisms mentioned above were incubated separately at 37±0.1°C for 24 h by inoculation into nutrient broth (Beef extract 1gm, yeast extract 2gm, Sodium Chloride 1gm, Peptone 5gm, distilled water 1 It). The culture suspensions were prepared and adjusted by comparing against 0.4-05 McFarland turbidity standard tubes. Nutrient Agar (NA) medium (Beef extract 1gm, yeast extract 2gm, Sodium Chloride 1gm, Peptone 5gm, Agar-Agar 20 gm, distilled water 1 It.) was used throughout the investigation for the growth of microorganisms. The medium was autoclaved at 121.6°C for 30 minutes. Nutrient Agar medium (10ml) was poured into each sterilized petri dish (9cm). The plates were left over night at room temperature to check for any contamination to appear. Bacterial lawns were prepared by distributing 100 µl nutrient broth culture of each bacterium homogenously over the petri dish medium.

Agar-wells of 8mm diameter were prepared with the help of stainless steel cork borer. One well was prepared in each nutrient agar plate. For investigating bacterial activity, the well in each plate was loaded with 20, 40, 60 and 80% concentrations prepared separately by dissolving extracts in requisite amount of corresponding solvent (methanol, ethanol and distilled water in control sets), agar wells were filled with bare corresponding solvent only.

Plates inoculated with bacterial culture were incubated at 37±0.1°C for 24 h. All determinations were done in triplicates. At the end of incubation period, inhibition zones formed on medium were evaluated. For evaluation, diameter of bacterial colonies of treated and control sets were measured in mutually perpendicular direction on second day. Percentage inhibition of radial growth of bacteria was calculated after subtracting the value of treated/tested extracts from control as standard (Hemasphenpagam N and Selvaraj T, 2010).

% Inhibition of bacterial colony= $\dfrac{dc - dt}{dc} \times 100$

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[2] **20%** = 200 µl ss + **800 µl of** dw
[3] **40%=** 400 µl ss + 600 µl of dw
[4] **60%** = 600 µl ss + **400 µl of** dw
[5] **80%** = 800 µl ss + **200 µl of** dw

Where, dc= average diameter of bacterial colony in control sets.
dt = average diameter of bacterial colony in treatment sets.

Statistics

For extract (methanol, ethanol and water) from mushroom samples, three samples were prepared for assaying every antimicrobial attribute and component. The experimental data was subjected to an analysis of variance for a completely random design, as described by Stell, Torrie and Dickey (1997) to determine the significant difference.

III OBSERVATIONS

Antimicrobial activity of Ramaria botrytis

To test the antimicrobial activity of Ramaria botrytis, four concentrations (20, 40, 60 and 80%) of extracts prepared in three different solvents (water, methanol and ethanol) were screened against five pathogens comprising both fungal and bacterial pathogens i.e. P. infestans, A. alternata, F. sambucinum, E. coli and S. aureus. The circular growth of each pathogen in petriplates was measured after ten days of incubation at a temperature of 25 ± .2^{0}C. The mean % growth inhibition of extracts is presented in Table 1 and Plate –XIV and XV (Petriplates 1.1-1.14).

It is evident from Table 1 that all the three extracts i.e. aqueous extract, methanol extract and ethanol extract were effective in checking the growth of all the five test pathogens, except aqueous extract which was found totally ineffective against E.coli. Most interesting findings were that of methanol and ethanol extracts in the concentration range of 40-80% inhibited growth of P. infestans completely 100% inhibition). In case of remaining four pathogens (i.e. A. alternata, F. sambucinum, E. coli and S. aureus), inhibition increased with increase in concentration of different solvent extracts (table 4.6). Maximum growth inhibition against all five pathogens was observed in methanol extract at a concentration level of 80% and followed by ethanol and aqueous extracts at the same level of concentration. On comparing the antifungal and antibacterial activity it is revealed that all the extracts extracts.(except, aqueous extract at all concentrations remained totally ineffective against E.Coli.) proved very effective against fungal pathogens as compared to bacteria. Similarly, comparison between two bacteria i.e. S. aureus and E. coli it was revealed that all the extracts were more effective against S. aureus than E. coli.

Therefore, it is concluded that all the three solvent extracts of Ramaria botrytis inhibited the mycelia growth of all the five test pathogens to different extents. Though, ethanol extract at a concentration of 80% was quite effective but methanol extract at a concentration level of 80% proved really very effective against all the pathogens.

Table 1: Antimicrobial activity of Ramaria botrytis

Extract	Concentration in %	%age growth inhibition of test pathogens by different extracts of Ramaria botrytis				
		P. infestans	A. alternata	F. sambucinum	E-coli	S. aureus
Aqueous extract	20	16.89 ± .84	13.78 ± .39	9.67 ± .34	-	9.11 ± .19
	40	31.89 ± .51	27.11 ± .84	22.00 ± .67	-	15.00 ± .33
	60	41.55 ± .39	37.36 ± .34	41.44 ± .51	-	19.55 ± .69
	80	53.33 ± 1.15	53.78 ± .39	53.67 ± .34	-	27.00 ± .33
Methanolic extract	20	81.57 ± .51	26.89 ± .19	28.22 ± .34	15.22 ± .69	11.89 ± .84
	40	100.00 ± .00	45.33 ± 1.34	52.22 ± .19	24.45 ± .39	25 .22 ± 1.07
	60	100.00 ± .00	78.00 ± .37	66.33 ± .34	36.22 ± .39	31.89 ± .84
	80	100.00 ± .00	87.22 ± .51	88.33 ± .34	40.78 ± .19	38.00 ± .00
Ethanolic extract	20	76.44 ± .19	21.44 ± 1.26	16.33 ± .34	12.78 ± .69	9.33 ± .58
	40	100.00 ± .00	40.78 ± .19	36.33 ± .34	22.56 ± .51	18.44 ± .51
	60	100.00 ± .00	61.22 ± .69	54.33 ± .34	31.22 ± .69	25.55 ± .39
	80	100.00 ± .00	81.67 ± 1.00	82.45 ± .39	37.67 ± .58	35.33 ± 1.15

Each data represents the mean of 3 replicates ± S.D.

PLATE-XIV

Showing the fungitoxic activity of different solvent extracts of Ramaria botrytis.

1.1 Petriplates showing growth inhibition of Phytophthora infestans by aqueous extract of Ramaria botrytis.
1.2 Petriplates showing growth inhibition of Phytophthora infestans by methanolic extract of Ramaria botrytis.
1.3 Petriplates showing growth inhibition of Phytophthora infestans by ethanolic extract of Ramaria botrytis.
1.4 Petriplates showing growth inhibition of Alternaria alternata by aqueous extract of Ramaria botrytis.
1.5 Petriplates showing growth inhibition of Alternaria alternata by methanolic extract of Ramaria botrytis.
1.6 Petriplates showing growth inhibition of Alternaria alternata by ethanolic extract of Ramaria botrytis.
1.7 Petriplates showing growth inhibition of Fusarium sambucinum by aqueous extract of Ramaria botrytis.
1.8 Petriplates showing growth inhibition of Fusarium sambucinum by methanolic extract of Ramaria botrytis.
1.9 Petriplates showing growth inhibition of Fusarium sambucinum by ethanolic extract of Ramaria botrytis.

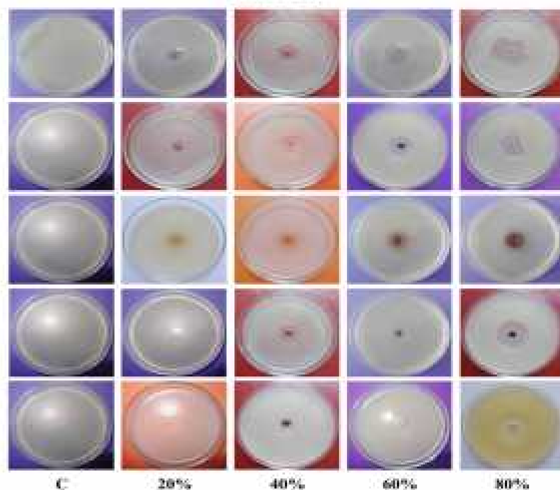

PLATE-XV

Showing bactericidal activity of different solvent extracts of *Ramaria botrytis*.

1.10 Petriplates showing growth inhibition of *Escherichia coli* by methanolic extract of *Ramaria botrytis.*
1.11 Petriplates showing growth inhibition of *Escherichia coli* by *ethanolic* extract of *Ramaria botrytis.*
1.12 Petriplates showing growth inhibition of *Staphylococcus aureus* by aqueous extract of *Ramaria botrytis.*
1.13 Petriplates showing growth inhibition of *Staphylococcus aureus* by methanolic extract of *Ramaria botrytis.*
1.14 Petriplates showing growth inhibition of *Staphylococcus aureus* by *ethanolic* extract of *Ramaria botrytis.*

IV DISCUSSION

As natural products have potential of containing therapeutic agents against infectious diseases (Clardy et al., 2004). Natural products, either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drugs but only a minute portion of the available diversity among fungi, marine flora and fauna, bacteria and plants has yet been explored for such purposes (Cos et al., 2006). The use of plant extracts (including macro fungi: mushrooms) for antimicrobial activity is enjoying great popularity since 1990's when people realised that effective life span of antibiotics and other synthetic chemicals is limited and over dose and misuse of these chemicals is causing microbial resistance (Alam et al., 2009). In this context, mushrooms are not only source of nutrients but also could be used to prevent diseases such as hypertension, hypercholesterolemia, cancer, skin-boils, fibrosis, diabetes, urinary and pulmonary infections in man (Wasser and Weis et al., 1999a; Bobek et al., 1995).

Fungal pathogens are also significant destroyers of food stuffs during storage, rendering them unfit for human consumption by retarding their nutritive value and sometimes by producing mycotoxins. Approximately, 20-40% of cereals world-wide are contaminated with mycotoxins produced by different fungi during storage (Kumar et al., 2007). One third of the world's potential food supplies are estimated to be lost due to pre and post-harvest pathogens and diseases. According to FAO estimates, potential losses world-wide are 35% (Odhiambo, 1985).

Chemical control measures against plants have a long history. Already in nineteenth century and even earlier chemicals containing copper, sulphur or phenolic compounds were used to control various plant diseases (Backhaus, 2009). Fungicides are usually applied as effective, dependable and economical control measures to control fungal diseases. However, the indiscriminate use of chemical fungicides has resulted in several problems, such as toxic residues in food, water and soil and degradation of the ecosystem, leading to the fear that their regular use may harm the environment further. Hardly 0.1% of the agro-chemicals used in crop protection reach the target pathogen leaving the remaining 99.9% to enter environment to cause a hazard to non-target organisms including humans, animals and to environment (Pimentel and Levitan, 1986; Varma and Dubey, 1999). According to WHO estimates, approximately 0.75 million people are becoming ill every year with agrochemicals poisoning, further the resistance of pathogens to fungicides has rendered certain fungicides ineffective, giving rise to new physiological races of pathogens.

Basic research for over more than 40 years in biology and biochemistry has made it possible to envisage not only how new pesticides may be synthesized but also has generated a completely new approach to the production of plants using secondary plant products which may be toxic to a specific pathogen and harmless to humans and animals. Pesticide plants have been in nature for millions of years and their bioactive compounds are not having any ill or adverse effect on the ecosystem. They have distinct advantage in management of diseases caused by pathogens. Plants have natural potential to withstand the aggressiveness of pathogenic species. A wide spectrum of secondary substances is contained in higher as well as lower plants. The total number of plant chemicals may exceed 4000 and out of these 1000 are secondary bioactive metabolites. These bioactive metabolites act as a major defence mechanism for plants (Tripathi and Shukla, 2010).

The preservative nature of some plant extracts have been known for centuries and has been reported from higher as well as lower plants (especially mushrooms) (Datar, 1999). The antimicrobial metabolites are contained in the phytochemical constituents (alkaloids, saponins, tannins, chalcones, tocopherols, beta-glucans, flavonoids, polysaccharides and polyphenols etc.) of the plants (Edeoga and Mbaebie, 2005). Antimicrobial activities have been linked to the presence of bioactive compounds which sometimes serve to protect plants themselves against bacterial, viral and fungal infections as well as exhibiting their antimicrobial properties against these organisms (El-Mahmood and Amey, 2007). Therefore, these days plant extracts have assumed a special significance as an eco-friendly method for plant disease management. Further, mushrooms are proving most promising agents as the antimicrobial substances. Edible and non edible mushrooms have the potential to be developed into bio control agents for the control of plant as well as human and animal diseases (JinTong et al., 2010).

Findings of the present investigation are in agreement with the work of (Iftekhar, 2011; Centko et al., 2012). Antimicrobial activities of Ramaria flava (Tourn: Battarra) Quel.extract obtained with ethanol were investigated in this study. The ethanol extract of R. flava inhibited the growth of Gram Positive bacteria better than Gram negative bacteria and yeast. The crude extract elicited minor antibacterial activity against Pseudomonas aeruginosa, Escherichia coli, Morgnella morganii, and Proteus vulgaris. The antibacterial profile of R. flava against tested strains indicated that Micrococcus flavus, Micrococcus luteus and Yersinia enterocolitica were the most susceptible bacteria of all the test strains (Gezer et al., 2006). Likewise, four butenolides, ramariolides A-D (1-4), have been isolated from fruiting bodies of the coral mushroom Ramaria cystidiophora.

The major metabolite, remariolide A(1), which contains an unusual spirooxiranobutenolide moiety revealed in-vitro antimicrobial activity against Mycobaterium smegmatis and Mycobacteruim tuberculosis.

V CONCLUSION

In the present investigation, it was considered worthwhile to find out the antimicrobial (antibacterial and antifungal) properties of extracts of Ramaria botrytis. Since, mushrooms are perishable items, dried mushroom parts (fruiting bodies with stalk) have been used for their fungal toxicity and bactericidal property against test fungal and bacterial pathogens. In present study extracts of Ramaria botrytis (dried powder) prepared in three different solvents separately i.e. methanol, ethanol and water were screened at the concentrations 20%, 40%, 60% and 80% against three test fungal pathogens.

Phytophthora infestans, Alternaria alternata and Fusarium sambucinum and two bacteria Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). It was observed that growth inhibition took place at every concentration studied i.e. 20, 40, 60 and 80% concentration in every extract with different solvents, except aqueous extract which remained totally ineffective against E.Coli. at all concentrations. Maximum growth inhibition was observed at 80% concentration in every extract with different solvents. Tables 1 clearly put forward that Ramaria botrytis under investigation exhibited antimicrobial activity against every test pathogen Growth inhibition increased with increase in the concentration level. Therefore, maximum inhibition was observed at 80% concentration while minimum at 20% concentration.

Although, inhibition at 20% concentration was negligible but concentrations at 40%, 60%, and 80% levels significantly inhibited the growth of fungal pathogens;Phytophthora infestans, Alternaria alternata and Fusarium sambucinum. Moreover, on comparing the % inhibition growth of test pathogens, it was found that maximum growth inhibition was exhibited by methanol extract followed by ethanol and aqueous extracts. Phytophthora infestans was found most sensitive pathogen against methanol and ethanol extracts as its growth was totally inhibited even at 40% concentration. Aqueous extract was also fairly significant as it inhibited its growth up to 40% at 80% concentration. All the extracts (except aqueous extract against E. coli) proved very effective in controlling the growth of both tested bacteria. But growth inhibition was more pronounced against Staphylococcus aureus as compared to Escherichia coli.

The results of the present investigation are also in agreement with information available in the literature. Previous studies inferred that mushrooms have great potential to be used as source of nutritionally functional food and a source of biologically active, physiologically beneficial and nontoxic medicines (Wasser, 1999a). Many previous findings depicted the mushrooms as a source for the development of medicines and drugs due to their pharmacological effects against pathogenic microbes and drugs due to their pharmacological effects against pathogenic microbes (Jonathan and Fasidi, 2003; Gbolagade et al., 2005; Gezer et al., 2006). It is estimated that approximately 50% i.e. 5 million metric tons of cultivated edible mushrooms contains the functional therapeutic properties. The available literature confirms the anti-microbial property of macro fungi: Tricholoma giganteum, Lentinula edodes, Lentinula boryana, Lactarius deliciosus, Lactarius indigo, Podaxis pistillaris, Russula paludosa, Russula delica, Pleurotus sajor-caju, Pleurotus eryngii, Pleurotus florida, Pleurotus pulmonarius, Pleurotus citirinopileatus, Pleurotus villosus, Oudmensiella mucida, Cantharellus cibarius, Ramaria botrytis, Ramaria cistidiophora, Agaricus bisporus, Agaricus bitorquis, Agaricus blazei, Hygrophorous chrysodon, Armillariella mellea, Ganoderma lucidum, Flammulina velutipes, Hypsizygus marmoreus, Volvariella volvacea, Armillariella tabescens against fungal and bacterial pathogens.

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