WINSOME PUBLISHING
Global Journal of Infectious Diseases and Health Insights ISSN:3064-707X

Research Article Open Access

Antibacterial Agent from Lignicolous Macrofungi

Dr. Rehan Haider PhD[1*], Dr. Geetha Kumari Das Ph. D[2], Dr Zameer Ahmed PhD[3], Dr Sambreen Zameer[4]

[1]Head of Marketing and sales Riggs Pharmaceuticals, Department of Pharmacy, University of Karachi

[2]Dr. Geetha Kumari Das Ph.D, GD Pharmaceutical Inc OPJS University Rajasthan

[3]Assistant Professor, Dow University of Health Sciences Karachi Pakistan

[4]Associate Prof Department of Pathology, Dow University of Health Sciences Karachi Pakistan

Corresponding Author: Dr. Rehan Haider PhD[1*]

Received date: October 27, 2024; **Accepted date:** October 30, 2024; **Published date:** November 21, 2024

Citation: Dr. Rehan Haider PhD[1*], Antibacterial Agent from Lignicolous Macrofungi, **Global Journal of Infectious Diseases and Health Insights**, vol 1(2). DOI: 10.9567/ISSN.2024/WSJ.92

Abstract

Lignicolous macrofungi, typically referred to as timber-decaying fungi, have emerged as a substantial source of bioactive compounds with antibacterial homes. This observation reviews the antibacterial retailers isolated from numerous lignicolous macrofungi, highlighting their capacity applications in preventing antibiotic-resistant pathogens. The antibacterial efficacy of extracts from these fungi is attributed to a various array of secondary metabolites, which include phenolic compounds, terpenoids, and polyketides. The mechanisms of motion for those bioactive compounds also are mentioned, demonstrating their capability to disrupt bacterial mobile walls, inhibit DNA synthesis, and interfere with protein manufacturing. Furthermore, the exploration of lignicolous macrofungi as biotechnological assets provides opportunities for the improvement of novel antibacterial tablets. This evaluation additionally emphasizes the significance of sustainable harvesting practices to make certain the conservation of fungal biodiversity even as facilitating bioprospecting efforts. The findings advise that lignicolous macrofungi constitute a promising avenue for the discovery of new antibacterial sellers, especially in mild of the growing worries concerning antibiotic resistance. Future research has to be aware of the isolation and characterization of these compounds, in addition to the elucidation of their pharmacological mechanisms. On average, the antibacterial marketers derived from lignicolous macrofungi may want to play a crucial role in the development of effective cures in opposition to multidrug-resistant bacterial infections.

Keywords: Antibacterial agents, lignicolous macrofungi, bioactive compounds, antibiotic resistance, sustainable harvesting, bioprospecting

Introduction

In ancient times, mushrooms had been prized as meals as well as a supply of drugs, giving upward push to a growing hobby these days ("useful food"). range of macrofungi is of a medicinal importance and represents an infinite source of secondary metabolites of excessive medicinal value even as a huge wide variety of biologically lively molecules are recognized in lots of species of macrofungi in the course of the sector (Wasser & Weis, 1999; Kitzberger et al., 2007; Barros et al., 2007; Turkoglu et al., 2007; Kim et al., 2008; 2007; Wasser, 2011).{1,2,3,4,5,6 }Further, of Importance is the amount of produced substances particularly, they should be simple for production (business synthesis) or there ought to be enough raw cloth for extraction of active molecules.

Such molecules, if chemical businesses accountable for the organic hobby are regarded, need to serve as basic compounds for the synthesis of new molecules. Lignicolous macrofungi explicit widespread biological results, consisting of antibacterial interest (Hur et al., 2004; Ishikawa et al., 2005; Kalyoncu et al., 2010){7,8,9 }and their secondary metabolites may be effortlessly extracted and identified. It's been discovered that secondary metabolites are very divergent in structure and play no critical position in their boom and replica, but in all likelihood have a feature in the biochemical evolution of a species ensuring its survival (Engler et al., 1998) {10}. The presence of these compounds in macrofungi is genetically

determined, however also varies as a function of ecological factors and the growth level of those organisms (Puttaraju et al., 2006){11}. The fungal metabolites of fruiting bodies often fluctuate from those of mycelia of submerged cultures or fermentation broth. Furthermore, biogenetic pathways are as a substitute depending on their habitats or geographic starting place. The chemical composition of fungal species drastically is predicated on the strains and websites (substrates) of the fruiting frame manufacturing. The degree of phenolic compounds seems to vary a whole lot depending on the location and pressure conditions (Kim et al., 2008). concerning this, more geographical regions and extra habitats should be analyzed in the future. A notable ability of these fungi is located in their use as dietary supplements, regardless of lively principle. Some of the products derived from mushrooms that are sold within the marketplace are untested and of suspicious exceptional. Since the natural style of existence emerged as increasingly popular around the sector, what approach return to organic, herbal food and drugs, many people lack an essential attitude to the so-known ecological merchandise. It would consequently be crucial to broaden food supplements and drugs based on natural sources, however with the necessary scientific confirmation of values of such products

Macrofungi

Macrofungi or mushrooms are not taxonomic classes, being maximum often used as terms for fungi with distinct fruiting our bodies, which can be normally fleshy and fit to be eaten, hypogeous or epigeous, huge sufficient to be visible with the naked eye, and picked by way of hand (Chang and Miles, 2002, Karaman et al., 2012).{12}

Lignicolous (wood-decaying) macrofungi, by and large belonging to the Polyporaceae own family, are without difficulty noticed, amassed, and identified in the discipline. Taxonomically, those fungi in particular belong to the phyla Basidiomycota and Ascomycota, along with approximately 20,000 acknowledged species, widely distributed on earth. Recent estimations advise that even greater than 1.5 million species of fungi exist on our planet and about 140,000 species belong to macrofungi. However, simplest 10% of them are explored and 16% are cultured (Chang & Miles, 2004; Mueller, payments & Foster, 2004)

1.2 Antibiotics and antimicrobial retailers

From the beginning until now, humankind has always been confronted with the problem of spreading of infectious diseases. Nowadays, more than a hundred and 150 compounds make an arsenal of antimicrobial materials used in the treatment of infectious illnesses. Antibiotics are described as low molecular weight natural products (secondary metabolites or idiolites) made via microorganisms, which are energetic at low concentrations in opposition to other microorganisms. There are estimations that among 12,000 antibiotics regarded, about 55% are produced by way of Streptomyces, 11% with the aid of different Actinomycetes, 12% from different organisms, and 22% from filamentous fungi (Inouye et al., 2004).{13} In its broadest definition, an antibacterial is an agent that interferes with the boom and reproduction of bacteria. In contrast to antibiotics, antibacterial aren't used as a medication for people or animals, but at the moment are most usually described as sellers used to disinfect surfaces and get rid of potentially dangerous microorganisms observed in products together with soaps, detergents, fitness and skin care products and family cleaners. Due to the fact Alexander Fleming`s discovery, in 1928, of the first antibiotic, called penicillin, produced with the aid of the mold Penicillium chrysogenum, an actual revolution in medicinal drugs with a new generation of antibiotics started. Later, the whole group of β-lactam antibiotics (penicillins and cephalosporins) turned into determined, followed by the aid of Waxman`s discovery of streptomycin derived from Streptomyces bacteria, used in a remedy for tuberculosis), and then tetracyclines, quinolones, antifungal metabolites, antiparasitic substances, and more currently antiviral tablets together with acyclovir. In 1971, the second considerable antibiotic cyclosporin A and C had been isolated from the fungal organism Hypocladium inflatum gams (Tolypocladium inflatum) that's the asexual state of the pathogen of beetles Elaphocordyceps subsessilis (Petch) G.H. Sung, J.M. Sung & Spatafora). Its immunosuppressive hobby was revealed in 1976 through J.F. Borel which became approved for use in 1983 allows you to reduce the risk of organ rejection in transplant surgical procedure (Upton, 2001 as cited in Giovannini, 2006).

1.3 Antibiotic resistance and addition perspectives

Nowadays, antibiotic resistance is a critical hassle and antibiotics are dropping their effectiveness what is, in particular, vital and features extreme threats for humans whose health is already compromised using pressure in the cutting-edge manner of life or by using contamination (HIV Patients, immunocompromised persons who are beneath chemotherapy). On the side the increasing use of antibiotics and antibiotic retailers, the resistance of bacteria to common and more often used antibiotics multiplied, resulting in low reply to the antibiotic treatment

The existence of multidrug-resistant disease once felt to be beneath control, increased as properly, tuberculosis, penicillin-resistant pneumonia, resistant malaria (the motive of dying of 1.1 million people in 1998), resistant traces of gonorrhea or dysentery due to Shigella and Salmonella (2.2 million deaths in 1998).

The public challenge about contamination has been accelerated, resulting in greater public use of a kind of antibacterial sellers designed to cast off ailment-causing organisms from external surfaces earlier than they could

enter the body. Today, antibacterial can also be impregnated into sponges, reducing boards, carpeting, and kid's toys. However, if used too frequently and indiscriminately, certain antibacterial dealers, those who go away trace chemical residues and that concentrate on specific approaches inside the lifestyle cycle of micro organism, may also pick for resistant traces \(http://www.tufts.edu/med/apua/about_issue/agents.shtml) . Furthermore, no new class of antibacterial substances has been developed to fight infectious diseases considering 1970 (WHO, 2000). it is consequently essential to find a few new compounds to fight in opposition to these resistant microorganisms. Then starts the parallel war in opposition to antibiotic resistance exhibited inside the continuous screening of recent natural resources of undiscovered antibiotics from the character. In this way, the ability of mushrooms has an excellent advantage, even in the evaluation of the bacteria. These days it is a good deal extra complex to locate new pharmaceutical lively substances via chemical synthesis than from existing and unexplored natural sources. Screenings of biological sports have made top-notch development in exploring the rich unlimited and undiscovered natural merchandise to use for manufacturing of pharmaceutical and agrochemical products (Anke, 1989). Many organisms had been studied as doubtlessly new resources of undiscovered bioactive additives, amongst which fungi from the phylum Basidiomycota gave promising outcomes. In the Forties, the pioneers in such studies had been Anchel, Hervey, Wilkins et al., and Florey et al. 1949, who tested extracts derived from fruiting bodies and mycelia cultures of greater than 2000 species, ensuing in isolation of a tricyclic diterpene antibiotic (pleuromutilin from Pleurotus mutilus). In the course of Nineties of the ultimate century many new systems and biological activities were detected (Anke, 1989). Since then, several research was finished. Nowadays we're witnessing very important conflict not only in opposition to microorganisms but additionally against another human disease which include cancer, viral, and different diseases.

1.4 Antimicrobial substances - Antibiotics from fungi and macrofungi

Microbial metabolites and their derivatives play an essential position in the improvement of drugs. the usage of these metabolites has grown notably during the last century, beginning with Fleming's discovery of penicillin (1924), in the beginning from Penicillium notatum filamentous micro-fungus, through Brotzu's discovery of cephalosporins from some other fungus, mold Cephalosporium acremonium (Acremonium chrysogenum now), till these days while the eastern clinics use 30 penicillin derivatives and approximately 49 derivatives of cephalosporin. Although the metabolites originating from fungi had been the main objectives of antimicrobial screening, these studies have been interrupted for a brief time by using Waksman's discovery of streptomycin (1945) originating from Actinomycetes. it's far believed that the reason for the smash helped with the aid of the reality that fungi frequently produce mycotoxins with reported cytotoxicity in humans and animals, and one example is the aflatoxin from the mold Aspergillus flavus, the maximum distinguished reason for continual hepatitis that ends in tumor malignancy.

However, in recent years the fashion has changed and fungal metabolites have once more attracted the eye of pharmacological studies. This could be seen from the records offering fungal metabolites an increasing number of essential as bioactive marketers and showing that the percent of medicines as opposed to the metabolites originating from actinomycetes are as follows (in step with the magazine of Antibiotics (I), Tokyo): 13 as opposed to 66% (1983), 16 as opposed to 74% (1990), 38 versus 53% (1994) and 47 as opposed to 44% (2000), at the same time as the proportion of metabolites originating from the microorganism remained at approximately 8%, besides 1983 while it became 21%. A comparable tendency was discovered for metabolites which can be registered as patents in Japan, displaying that the products from the fungi grew intensively: 11% (1983), over 21% (1990) to 36% (2000), and the products from actinomycetes decreased sharply from 74% (1983), over 66% (1990) to 48% (2000). In step with Tanaka and Omura (1993),{14} 43% of more than 8000 new microbial metabolites had been discovered thanks to Jap scientists. Its miles feasible that the abundance of secondary metabolites of fungi and actinomycetes, in comparison with microorganisms and yeasts, is associated with the characteristics of the environment terrible in nutrients. Dietary hindrance, in addition, induces secondary metabolism and the production of diverse compounds, to make the most scarce nutrients within the pleasant quantity feasible (Altered et al., 1999){15}. Taking into account the antibiotic screening, overview of Inouye et al., 2004 showed that the range of antifungal metabolites elevated notably, anticancer metabolites - fairly, even as the range of antibacterial metabolites reduced within the final ten years. However, the vastest boom turned into found in bioactive metabolites of the non-antibiotic mode of movement, especially concerning the screening of inhibitors of cholesterol synthesis, of which 93% originated from fungi (Yagisawa, 2000).

In this sense, it's miles taken into consideration that the eukaryotic fungal metabolites in motion in mammalian cells could have a ways fewer aspect results as compared with prokaryotic metabolites. Cultures of micro-organisms normally contain complex mixtures of different compounds, and small and huge molecular weights, which makes an instantaneous pharmacological screening greater difficult, thinking about the truth that could effortlessly be masked with the aid of the activity of different compounds in the aggregate. Being sessile organisms, which can be of

their natural surroundings continuously uncovered to affect of different competition (parasitic organisms), it isn't always unexpected that many antibiotics are isolated from fungi (Lindequist et al., 2005){16}. even though nowadays, still handiest compounds originating from micro-fungi or synthetic drug treatments have been used, literature statistics pointing to higher fungi, macro-fungi, and frequently Basidiomycetes as natural resources wealthy in new antimicrobial substances are once in a while determined (Suay et al., 2000). As potential new assets of natural antibiotics, lignicolous mushrooms once more grow to be difficult to observe (Smania et al., 2001){17}. The fact that human beings and animals proportion common microbial pathogens with fungi (E. coli, S. aureus, and P. aeruginosa) has brought about the notion that they produce compounds that can have similar consequences in humans (Zjawioni, 2004){18}. In Western Europe, the activity for this organization of fungi began with the invention of antibiotics (penicillin), whilst a group of scientists with their pioneering studies of new antibiotics originating from macrofungi Basidiomycota, led via M. Anchel, A. Hervey, WH Wilkins, and Kavanagh, commenced research of extracts and lifestyle mycelia and fruit frame of approximately 2000 species (Florey et al., 1949). This research has resulted in the isolation of antibiotics 3-cyclic diterpene pleuromutilin (Kavanagh et al., 1951) from Pleurotus mutilus species. Pleuromutilin has verified its antibacterial hobby by inhibiting bacterial protein synthesis via interacting with RNA (Lorenzen & Anke, 1998).{19} After that, the primary semisynthetic antibiotic tiamulin turned into produced together with valnemuline, utilized in veterinary medicine (Egger & Reinshagen, 1976) for the treatment of Mycoplasma infections in animals (Lorenzen & Anke, 1998).

Much research has shown that macrofungi produce many interesting pharmacological materials. Through evaluating the range of studied fungi with the ones whose chemical and pharmacological consequences are completely unknown, we realized that a completely small, even insignificant fraction of probably active fungal substances is recognized. For example, the illustrative instance is the species Ganoderma lucidum, witnessing that every species incorporates many distinct lively additives. Similarly, the production of certain secondary metabolites might also depend on the characteristics of the lines (isolates) or traditional conditions. Therefore, many scientists dealing with this hassle are in reality looking for new energetic compounds to be used within the Destiny. Its miles clean that simplest a small variety of lively compounds studied in vitro or in vivo on animals as biological fashions fit the wishes of allopathic medication, described using chemical composition, precise dosing, toxicology, pharmacodynamics, and medical studies.

Macrofungi want antibacterial and antifungal compounds to continue to exist in their natural environment. Considering fungi and human beings percentage not unusual microbial pathogens (e.g. E. coli, S. aureus and P. areuginosa), antimicrobial compounds that can be produced through fungi in opposition to microorganisms, can benefit humans (animals). Compounds of the special activity are the ones that show off antibacterial activities in opposition to multiresistant bacterial strains (methicillin-resistant S. aureus – MRSA or vancomycin-resistant Enterococcus – VRE). In keeping with a recent biological assessment, more than 75% of screened polypores showed sturdy antimicrobial hobby inhibiting ordinarily Gram-nice bacterial strains (B. subtilis, S. aureus and M. flavus). It has been said that new sesquiterpenoid hydroquinones produced using some species of the Ecu Ganoderma genus, named vancomycin, inhibit the increase of methicillin-resistant S. aureus and different bacteria (Mothana et al., 2000). based totally on our effects of antibacterial screening, 60% methanol and 55% chloroform extracts reached a considerable antibacterial hobby, giving the diameter of the inhibitory zone (>15mmØ) in opposition to one or more target organisms. Gram–bad bacteria have been less touchy to the applied extracts than Gram-tremendous ones, besides G. lucidum ethanolic extract (25mg/ml) in opposition to P. aeruginosa (h) and E. coli (ATCC 25922). 3 extracts of lignicolous macrofungi P. betulinus, C. versicolor and G. lucidum confirmed an extensive variety of sports towards all tested Gram-tremendous and some Gram-bad microorganisms, accomplishing MIC values in particular at an awareness of 17.5mg/ml. In contrast to methanol, chloroform extracts did now not show awareness dependence even as the idea of a dose-reaction phenomenon- hormesis (low dose stimulation and high dose inhibition) can be used for clarification of this phenomenon. The proper composition of tested extracts of fungi is unknown and may simplest be assumed that the effect of crude extracts, which might be attention dependent, is an effect of complicated interactions among cells and combos of compounds in the extracts (Karaman et al., 2009a).{20}

In a current screening of antibacterial pastime of water and methanol crude extracts of the species Meripilus giganteus towards nine species of Gram-nice and four species of Gram-negative bacteria, the most active extract became methanolic extract, inhibiting all the Gram–fine bacteria (typically S. aureus, Rh. equi, Bacillus) and handiest Gram-poor ones, C. perfringens and P. aeruginosa, ATCC strains (Karaman et al., 2009b)[21} The animal strains showed to be the most inclined analyzed lines, indicating a possible application of this fungus in opposition to Gram effective bacterial infections in animals. Seeing that water extract exhibited best a slim antibacterial effect, we assumed that the acquired consequences couldn't be attributed to the compounds like proteins or polysaccharides. Those results are in settlement with the literature information for similar

polypore fungi (Lindequist et al., 2005; Zjawioni, 2004), proven sterols, and prostanoid .terpenoids in addition to phenolic compounds as the primary active components chargeable for the received hobby (Turkoglu et al., 2007; Barros et al., 2007; Elmastas et al., 2007).{22 }

1.4.1 Antiviral materials

Provided antiviral activity of fungi is related to their complete, complicated extracts, but additionally to the remoted compounds. dealers remoted from fungi can directly motive the inhibition of viral enzymes, the synthesis of viral nucleic acid, or adsorption and absorption of virus in mammalian cells. The most often small molecules are lively inside the direct antiviral effect, while the oblique effects are mediated by means of antiviral activity immunostimulative polysaccharides and different complex molecules (Zjawioni, 2004).

1.4.1.1 Low molecular weight compounds with antiviral pastime

Several triterpenes from G. lucidum (ganoderiol F, ganodermanontriol and ganoderic acid and B) are energetic antiviral retailers against HIV-1 virus. In vitro antiviral activity of influenza viruses type A and B became observed in extracts of mycelium of mushroom Kuehneromyces mutabilis (Schaeff.: Fr.) (Singer & AH Sm.), while the extract and two isolated phenolic components from the mushroom Inonotus hispidus (Bull.; Fr.) P. Karst, as well as ergosterol peroxide, are found in many unique fungal species. 1.4.1.2 excessive molecular weight compounds with antiviral activity Water-soluble lignins remoted from Inonotus obliquus (Pers.: Fr.) Pilate, inhibit HIV protease with IC 50 price of 2.5 mg/ml. Anti-HIV hobby is recorded for the submerged lifestyle media of L. edodes and water-soluble lignin isolated from the same fungus. Protein polysaccharide complicated PSK and PSP from Coriolus versicolor, also shows antiviral pastime on HIV and cytomegalovirus in vitro. Inhibition of HIV-1 opposite transcriptase is caused by velutin, protein from Flammulina velutipes, which inactivates ribosomes. MD fractions of Mushroom Grifola frondosa confirmed general development of situation of the patients (85%) who had numerous symptoms of HIV and different secondary diseases (Zjawioni, 2004).

1.4.2 Antifungal materials

Compounds with antibacterial and antifungal interest of mushrooms assists in their survival in their environment. Those substances can be very beneficial inside the treatment of human infections, but the authentic antibiotic therapeutics in the world market can be handiest determined originating from microfungi so far. Opportunistic fungal infections are continually a big problem, in particular in immunocompromised sufferers receiving chemotherapy or in cases of transplantation of organs or bone marrow, in addition to in HIV contamination. Over the last ten years, the interest in compounds that display antifungal pastime has been accelerated. Amongst them the ordering (tricyclic diterpene glycoside) turned into for the primary time remoted in 1971 (Hauer and Sigg as stated in Inouye et al., 2004), and slightly more potent zofimarin was remoted for the first time in 1987 (Ogita et al. 1987 as cited in Inouye et al., 2004). similarly, suggestive is xylan (compound SCH57404) remoted from the lignicolous fungus Xylaria sp. (Schneider, 1995). Many derivatisations of sordarin antibiotics have been completed in studies corporations of the Glaxo Smith Kline company via biotransformation with Streptomyces avermitilis, what resulted within the synthesis of GM237354 (Herreros et al., 1998), with the MIC of ninety% that become 0.1/2 mg/ml for isolates of C. albicans and 0.12 for C. tropicalis. Further improvement of these compounds has led to the azasordarin institution in which the sugar component is replaced through N substituted morpholine (Herreros et al., 2001 as referred to in Inouye et al., 2004).

Numerous antifungal metabolites with steroid shape were also isolated from fungi. A25822 A and B from Geotrichum (Gordee and Butler, 1975 as stated in Inouye et al., 2004) and from Wallemia Sebi; Mer-NF8054 A and X from the genus Aspergillus. The maximum famous triterpene, flavonol remoted from basidiomycetous Favolashia sp. (Anke et al., 1995 as mentioned in Inouye et al., 2004) is a metabolite that exhibited antifungal pastime against Ascomycetes, Basidiomycetes, Zygomycetes, and Oomycetes, however, did no longer display antibacterial activity.

Researchers of Merck institution have found four acidic terpenoids from filamentous fungi: Serotonin A (from Trichoderma kongsi), secosteroid (from Ascotricha amphitrite) arundifungin (steroid from Arthrinium arundinis) and enfumafungin (pentacyclic terpenoid from mildew Trichoderma kongsi), secosteroid (from ascomycetous Ascotricha amphitrite), arundifungin (steroid from mold Arthrinium roundings) and enfumafungin (pentacyclic terpenoid from Aureobasidium), which had been found to affect the biosynthesis of β-D-glucan however now not the biosynthesis of steroids. Among them the high-quality antifungal pastime on Candida species and species of Aspergillus genera showed enfumafungin.

1.5 Chemical nature of antibacterial sellers

A huge variety of pharmacologically energetic materials like sesquiterpenes (Abraham, 2001)[,23} hydroquinones (Mothana et al., 2000){24}, polysaccharides and complexes of polysaccharide-peptide (Liu, 1999), lanostanoide triterpenoids (Shiao, 1992, Leon et al., 2004){25} Steroids (Smania, 2003), nucleosides, alkaloids, and nutrients (Paterson, 2006) from fruit bodies of polypore fungi were detected. Recent studies reported phenolic compounds

(Turkoglu et al., 2007, Paterson, 2006, Ribeiro et al.,

2007)26,27} as the primary active Antioxidative additives in fungal extracts (Kityberger et al., 2007; Barros et al., 2007). It is assumed that antibacterial results exhibited by way of fungal extracts of different polarities may be related to a standard effect of phenolic compounds (e.g. phenolic acids: caffeic acid, ellagic acid; flavonoids, hydroquinones) detected in similar extracts of the species G.lucidum, F.velutipes, P.ostreatus, or natural acids (oxalic, malic) formerly detected in L. sulfurous and F. hepatica, in addition to terpenoids.

1.5.1 Product of the number one metabolism

Polysaccharides. Polysaccharide molecules that shape an essential part of the fungal cell wall additionally exhibit antimicrobial homes (Stamets, 2002). Polysaccharides are the maximum crucial additives of fungal bioactive substances, tested to offer many clinical and therapeutic possibilities (Fan et al., 2006){28}at the same time as their antibiotic effect is regularly particular to positive microorganisms (Stamets, 2002){29}. Most of these compounds belong to glucans or heteroglycans (Fan et al., 2006). it's miles believed that the antibacterial and antifungal effects of β-glucan are based totally on the activation and strengthening of the immune reaction, and their use is recommended in aggregate with different antibiotics and immunostimulators in the prevention and remedy of infectious sicknesses, particularly immunocompromised people (Chen & Seviour, 2007){30}.

Proteins and polypeptides. Proteins that act inhibitory on microorganisms are found regularly in organisms of plant and animal species, while their presence is rare in fungi (Wang & Ng, 2006). It's far believed that those proteins are frequently positively charged, and that the mechanism in their motion is found by forming ion channels in the cell membranes of microorganisms as well as by using aggressive binding to host mobile polysaccharide receptors (Cowan, 1999). Proteins and peptides are isolated from macrofungi whose antimicrobial effect is confined to a small number of general phytopathogenic species (Table1)

Compound	Origin/Source	Biological Activity	Reference
Extracellular Polysaccharides (noncellulose β-glucans)			
Lentinan	*Lentinus edodes* mycelial extract	Antifungal: *Candida albicans*; Antibacterial: *Mycobacterium tuberculosis*, *Listeria monocytogenes*, *S. aureus*, *M. luteus*, *B. cereus*; Antiviral: Herpes simplex-a type 1	Stamets, 2002; Kitzberger et al., 2007; Chen & Seviour, 2007
Schyzophyllan (SPG)	*Schyzophyllum commune*	Antifungal: *Candida albicans*; Antibacterial: *S. aureus*	Stamets, 2002
Krestin (PSK)	*Trametes versicolor*	Antifungal effect: *C. albicans*	Stamets, 2002; Kitzberger et al., 2007
Grifolan (GRN)	*Grifola frondosa*, *Lepista nuda*		
Intracellular Polysaccharides			
- Containing 1,6-α-D-galactopyranosyl units, substituted on O-2 position with α-L-fucopyranosyl or 3-O-α-D-manopyranosyl-α-L-fucopyranosyl units. Found only in fungi and considered a type of reserve material			*Fan et al., 2006*
Fucogalactan CMP3	*Coprinus comatus*	Not yet investigated (*C. comatus* showing antibacterial activity)	Fan et al., 2006
Fucogalactan	*Ganoderma applanatum*		

Manofucogalactans	*F. velutipes*, *Polyporus pinicola*, *P. fomentarius*, *P. igniarius*	Not yet investigated; considered as reserve material	Fan et al., 2006
Fucomanogalactans	*Laetiporus sulphureus*		
Ganoderm in	Protein, molecular weight ≈15 kDa from *Ganoderma lucidum*	Antifungal to phytopathogens *Botrytis cinerea*, *Fusarium oxysporum*, *Physalospora piricola*	Wang & Ng, 2006
Pleurostrin	Peptide, molecular weight of 7 kDa from *Pleurotus ostreatus*	Antifungal effect: *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola*	Chu et al., 2005
Lyophyllin	Aqueous solution of *Lyophyllum shimeji*	Antifungal effect: *Mycosphaerella arachidicola*, *Physalospora piricola*	Takakura et al., 2001; Wang & Ng, 2006
Trichogin	Peptide from *Tricholoma giganteum*	Antifungal activity against *Fusarium oxysporum*, *Mycosphaerella arachidicola*, *Physalospora piricola*; inhibitory effect on HIV-1 reverse transcriptase	Guo et al., 2005
Eryngin	Peptide, molecular weight of 10 kDa from *Pleurotus eryngii*	Inhibition of *Fusarium oxysporum*, *Mycosphaerella arachidicola*; N-terminal shows similarity to antifungal protein Lyophyllin	Wang & Ng, 2004
Agrocybin	Peptide, molecular weight of 9 kDa from *Agrocybe dura*, *A. cylindracea*	Antibacterial effect against Gram+ and Gram- bacteria: *B. mycoides*, *B. subtilis*, *E. coli*, *Klebsiella pneumoniae*, *Mycobacterium pheli*, *M. smegmatis*, *Photobacterium fischeri*, *P. aeruginosa*, *S. aureus*; Antifungal effect: *Aspergillus niger*, *Gliomastix convoluta*, *Memnoinella echinata*, *Myrothecium verrucaria*, *Penicillium notatum*, *Phycomyces blackesleeanus*, *Stemphylium consortiale*, *Trichomonas mentagrophytes*	Kavanagh et al., 1950; Ngai et al., 2005

Table 1. Polysaccharides, proteins and peptides from macrofungi with antimicrobial effect

Dietary fibers. Excessive molecular weight materials that are excreted without digestion and absorption from the human body are referred to as nutritional fibers (Mizuno, 1999). Mushrooms include those materials, that are composed of β-glucan, chitin, and heteropolysaccharide (pectin substances, hemicellulose, polyuronidase, etc..) within the variety of 10-50% in dry weight of the substance. Seeing that they take in dangerous substances, hindering their intestinal absorption, nutritional fibers are powerful in preventing colon and rectal cancers (Mizuno, 1999). Lectins. Lectins (Latin legere = to take, to pick) are defined as carbohydrate-binding proteins of non-immune origin which agglutinate cells or precipitate polysaccharides or glycoconjugates (Kawagishi, 1995). Many species of flowers, animals, and microorganisms incorporate lectins, but the fungal lectin remains not explicitly defined. To date, several lectins were remoted from mushrooms of the genus Polyporales: Grifola frondosa (GFL), Fomes fomentarius (FFL), and Ganoderma lucidum (GLLs). A few are isolated from the fruit bodies and a few from the mushroom mycelium. GFL is cytotoxic to HeLa cells, and its hobby is defined via the binding of lectins to carbohydrate elements of the cell with the aid of preventing aggregation of cells (Wasser & Weis, 1999).

1.5.2 products of secondary metabolism

Secondary metabolites produced via a huge range of macrofungi have high-quality healing significance. Those compounds arise as intermediate products of number one metabolism, however maximum of them are categorized in keeping with the 5 fundamental metabolic resources (desk 2,3,4). The most effective pathways of synthesis of secondary metabolites are polyketide and mevalonate pathways (Zeidman et al., 2005, from Giovanni, 2006).

1.5.2.1 Phenolic compounds

Phenols are certainly one of the most important instructions of secondary biomolecules, which can be characterized through the presence of aromatic rings with hydroxyl groups bonded immediately to an aromatic hydrocarbon organization. although they are first diagnosed in flora (Cowan, 1999), their presence was also observed in fungi (Barros et al., 2008, Mattila et al., 2001, Karaman, 2002, Karaman et al., 2012a). In current years, there was a causal

courting among the overall content material of these compounds with organic sports recorded in a huge wide variety of macrofungi (Barros et al., 2007), which include anti-inflammatory, antiallergic, anticancer, antihypertensive, antirheumatic and antibacterial interest. Antimicrobial residences of phenolics are explained via the presence of phenol hydroxyl companies, whose range is in correlation with their toxicity closer to microorganisms (Cowan, 1999). The feasible mechanisms of their movement consist of inhibition of extracellular microbial enzymes, deprivation of the substrates required for microbial increase, or direct movement on microbial metabolism through inhibition of oxidative phosphorylation, sulfhydryl corporations, and some non-unique interactions (Cowan, 1999).

it has been shown that the antimicrobial effects of extracts of mushroom Lactarius deliciosus, Sarcodon imbricatus and Tricholoma portentosum immediately correlated with the total content material of phenols and flavonoids in them (Barros et al., 2007). Extracts of all 3 fungi showed antibacterial effects on Bacillus cereus and antifungal to Candida neoformans, even as the extract of mushrooms Lactarius deliciosus and efficiency proven in opposition to P. aeruginosa and Candida albicans. High content of phenols has been recorded in lignicolous fungi Meripilus giganteus, G. lucidum, and Flammulina velutipes in the shape of coumarins and tannins, as well as In Ganoderma applanatum, where they were detected in the form of coumarins, flavonoids, and tannins (Karaman, 2002, Karaman et al., 2005). Data on the antimicrobial action of these Fungi also exist (Karaman et al., 2010). Analyses of extracts of the genus Ganoderma species shown the presence of polyphenolic compounds, and the antimicrobial properties of these mushrooms explain the activity of compounds of hydrohynon composition - vancomycin A and B (Ofodile et al., 2005 as cited in Mothana et al., 2000). High concentrations of phenolic acids (> 1.0 mg / g), mainly a high concentration of gallic acid and protocatechuic could be interpreted as anti-microbial activity of the following species: L. sulfurous, F. hepatica, P. ostreatus, F. velutipes, and partially M. giganteus, which in antimicrobial screening showed moderate activity (Karaman, 2009b). Further studies of mechanisms of antimicrobial components originating from mushrooms could be suggested, including the influence of the protein compounds and organic acids such as oxalic acid, which accumulates in the fruit bodies of brown rot mushrooms, but also malic acid, ellagic acid, or some other compounds. Flavonoids are hydroxylated phenolic compounds (C6-C3 units associated with the aromatic core) and antimicrobial activity can be explained by their ability to create complexes with extracellular soluble proteins and polypeptides that build cell walls of microorganisms, as well as disruption of the function of the cell membrane (Cowan, 1999).

Only a few data dealing with the detection of flavonoids (rutin, chrysin, naringin, myricetin, and quercetin) in tericolous (Turkoglu, 2007; Baros et al., 2007) lignicolous fungal species (Kim et al., 2008, Jayakumar et al., 2009). Since flavonoids are phenolics that generally occur in plants acting as antioxidants, antimicrobials, photoreceptors, feeding repellants, or UV protectors (Pietta, 2000) we assume that the presence of these metabolites in TP of fungi that generally are in tight connection with wood could have an impact on the expressed bioactivity. Recent studies conducted with mushrooms showed a positive correlation between the TP and antioxidant capacity (Turkoglu, 2007, Ribeiro, 2007), possibly due to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals. Plotting TP content versus antibacterial activity (Karaman et al., 2010), revealed a good positive correlation between these two parameters, showing higher values for MeOH than CHCl3 extracts against most of the bacteria. By comparing different strains of the same bacteria (S. aureus) it was concluded that the effect of TP on the antibacterial activity may be strain-specific.

Worthy of note is the antibacterial activity of fungi against the multidrug-resistant strains of bacteria. New sesquiterpenoid hydroquinone from Ganoderma Pfeiffer Bres., called ganomycin (Mothana, et al., 2000) inhibits methicillin-resistant strains of Staphylococcus aureus and the growth of other, mainly Gram-positive bacteria. In addition, sterol-type compounds, isolated from the species G. applanatum such as 5α-ergot-7-en-3β-ol, 5α-ergot-7, 22-Dien3β-ol, 5.8-epidioxy-5α, 8α-ergost-6,22-dien-3β-ol and another new lanostanoid showed weak activity against many Gram + and Gram - bacteria. Oxalic acid is one of the substances responsible for the antimicrobial effects of mushroom Lentinula edodes (Berk.). Chloroform extract of mycelium L. edodes has bactericidal properties (Hirasawa et al., 1999). Tannins are complex polyphenolic compounds that are divided into two groups: Hydrolyzed (esters of phenolic acids and sugars), and condensed (constructed from flavonoid monomers). The antimicrobial activity of tannins is expressed due to their ability to link amino acids in proteins, inactivate adhesions, and enzymes, and transport proteins of cells. Membranes of microorganisms (Cowan, 1999), as well as the formation of complexes with metal ions (Biradar et al., 2007). In addition, tannins could form complexes with polysaccharides, affecting microorganisms.

The equivalent of tannic acid was detected in extracts of shiitake mushrooms (Lentinus edodes), which show the antibiotic effect against bacteria M. luteus and B. cereus and the fungus Candida albicans, while against the strains of E. coli and S. aureus did not show the same activity (Kitzberger et al., 2007). While the focus of previous micro chemical (gr. myces=fungi) analysis of Pleurotus ostreatus was mainly put on the vitamins and minerals content,

indicating a high nutritional value of mushrooms (Mattila et al., 2005), recent research revealed its exceptional antimicrobial and antioxidant effects that are associated with the presence of terpene and phenolic compounds (Iwalokun et al., 2007). The presence of phenols in the form of pyrocatechols, and flavonoids in the form of quercetin, was noted in extracts of the fungus Laetiporus sulphureus, which explains its strong antioxidant properties (Turkoglu et al., 2006). This study also shows that ethanol extract of L. sulphureus exhibits Strong antibiotic effect against Gram-positive bacteria (B. subtilis, B. cereus, M. luteus and M. flavors) and the yeast Candida albicans, while its activity against Gram-negative bacteria is much lower.

Coumarins are phenolic compounds of characteristic odor, and, according to the chemical structure, they are lactones built from the benzene and pyrone ring (Cowan, 1999). Despite the antiviral activity of some coumarins and the evidence of their inhibitory effect on the fungus Candida albicans in vitro conditions (Cowan, 1999), data on antimicrobial activity of these compounds are scarce. The presence of coumarin in fungi has been established in most genera of the Xylariaceae family (Ascomycetes) (Whalley et al., 1999), as well as in certain fungi belonging to lignicolous basidiomycetes based on preliminary TLC profiling (Karaman, 2002).

Other agents with weak antibacterial effects found in macrofungi are steroids like 5αergosta-7,22-dien-3β-ol or 5,8-epidioxy-5α,8α-ergosta-6,22-dien-3β-ol, isolated from Ganoderma applanatum (Pers.) Pat., proved to be weakly active against several Gram-positive and Gram-negative bacteria and organic acids like oxalic acid proved to be responsible for the antibacterial effect of Lentinula edodes (Berk.) Pegler against S. aureus and other bacteria. Other, non-phenolic, compounds including terpenoids (Leon et al., 2004) and polysaccharides (Tseng et al., 2008) have also been designated as mushroom antioxidants or antimicrobials.

1.5.2.2 Terpenoid compounds

Terpenes are a broad class of lipophilic secondary metabolites whose general chemical structure is C10H16. In nature they appear as diterpenes, triterpenes, and tetraterpens (carotenoids) - C20, C30, C40, as well as the hemipterans and sesquiterpens - C5, C15. If include additional elements (mostly oxygen within the hydroxyl and carbonyl groups), they are called terpenoids (Cowan, 1999). Terpenoids originate from simple acyclic compounds, isoprene, and mevalonic acid, and their structure may be acyclic, monocyclic, or bicyclic. Their structure is isopentenyl-pyrophosphate (IPP), whose synthesis is realized in two ways and pathways of synthesis of higher isoprenoids continue after the Isomerization of IPP in

Many of these species have found wide application in the prevention and treatment of various diseases due to the

DMAPP. For all animal and fungal cells, the characteristic is the mevalonic pathway of isopentenyl-pyrophosphate synthesis, while most plants, bacteria, actinomycetes and protozoa have a non-mevalonic mode of synthesis (Inouye et al., 2004).

One of the many functions of these compounds is their antimicrobial activity, but the mechanism of action of terpenoids on microorganisms is not fully understood (Cowan, 1999). According to their lipophilic nature, it is assumed to act by disrupting membrane functions of microbial cells (Cowan, 1999), and some authors believe that they may cause increasing non-specific cell membrane permeability for the antibiotic molecule (Byron et al., 2003). Though plant organisms are thought to be the largest source of triterpenoids, in recent years more and more data indicate the presence of these compounds in some representatives of macrofungi (He et al., 2003, Akihisa et al., 2005; de Silva et al., 2006;

Abraham, 2001, Deyrup et al., 2007).

Sesquiterpenes. One of the many strategies that representatives of the higher fungi use to protect themselves against several parasites that feed on their fruit bodies is the production of toxins. Interestingly, many of these toxic chemicals suit sesquiterpenes (Abraham, 2001). For most Basidiomycota fungi the presence of sesquiterpens of protoiludane type is characteristic, which originate from humulene, compounds present in a rare fungus, formed by cyclization of farnesyl-pyrophosphate. Of the few ways of humulene transformation, the most important pathway of synthesis of protoiludane, a tricyclic compound which, due to the high reactivity caused by the presence of cyclobutane, is further transformed into a series of compounds. Some of these sesquiterpenes show interesting biological activity and are considered to be a very interesting object of study in terms of medical chemistry. Several groups of sesquiterpenes originating from higher fungi show a greater or lesser antimicrobial effect (Tables 2, 3). It is interesting to note that some representatives of the genera Russula and Lactarius synthesize sesquiterpene alcohols that are esterified with fatty acids. These esters do not show strong antibiotic activity, but in the case of mushroom fruit body injury, leads to cleavage of ester bonds and release of alcohols that are highly reactive and therefore very toxic to microorganisms. Therefore, the mentioned esters may be considered as pro-medicines or precursors of compounds that in metabolic processes are transformed into an active form. Triterpenes. Compounds of triterpene composition are found in many mushroom extracts which showed some antibiotic properties. Genus Ganoderma contains about 200 species known for the production of triterpene compounds.

numerous biological activities based on the presence of triterpene components (Ofodile et al., 2005).

Although thought to be active against bacteria just due to the presence of triterpenes in these fungi, some data disagree with such opinions, giving the example of seven different triterpenes isolated from a Vietnamese species G. callosum, which showed no antimicrobial effect but exhibit strong anti-inflammatory activity (Ofodile et al., 2005). Most triterpenes synthesized by species of the genus Ganoderma belong to the lanostane type (de Silva et al., 2006). Over 100 compounds from this group have been identified, among them a few newly discovered (Akihisa et al., 2005; de Silva et al., 2006, Jian et al., 2003, Kamo et al., 2003). The review of triterpene compounds isolated from macrofungi is given in Table 3.

An overview of other compounds isolated from macrofungi, which exhibit antimicrobial activity is shown in Tab. 4

Compound	Origin	Name or Chemical Structure	Effect (Activity)
Caryophyllene	*Naematolin, Hypholoma fasciculare*	Weak antibacterial	
Collybial	*Collybia confluens*	α,β-unsaturated aldehyde	Low antifungal, high antibacterial (*Bacillus* sp.), high antiviral, cytotoxic, non-selective antibiotic
Protoilludanes	-	Esters of protoilludanol	-
Marasmanes	-	-	-
Hydrogrammane	-	Modified marasmic sesquiterpenes	-
Cucumanes	-	-	-
Armillyl Orselinate	*Armillaria mellea* (similar to *A. tabescens*)	Chlorinated derivatives	Prevents thrombocyte aggregation, cytotoxic, antimicrobial
Melleolides	-	B, C, D, E, F, G, H, I, J (everniate-armillarin)	High antibacterial, antifungal & cytotoxic
Radulon A	*Radulomyces confluens*	-	-
Lentinellic Acid	*Lentinellus*	Methyl esters of lentinelic acid	-
Marasmic Acid	*Marasmius conigenus*	Hydroxy derivative of marasmic acid	Low antifungal, high antibacterial, cytotoxic
Pilatin	*Flagelloscypha pilatii*	Contains many Basidiomycota by damage of fruiting bodies	Low antifungal, high antibacterial, cytotoxic
Velutinal	-	Fatty acid esters	-
Hydrogrammic Acid	*Clitocybe hydrogramma*	-	Antibacterial against *Bacillus* sp., no activity against *E. coli* and fungi
Fomannosanes	*Fomes annosus*	Fomanosin, Illudosin	-
Illudanes	*Omphalotus olearius*	Illudin S, Illudin M	Cytotoxic, antibiotic (*S. aureus*), antifungal
Isoilludanes	*Lampteromyces japonicus, Omphalotus olivascens*	Illudin F, G, H, C2, C3, etc.	Antiviral (inhibits reverse transcriptase of viruses causing leukemia in rats), high cytotoxic, mutagenic

Clitocybe Candicans *Clitocybe candicans* - Weak antibiotic activity on *B. subtilis*

Clavicoronic Acid *Clavicorona pyxidata* - Bactericidal, phytotoxic

Leaianafulven *Mycena leaiana* - Anticancerogenic properties

Table 2. Antimicrobial effects of sesquiterpenoids originated from macrofungi (according to Abraham, 2001)

Compound Name or Chemical Structure	Origin	Effect (Activity)
Hirsutanes	*Stereum hirsutum*	-
Triterpenoids	*Lanostane-type*	Antimicrobial activity, common in plants and lichens
Pleurotelanes	*Pleurotus hypnophilus*	Created by modification of hypnophillin; includes pleurotelic acid and pleurotellol
Cucumanes	*Macrocystidia cucumis*	-
Merulanes	*Merulius tremellosus* (culture)	Includes merulidial and meruliolactone
Isolactaranes	-	-
Hirsutic Acid C	*Stereum hirsutum*	-
Complicatic Acid	*Stereum complicatum* (culture)	-
Hypnophillin	*Pleurotus hypnophilus, Coriolus consors*	Antifungal and cytotoxic activities
Coriolin A, B, C	*Coriolus consors*	-
1-Desoxyhypnophyllin	*Lentinus crinitus*	-
Gloeosteretriol	*Gloeostereum incarnatum* (culture)	-
Incarnal (dehydro-hirsutanol A)	*Gloeostereum incarnatum*	-
Meruliolactone	*Merulius tremellosus*	-
Stereopolide and Dihydrostereopolide	*Stereum purpureum* (culture), *Merulius tremellosus*	-
Lanostane-type Terpenes	*G. applanatum, G. lucidum*	Includes lanostane and ergostane derivatives, has fatty acids and antimicrobial properties
Triterpenoid Lactones	*Fomes cajanderi*	Includes fomlactons A, B, C; known for antimicrobial and cytotoxic effects
Triterpenic Glycosides	*Kolocosides A, B, C, D*	-
Fuscoatroside	-	-
Enfumafungin	-	-
WF11605	*Xylaria* from Hawaiian Islands	-
Triterpenoid Saponins	*P. ostreatus*	Antimicrobial effects

Compound Name or Chemical Structure	Origin	Effect (Activity)
Favolon	*Favolaschia*	Antimicrobial; contains variable cyclic structures and substitution methods

Table 3. Antimicrobial effects of sesquiterpenoids and triterpenoids from macrofungi (according to Abraham, 2001)

1.6 Extraction methods

Extraction procedures are important in assessing the good antibacterial activities of extracts. Macrofungi are commonly collected either randomly or by locals in geographical areas or forest habitats where the fruiting bodies are found. Initial screenings of fungi for possible antibacterial activities usually begin by using crude aqueous or alcohol extractions. Since the majority of the identified components of mushrooms are active against microorganisms, they are mostly obtained through initial ethanol or methanol extraction.

Water-soluble compounds, such as polysaccharides and polypeptides, including lectins, are commonly more effective as inhibitors of virus adsorption and cannot be identified in the screening techniques commonly used. Tannins and terpenoids are occasionally obtained by treatment with less polar solvents.

For alcoholic extraction, the intact mature fruiting bodies or their segments are brush-cleaned, air-dried to constant mass pulverized, and then soaked in methanol or ethanol for extended periods (24-72h). The resultant filtrated extracts are then filtered and washed, concentrated under reduced pressure at low temperature to avoid destroying any thermo-labile antimicrobial agents present in the extract, and redissolved in the alcohol (or 5% DMSO) to a determined concentration. Water extractions, generally used distilled water, blending of slurry, filtration, and centrifugation (approximately 15,000 for 30 min) multiple times for clarification

Compounds	Origin/Source	Biological Activity	Reference
β-methoxyacrylates, strobilurins, and oudemansins	*Oudemansiella mucida, Xerula malanotricha, Xerula longipes*	Antifungal activity against a large number of saprotrophic and phytopathogenic fungi, inhibiting respiration	Anke et al., 1979; Anke et al., 1983
Polyenes (xerulin, dihydroxerulin, and xerulinic acid)	*Xerula malanotricha*	Antimicrobial, anticancer, antiviral, and anti-inflammatory activity; inhibition of cholesterol biosynthesis; cytotoxic effects	Negishi et al., 2000; Kuhnt et al., 1990
Agrocybolacton	Cultures of *Agrocybe* genus	Moderate antibacterial activity against Gram-positive bacteria *Bacillus subtilis* and *Mycobacterium smegmatis*	Rosa et al., 2003
Lentionine (1,2,3,5,6-enthatiocyclocheptane) and its disulfide derivative	*Lentinus edodes*	Antibacterial and antifungal effects	Hirasawa et al., 1999
Cinnabarine	*Pycnoporus cinnabarinus*	Antibacterial (against *Bacillus subtilis* and *Staphylococcus aureus*) and antifungal effects	Shitu et al., 2006
Laschiatrion	Cultures of *Favolaschia* genus	New antibiotic with steroid skeleton; in vitro antifungal activity against some human pathogens; no detected antibacterial or cytotoxic effects	Anke et al., 2004

Table 4. Other compounds from macrofungi with antimicrobial activity

1.7 Evaluation of antibacterial activity

1.7.1 Techniques used in research of new substances

Basidiomycota and fungi in general, represent an inexhaustible source of new substances, even though each species contains hundreds of active metabolites. Therefore, test systems for research of new substances must be fully simplified, fast, efficient, and as cheap as possible (Hostettmann et al., 1997, as cited in Giovanni, 2006). In addition, biotests (bioassays) must be sufficiently sensitive to detect the activity of substances in low concentrations, in the so-called solid (crude) extracts. Crude products can be used in antimicrobial testing disc-diffusion and broth-dilution assays to test for antibacterial properties including bioautography according to standard procedures (NCCLS or CLSI procedures). The use of standard cultures of familiar characteristics is recommended though several precautions have to be taken into account. In a recent study, the differences between the two screening methods applied were not statistically significant (t-test at level $p < 0.05$). Both Meripilus extracts analyzed (water and methanol) showed wider inhibition zones in the disc-diffusion method, indicating that it is more appropriate for the testing of polar extracts (Karaman et al., 2009b). Similar results were

confirmed for extracts of the genus Fomes although showed broader inhibitory zones using the method of "wells", compared with inhibitory zones obtained by the disc-diffusion method. For other extracts, however, the disk-diffusion method could be recommended, indicating that the

polarity of active substances in the extract influences on results obtained in the particular method applied.

MIC and MBC determination is used to quantify antimicrobial activity using the two-fold dilution method according to CLSI guidelines. The MIC is defined as the lowest concentration preventing visible growth while the complete absence of growth is considered as the MBC. The lower MIC or MBC values concerning the extract concentration indicate a higher activity, implying better quality of the extract. To confirm MBCs, aliquots of the experimental suspensions (100µl) could be subcultured on Müeller Hinton agar plates incubated overnight.

A potent source of antibacterial agents is the species M. giganteus (50mg/ml), showing high activity against both groups of bacteria reaching MIC values in a wide range of concentrations (<17.5 -1125µg/ml). Various activities have been detected among different strains of S. aureus, indicating that fungal extracts are target-specific on an intraspecific level (strain specific).

The antibacterial assay may be performed in 96-well micro-plates instead of tubes. If 5% DMSO is applied for dissolving a negative control with 0.5% DMSO must be used to ensure that DMSO did not affect bacterial growth. Results are recorded after incubation at 35-37ºC for 18-24h and all the samples should be tested in triplicate. Bioautography is one of the most effective tests for the detection of antimicrobial metabolites, because it localizes the place of the active component, therefore enabling the isolation of the active component precisely. Bioautography may be the direct, when microorganisms grow directly on the TLC plate, then contact, when the active compound is transferred from the TLC plates to inoculated agar and agar-spill-over (so-called immersion bioautography), when the inoculated agar medium is spilled over the TLC plate (Rahalison et al., 1991). In the bioautography agar overlay method, the drug to be evaluated is adsorbed onto the TLC plate and the inoculum is laid onto the plate as a very thin layer (1 mm). The advantage of this method is that the amount of sample being used is very small and that the fractionalization of the crude extracts on its different components simplifies the identification of the active compound.26

In our recent work, the TLC chemical profile of the analyzed species of lignicolous macrofungi showed that they are rich in phenols, although the differences in the number and quality of the extracted compounds have been noticed. Comparing the TLC profiles, fungi can be classified into three groups according to the obtained retention factor e.g. RF values representing the distance traveled by the compound divided by the distance traveled by the solvent: 1) three species: C. versicolor, G. lucidum, and G. applanatum contain compounds with similar (Rf = 0.68, Rf = 0.69, Rf = 0.70, respectively), 2) five species M. giganteus, L. sulphureus, F. velutipes, F. hepatica, and P. ostreatus showed a small amount of evaluated compounds and intense fluorescence at the start line after the spraying, 3) the species P. betulinus expressed with three spots in the MeOH extracts (Rf = 0.62, Rf = 0.65, Rf = 0.68), which extinguished fluorescence in the UV 254th (Karaman, 2009c). Furthermore, we made slight modifications to the standard procedure of bioautography in the same study using the following: soft (top) agar (0.7% Nutrient agar) which was mixed with a freshly prepared inoculum of bacteria (0.5Mac Farland optical density) and with the aqueous solution of tetrazolium red dye 0.1% w/v (1mg/ml)- 2,3,5-triphenyl tetrazolium chloride (TTC, Sigma) (3:1:0.1). The strain S. aureus was used as the indicator organism. Amoxicillin (64µg/ml) was used as positive control. Approximately 10µl of the solution of

Each extract was applied on a TLC plate (silica gel 60, F 254, DC-Plastikfolien, 0.2 mm thick, Merck, Germany) for about 2h, equally prepared as a reference plate for chemical analysis. Bioautography test plate was developed in the same tank using the pre-determined mobile phase which was removed from the plate by drying with a stream of cool air from a heating gun. Separated spots were visualized under UV light and marked by pencil (Figure 2A).

Developed plates were placed upside-down in the Petri dishes containing bottom agar (nutrient agar, Torlak, Belgrade). Soft agar (07% Nutrient agar) was melted and poured into sterile tubes (100 ml) in which the dye and bacteria were added quickly. That mixture flowed over the chromatograms in the petri dishes. After the agar had solidified, the plates were inverted and incubated at 35ºC for 24h. The clear zones on the chromatogram indicate areas of inhibition zones on the red background where bacteria are present. Comparing clearing zones with reference TLC plate according to Rf values the most active components of crude fungal extracts could be approximately detected (Fig. 1B).

Bioautography results showed many antibacterial compounds against animal strains of S. aureus that was mostly present in the polar region of the bioautogram. According to detected clearing zones, chloroform extracts were more active corresponding to more detected UV absorptive substances along the chromatogram. However, these substances were not active in methanolic extracts on bioautograms for C. versicolor and P. betulinus.

Developing system: toluene-ethyl acetate – 90% formic acid (5:4:1 v/v/v). Detection: 366 nm UV light without spraying. Extracts: lane 1- M. giganteus (MeOH), lane 2- L. sulphureus (MeOH), lane 3- C. versicolor (MeOH), lane 4- F. velutipes (MeOH), lane 5- G. lucidum (EtOH), lane 6- G. applanatum (MeOH), lane 7- P. tigrinus (MeOH), lane 8- P. betulinus (MeOH), lane 9- P. ostreatus (MeOH), lane 10- F. hepatica (MeOH), lane 2´- L. sulphureus (CHCl3), lane 3´-

C. versicolor (CHCl3), lane 4´- F. velutipes (CHCl3), lane 6´- G. applanatum (CHCl3), lane 7´- P. tigrinus (CHCl3), lane 8´- P. betulinus (CHCl3) B: Bioautogram of extracts for S. Aureus. Extracts: lane 1- M. giganteus (MeOH), lane 2- L. sulphureus (MeOH), lane 4- F. velutipes (MeOH), lane 3- C. versicolor (MeOH), lane 6- G. applanatum (MeOH), lane 5- G. lucidum (EtOH), lane 7- P. tigrinus (MeOH), lane 8- P. betulinus (MeOH), lane 9- P. ostreatus (MeOH), lane 10- F. hepatica (MeOH), lane 2´- L. sulphureus (CHCl3), lane 3´- C. Versicolor (CHCl3), lane 4´- F. velutipes (CHCl3), lane 6´- G. applanatum (CHCl3), lane 7´- P. tigrinus (CHCl3), lane 8´- P. betulinus (CHCl3).

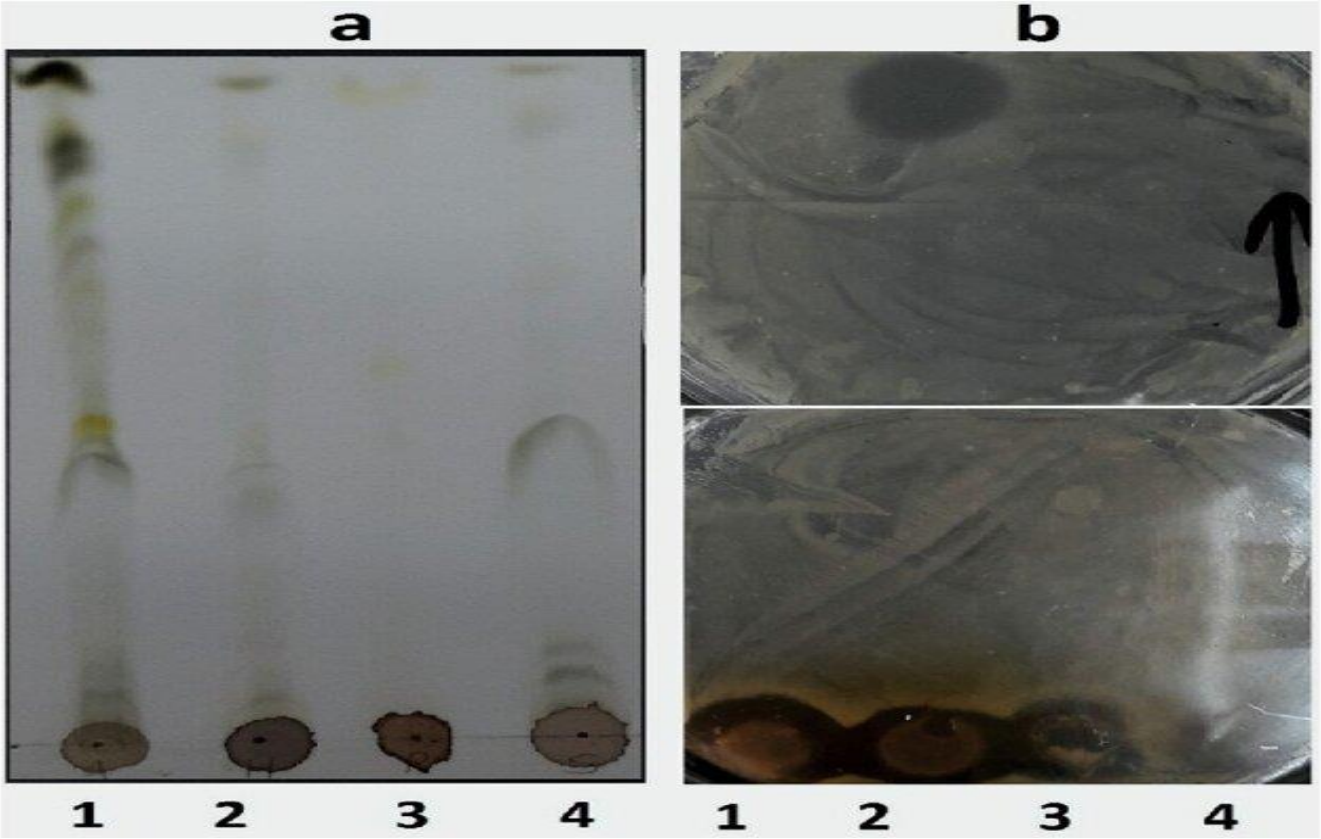

Fig. 1. A: TLC separation of crude extracts (methanol - MeOH and chloroform - CHCl3) of selected lignicolous species prepared for bioautography assay and B: bioautogram of extracts for Gram-positive bacteria S. aureus, animal strain

1.8 Target organisms

Bacillus subtilis is a Gram + bacteria, non-pathogenic to humans, and can be used as a model organism in similar tests, since the representative of the same genus, bacteria B. anthracis is responsible for the disease anthrax, which is characterized by the appearance of edema, hemorrhage, and tissue necrosis. It is common in some animals, often used as a biological weapon in bioterrorism. If an extract shows activity against B.subtilis, it is possible to be active against B. anthracis and possibly against other pathogenic Gram + bacteria such as species of the genera Staphylococcus and Streptococcus. Escherichia coli. E. coli, Gram bacteria, inhabits the gastrointestinal tract of humans and warm-blooded animals, making their normal indigenous microflora. In immuno-suppressed patients, however, it can cause infections, sometimes fatal (Giovannini, 2006). Gram - bacteria cause more problems than Gram +, as a result of their different cell wall structure.

Since penicillin and cephalosporin antibiotics belong to the group that acts at the level of cell wall synthesis, the exploration of new types of antibiotics is very important for a group of Gr- organisms. C. albicans belongs to Deuteromycota, representing yeasts forming pseudo-mycelia. It lives as a part of the normal human microflora, especially in the mucosa of the mouth and vagina. In immuno-suppressed individuals (AIDS, chemotherapy, inadequate nutrition, and poor hygiene), or after prolonged use of antibiotics, it can cause a disease called candidiasis, which is the most commonly caused by C. albicans as the most widespread species. It may affect almost any tissue, starting with simple children's thrush, and ending with systemic infections. Most commonly it is manifested in the form of slimy mucus. C. albicans, is a very convenient target organism in the detection of new antifungal drugs

2. Determination of active substances

In the last decades of the 20th century, the study of macrofungi was intensified, including the research of structurally different metabolites (polysaccharides, glycoproteins, proteaglucans, terpenoids, fatty acids, proteins, lectins, etc..) originating from the primary or secondary metabolism of fungi, as well as different biological activities that they express.

Metabolites from fungal fruit bodies or spores themselves are substantially different from those that come from the extracellular liquid of the medium in which submerged mycelium was grown or from cells of the culture. Since the phenomenon of multidrug resistance of microorganisms is on the rise, the studies of macrofungi increased in range, even though they are very slow-growing organisms. The value of macrofungi and the dietary supplements, originating from these organisms, grows each year on the world market. They are very safe and considered as the factors useful in the daily diet, especially for people suffering from various diseases.

Natural-products chemists further purify active chemicals from crude extracts by a variety of methods. The chemical structures of the purified material can then be analyzed. Techniques for further chemical analysis include chromatography, bioautography, radioimmunoassay, various methods of structure identification, or modern techniques such as atom bombardment mass spectrometry, Gas chromatography-mass spectrometry, highperformance liquid chromatography, capillary zone electrophoresis, nuclear magnetic resonance spectroscopy, and X-ray crystallography.

Research Method

The research on the antibacterial properties of lignicolous macrofungi typically involves extracting compounds from various species of fungi that grow on wood. Common methods include solvent extraction, where organic solvents are used to isolate bioactive compounds, followed by in vitro assays to evaluate antimicrobial activity against specific bacterial strains. For instance, studies have shown that extracts from species like L. quercina exhibit significant antimicrobial effects, indicating the presence of pharmacologically active agents.

Results

The results from various studies indicate that lignicolous macrofungi possess a range of antibacterial properties. For example, research has demonstrated that certain Australian basidiomycetes contain secondary metabolites with notable antimicrobial activities. Additionally, specific compounds, such as flavonoids found in Ganoderma species, have been identified as potent antibacterial agents. The effectiveness of these extracts can vary based on the extraction method and the specific fungal species used.

Discussion

The discussion surrounding the antibacterial potential of lignicolous macrofungi highlights their role as a natural source of antimicrobial agents. The diversity of bioactive compounds found in these fungi suggests that they could be valuable in developing new antibiotics, especially in the face of rising antibiotic resistance. The findings emphasize the need for further research to explore the mechanisms of action of these compounds and their potential applications in medicine.

Conclusion

The presented results indicate that extracts from lignicolous macrofungi could be used in the prevention and treatment of Gram-positive bacterial infections resistant to antibiotics in animals (humans), although further toxicity assays (in vivo) must be performed before its application. The fact that fungi can have bactericidal properties with low cytotoxicity to the animal host underscores their usefulness as natural sources of human or veterinary medicines. Also, the results obtained should stimulate further studies of other, so far unexplored, species such as M. giganteus and P. tigrinus, since current knowledge of the antibacterial activities or chemical composition of their active agents is not capable of fulfilling the expectations.

Acknowledgment

The accomplishment of this research project would not have existed without the offerings and support of many things and institutions. We are intensely grateful to all those who performed a function for the benefit of this project We too thank my mentors, Naweed Imam Syed, Prof. Department of Cell Biology at the University of Calgary, and Dr. Sadaf Ahmed Psychophysiology Lab, University of Karachi, for their priceless recommendations and support during the entire study period. Their observations and knowledge assisted in forming the management concerning this project

Declaration of Interest I already acknowledge that I have no financial or additional private interest, direct or unintended, in some matter that raises or grants permission and contradicts my responsibilities as a director of my commissionManagement
ConflictsofInterest:
The authors reveal that they have no conflict of interest. Financial support and protection No Funding was taken to assist in the development of this study

References

1 Wasser. S.P & Weis, AL {1999} Medicinal features of stuff going on in taller Basidiomycetes mushroom Contemporary outlook{assessment}worlwide journal of Medicinal Mushroom,1,31-62

2Kitzberger, C S G, Tasmania, JrA, Pedrosa, R C & Ferreira S.R S {2007} Antioxidant and antimicrobial movements of shiitake{Lentinula edodes} extract acquired by way of fundamental solvent and fluids. Journal of Food Engineering 80, PP 631-638

3Barros, L.; Calhelha, R.C.; Vaz, J.A.; Ferreira, I.C.F.R.; Baptista, P.; Esteviinho, L.M. (2007).Antimicrobial motion and bioactive compounds of Portugese stormy tasty mushrooms methanolic extracts. European food research and era, Vol.225, pp. 151-156,

4Turkoglu, A., Duru, M.E., Mercan, N., Kivrak, I., Gezer, okay. (2007). Antioxidant and Antimicrobial exercises of Laetiporus sulphureus (Bull) Murrill. Food Chemistry, 101, pp. 267-273.

5Kim, M.Y.; Seguin, P.; Ahn, J.k.; Kim, J.J.; Chun, S.C.; Kim, E.H.; seo, S.H.; Kang, E.Y.; Kim,S.L. ; Park, Y.J. ; Ro, H.M. & Chung, I.M. (2008). Phenolic compound aggregation and antioxidant physical games of tasty and curative mushrooms from Korea. Journal of Agricultural and Food Chemistry; Vol.56, No.16, pp. 7265-7270.

6Wasser, S.P. (2011). Present day verdicts, future flows and not settled questions in research of healing mushrooms. Implemented Microbiology and Biotechnology, 89, pp. 1323-1332.

7Hur, J.M.; Yang, C.H.; Han, S.H.; Lee, S.H.; You, Y.O.; Park, J.C.; Kim, .J. (2004).Antibacterial impact of Phellinus linteus towards methicillin-resistant Staphylococcus aureus. Fitoterapia, Vol.75, pp. 602-605

8Ishikawa, N.; Yamaji, ; Ishimoto, H.; Miura, k.; Fukushi, Y.; Takahashi, ok.; Tahara, S.(2005). Manufacturing of enokipodins A, B, C, and D: a new group of antimicrobial metabolites from the mycelial civilization of Flammulina velutipes. Mycoscience, Vol.46, pp.39-45

9Kalyoncu, F.; Oskay, M.; Sağlam, H.; Erdoğan, T.F.; Tamer, A.U. (2010). Antimicrobial and Antioxidant endeavors of mycelia of 10 untamed sprout class. Magazine of Medicinal food. Vol.13, No.2, pp. 415-419.

10Engler, M.; Anke, T.; Sterner, O. (1998). Manufacturing of Antibiotics by Collybia nivalis,Omphalotus olearius, a Favolashia, and a Pterula elegance on everyday substrates.Zeitschrift für Naturforschung C, Vol.53, No.five-6, pp. 318-324.

11Puttaraju, N.G., Venkateshaiah, S.U., Dharmesh, S.M., Urs, S.M.N., Somasundaram, R. (2006). The antioxidant interest of Indigenous fit for human consumption Mushrooms. Magazine of Agricultural and food Chemistry 54, pp. 9764-9772.

12Karaman M, Novakovic M, Matavulj M. (2012). Fundamental fungal strategies in the restoration of the herbal environment. In: Vazquez, Silva editors. Fungi: kinds, environmental impact and function in ailment. Big Apple: Nova Technology Publishers Inc; in press.

13Inouye, S., Abe, S.h., Yamaguchi, H. (2004). Fungal terpenoid Antibiotics and Enzyme Inhibitors. In: Handbook of fungal Biotechnology. Arora D, editor, second Ed. New York: Marcel Dekker; pp. 379-400.

14 Tanaka, Y. & Omura, S. (1993): Agro active Compounds of Microbial origin. Annual evaluate of Microbiology, Vol. 47: 57-87

15Aldred, D., Magan, N., Lane B. S. (1999). Impact of water hobby and nutrients on boom and manufacturing of squalestatin S1 through a Phoma sp. magazine carried out Microbiology, Vol.87, No. 6, pp. 842-848.

16Lindequist, U.; Niedermeyer, T.H.J. & Julich, W.D. (2005). The pharmacological potential of Mushrooms. Evidence-based Complementary and alternative medication; Vol.2, No.3, pp. 285299.

17Smania, JrA, Monache, F.D., Logueric io-Leite, C., Smania, E.F.A., Gerber, A.L. (2001). Antimicrobial hobby of Basidiomycetes. Global Journal of Medicinal Mushrooms, three, pp. 87-93

18Zjawiony, J. (2004). Biologically energetic compounds from Aphyllophorales (polypore) Fungi. Magazine of herbal products; 67, pp. 300-310.

19Lorenzen, okay. & Anke, T. (1998). Basidiomycetes as an asset for new bioactive natural Products. Cutting-edge natural Chemistry, 2, pp. 329-364.

20Karaman, M., Mimica-Dukić, N., Knežević, P., Svirčev, Z., Matavulj, M. (2009a): Antibacterial Houses decided on lignicolous mushrooms and fungi from northern Serbia. Worldwide magazine of Medicinal Mushrooms, Vol.11, No.3, pp. 269-279.

21Karaman, M.; Kaišarević, S.; Somborski, J.; Kebert, M.; Matavuly, M. (2009b): organic activities of the lignicolous fungus Meripilus giganteus (Pers.:Pers.) Karst. Records of Biological Sciences, Vol.61, No.4, pp. 353-361.

22Elmastas, M.; Isildak, O.; Turkekul, I.; Temur, N. (2007). dedication of antioxidant Activity and antioxidant compounds in wild edible mushrooms. Journal of food Composition and analysis, Vol.20, pp. 337-345.

23Abraham, W.R. (2001). Bioactive sesquiterpenes produced by using fungi: are they beneficial for humans as nicely? Modern-day clinical Chemistry, 8: 583-606.

24Mothana, R.A.A.; Jansen, R.; Julich, W.D. & Lindequist, U. (2000). Ganomycin A and B, new Antimicrobial farnesyl hydroquinones from the Basidiomycete Ganoderma pfeifferi. Magazine of herbal products, 63, pp. 416-418.

25Leon, F.; Quintana, J.; Rivera, A.; Estevez, F. & Bermejo, J. (2004). Lanostanoide triterpenes From Laetiporus

sulphureus and apoptosis induction on HL-60 Human myeloid leukemia cells. Journal of natural products, 67, pp. 2008-2011.

26Paterson, R.R.M. (2006). Ganoderma –a therapeutic fungal biofactory. Phytochemistry, 67, pp. 1985-2001.

27Ribeiro, B., Valentao, P., Baptista, P., Seabra, R., Andrade, P.B. (2007). Phenolic compounds organic acids profiles and antioxidative houses of beefsteak fungus (Fistulina hepatica). Food Chemical Toxicology, 45, pp. 1805-1813.

28Fan, J. M., Zhang, J. S., Tang, Q. J., Liu, Y. F., Zhang, A. Q., Pan, Y. J. (2006): Structural Elucidation of an impartial

.

fucogalactan from the mycelium of Coprinus comatus. Carbohydrate Research, 341: 1130–1134.

29Stamets, P. (2002): Novel antimicrobials from mushrooms. Natural Gram, 54: 2-6.

30 Chen, J. Seviour, R. (2007): Medicinal significance of fungal - (1→ 3), (1→ 4) - glucans. Mycological studies, three: 635-652.