Screening Of Antimicrobial Activity Of Sesquterpenoid Crude Extract Of *Ganoderma*

Asghar.Sharifi[1], Seyed Sajjad Khoramrooz[1], Soheyla Jahedi[3,] Seyed Abdolmajid Khosravani[1]

1. Department of Microbiology, Medical Microbiology Research Center, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran.
2. Department of Parasitology and Mycology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran.
3. Farhangian University of Shiraz, Iran

khosravani2us@yahoo.com

Abstract: Antimicrobial activities of sesquiterpenoid of Ganoderma were tested against human pathogenic microorganisms. Four out of 11 species of Ganoderma showed good antimicrobial activity. Minimal inhibitory concentration was determined for the sesquterpenoid extract of Ganoderma Mazandaran Ganoderma lipsiense, Ganoderma multicornum and Ganoderma lucidum on selected microorganisms. Proteus mirabilis (MTCC 1429) Candida albicans (MTCC 1637), Klebsiella pneumonia (MTCC 432), Escherichia coli (MTCC 2064) and bacillus subtilus (NCIM 2010) were tested. Ganoderma lucidum extract showed maximal inhibition of Proteus mirabilis and was also active against Candida albicans, as was the extract of Ganoderma mazandaran. Lowest MIC values were 128 l/ ml demonstrated by sesquterpenoid extract of G. lucidium, and G. Mazandaran against B. subtilus and P.mirabilis. Further separation of the sesquterpenoid compounds need to be carried out to detect the bioactivity of specific compounds.

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1. Introduction

Antimicrobial activity is the ability of a substance to inhibit growth and reproduction or to kill microorganisms. A chemical, at low concentration, should have a broad spectrum of antimicrobial activity, which means that it should inhibit or kill many different kinds of microorganisms. Most pathogenic bacteria and fungi are susceptible to antibiotics or other antimicrobial agents and their response towards these antibiotics, however varies enormously (1). Antimicrobial agents include antibiotics and antimicrobial metabolites produced by one microorganism which inhibits the growth of other organism (2). Several antimicrobial metabolites have been isolated from mushrooms like Ganoderma and have a potent antiviral, bacterial and fungal activity (3-4). Ganoderma spp haves been economically important fungi, for over 4000 years particularly in the Far East countries, and used as antitumor activity (5-6). The reasons for the use of Ganoderma spp were to prevent, cure, treatment of cancer, diarrhea, and excessive salivation (7).

This report elucidates interesting chemical compounds extracted (sesquterpenoid), purified and identified from fruit bodies of Ganoderma as a bioactive agents tested against a selected isolates of microorganisms. The aims of the present study are to screen antimicrobial activities of sesquterpenoid extracts of Ganoderma on selected microorganisms.

2. Material and Methods

In this present work P. mirabilis MTCC 1429, C.albicans MTCC 1637, K. pneumonia MTCC 432, E.coli ATCC 2046, B. subtilis NCIM 2010 and S. aureus, were used as test microorganisms for sesquiterpenoid samples extracted from Ganoderma for their antimicrobial activity. Culture medium for bacteria: nutrient agar (1.0g beef extract, 2.0g yeast extract, 5.0g peptone, 5.0g sodium chloride, 20.0g agar, 1000 ml DW, pH was adjusted to 7.0-7.5).

Culture medium for Candida albicans was Yeast extract peptone dextrose agar(YEPD), (3.0g yeast extract, 10.0g peptone, 20.0g dextrose, and 15.0g agar, 1000 ml DW). The cultures were maintained as slants incubated at 37 C0. Sub culturing was done every two weeks for bacteria and yeast.

Preparation of inoculums:

A loop full of freshly isolated colonies of bacteria and yeast were suspended in 0.85% saline and/ or sterile distilled water.

Well Assay Method.

The well assay method was according to Barry (1986). In brief, the agar plates were prepared in accordance to the organism (as given previously). The plates were inoculated using a sterile cotton swab by spreading the inoculums evenly over the surface of the medium. Inoculums were left for few minutes to dry, with the lid closed at room temperature, wells were made with a cork borer

(6mm), and sample extracts of fungi were added to the wells (50µl in each well), also containing a well with positive control (methanol). The plates were incubated at 35-37 C for 18-24 hours. The activity was calculated by measuring the diameter of zone of inhibition (including the diameter of the well) to the nearest millimeter. The results of the test and control plates were compared.

Table 1. *Ganoderma* spp used:

Name of Species	Samples NO.
Ganoderma applanatum (Pers.) Pat.	GA-02.
Ganoderma capense	GA-06
Ganoderma chalceum	GA-39
Ganoderma lipsiense (Batsch.) Murill	GA-19
Ganoderma lucidum (Curtis; (Fr.) P Karst Var. lucidum.	GA-34, GA-38, GA-10
Ganoderma lucidum var. microsporus.	GA-16
Ganoderma multicornum(P Karst var.)	GA-28
Ganoderma multiplicatum (Mont.) Pat.	GA-12, GA-27
Ganoderma perzonatum (Murrill)	GA-36
Ganoderma Mazandaran(proposed new species).	GA-11
Ganoderma praelongum (Murrill)	GA-37
Ganoderma sp.	GA-K, GA-S
Ganoderma stipitatum (Murrill)	GA-07

Table 2: Antimicrobial Activity of Sesquterpenoid Extract From *Ganoderma* Samples Against Human Pathogen Microorganis

Samples	***P. mirabilis***	***C. albicans***	***K.pneumonia***	***S.areus***	***E. coli***	***B. subtilis***
G. mazandaran	25	24.6	17.6	20.6	24.67	28.3
G.lipsiense	25.3	24.3	16.67	22.6	19	21.67
G.multicornum	23.3	21.3	18.33	21.67	23	22.67
G. lucidum	31.3	27.3	21.67	29	30.67	32.67

For bacteria Nutrient Broth (NB) and for fungi Yeast

Table 3: Determination of the Minimum Inhibitory Concentration (MIC) of *Ganoderma* Samples against Human Pathogenic Microorganisms.

Sample	Name of microorganisms (MIC µg/ ml)					
	E. coli	***S.areus***	***K.pneumonia***	***P. mirabilis***	***C. albicans***	***B. subtilis***
G. mazandaran	64	64	64	32	64	32
G.lipsiense	64	64	64	32	32	32
G.multicornum	32	32	64	64	64	64
G. lucidum	32	32	32	32	32	32

Sample collection: Ganoderma spp were collected from different parts of Mazandaran province (Northern of Iran), brought to laboratory and air-dried in Department of Microbiology at Yasouj University of Medical Sciences, then it was ground and maintained in airtight plastic bag for further use (Table 1).

Identification:

The Ganoderma spp were identified using keys and morphological characters mentioned by Steyaert (8) and Ryvarden (9).

Sesquiterpenoid Extraction:

5gm of powder was extracted with 100ml (X 2) of chloroform overnight with initial warming. The filtrates were combined and evaporated under vacuum. The residue was dissolved in 25ml of ethanol (95%) and 25ml of lead acetate (4% aqueous). The solution was evaporated under vacuum; the resulting residue was dissolved in chloroform and again evaporated to dryness under vacuum. The residue was collected, weighed, dissolved in methanol and used for further TLC analysis. Solvent System: Chloroform: Methanol (9:1). (10)

Minimum inhibitory concentration (MIC):

The lowest concentration of the antimicrobial extract inhibiting the visible growth after overnight incubation is denoted as MIC. MIC of

the extract for bacteria was determined using broth dilution method. To determine Minimum Lethal Concentration (MLC), a known quantity of inoculum from each of the tubes of broth that showed no visible turbidity is sub-cultured to solid agar plate. The lowest concentration of antimicrobial agent that allowed less than 0.1% of the original inoculum to survive is said to be the MLC. The results of MIC are usually the same results of MLC, or one tube before MIC.

Potato Dextrose Broth (YPDB) was used. The solutions, the methanol solution with the extract, were serially diluted in respective media to obtain dried extract concentrations of 128, 64, 32, 16, 8, 4 and 2 mg ml-1. The experiments were performed in triplicate and analyzed by SPSS.

Tested cultures in this study were P. mirabilis, C. albicans, K. pneumonia, , S.aureus, E. coli and B. subtilis The cultures were maintained as slants, which were incubated at 37 0C. The sub culturing was done every two weeks. Each experiment was done in triplicate and analyzed by ANOVA test.

3. Results

The sesquiterpenoid extract of Ganoderma Ganoderma Mazandaran, Ganoderma lipsiense, Ganoderma multicornum and Ganoderma lucidum, from Mazandaran, Iran, were tested for antimicrobial activity by the disc diffusion agar method.

Strong = zone of inhibition equals or greater than 21mm

Moderate = zone of inhibition equals 11 mm to 20 mm

Weak = zone of inhibition equals or less than 10 mm

Data represented in Table 2 showed that, G. lucidium strongly inhibited the growth of E.coli, P.mirabilis, and B.subtillus with inhibition zone diameters of (30.69 mm), (31.3, mm), (32.67 mm) respectively. Similarly, the effect of commonly used antibiotics (for fungi we used fluconazol, and for bacteria nitrofurantoine, trimethoprim sulfumethoxazol, amikacin, tetracycline, penicillin, gentamycin, cefalotine, and polymixin B were used) was tested against these microorganisms and showed that they were highly resistant to at least one antibiotic ($P< 0.01$).

Ganoderma mazandaran showed maximum zone of inhibition of 28.3mm on B. subtillus and minimum zone of inhibition(17.6mm) on K.pneumonia, while Ganoderma lipsiense showed 23.3mm, 22.67mm and 21.3mm zone of inhibition on P.mirabilis B. subtilis and C.albicans respectively, G. multicornum showed the maximum zone of inhibition(23.3mm) by for P.mirabilis.

The MIC value of sequiterpenoid extract of G.lucidium against P.mirabilis, E.coli .S. aureus, K. pneumoniae and C.albicans (Table 3) was 32 µg/ml. Our results also indicates that the MLC values for P.mirabilis and C. albicans was 64µg / ml respectively. The MIC of G.mazandaran was 32 µg/ml by P.mirabilis and 64µg / ml in the other present microorganisms, Ganoderma lipsiense showed that MIC=32µg / ml on E.coli and S.aureus and 64µg / ml on other microorganisms, MIC= 32µg / ml by G. lipsiense against P. mirabilis,B.subtilus and C. albicanse and 64 µg / ml on other present microorganisms.

4. Discussions

Research for novel antibiotic is of utmost importance since most microorganisms have developed resistance to many antibiotics. The present work was carried out using extract from 11 Ganoderma spp. to search for novel compounds. Although, a few reports on bioactive compounds of Ganoderma spp are available (11-12). The results obtained in our study clearly indicate that extracts of mushrooms belonging to Ganoderma spp. possess potent antimicrobial activity.

Our present study revealed that purified sesquiterpenoid extract of G. lucidium exhibited an inhibitory effect against bacteria and fungi. These findings are in concomitant with other studies (13-14) G.stipitatum was active against Gram negative and Gram positive bacteria.

Apparently, the sesquiterpenoid extract of G.Mazandaran, G.lipsiense, G. multicornum and G. lucidum were potent and effective against the fungal isolates (C. albicans) since zone of inhibition of growth (23.3 mm to 31.3 mm) was observed in contrast with earlier findings regarding the antibiotics (14). In contrast Smala et al. (15) stated that G. annulare produces applanoxidic acid which showed a weak activity against the dermatophyte T. mentagrophyes.

According to Gao et al (16), G. lucidum and other Ganoderma species, more often in combination with chemotherapeutic agents, have been used to treat various bacterial diseases. They have suggested that the sesquiterpenoid components play an important role in its bioactive principle. Therefore it could be concluded from our results G. Mazandaran, G. lipsiense, G. multicornum and G. lucidum spp could be employed to combat several diseases caused by pathogenic microorganisms. Nevertheless, there is still more mushrooms needed to be examined for their potentiality activities against bacteria and pathogenic fungi.

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Corresponding Author:
Dr. Seyed Abdolmajid Khosravani
Department of Microbiology, Medical Microbiology Research Center, School of Medicine, Yasuj University of Medical Sciences, Yasuj, Iran.
E-mail: khosravani2us@yahoo.com

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