ISSN 2395-650X

International Journal of Life Sciences Biotechnology Pharma Sciences

IJLBPS

www.ijlbps.org

E-mail: editorijlbps@gmail.com editor@ijlbps.org

International Journal of Life Sciences Biotechnology Pharma Sciences

Volume2 Issue4,Oct2014

Ganoderma terpenoid extracts have antimicrobial activity

Kaul Shefali

Abstract

Researchers tested the antimicrobial effects of nine Ganoderma species from the Western Ghats of Maharashtra (INDIA) by extracting their terpenoids (sesquiterpenes, diterpenes, and triterpenes). In addition, seven commonly used antibiotics were tested for their efficacy against the sample microbes. The well assay technique was used for the tests. When compared to conventional antibiotics, the activity of sesquiterpenoid extracts was much higher against gram-positive and gram-negative bacteria and Candida albicans, while that of triterpenes and diterpenes was much lower. Diterpene and triterpene extract failed to kill Candida albicans. Sesquiterpenoid extract from Ganoderma samples was the only one to exhibit antibacterial activity among the other terpenoid extracts. Antimicrobial agents may be found in ganoderma samples; they are particularly promising in G. lucidum, G. chalceum, and G. stipitatum.

Keywords: *Ganoderma*, Antimicrobial, Terpenoids, Western Ghats.

Introduction

Anticancer, antimalarial, and anti-diabetic capabilities are only some of the medical benefits associated with the ganoderma mushroom's bioactive components. In traditional Chinese medicine, this fungus is often used to treat a wide range of illnesses. It is a well-studied genus in the aphyllophorales family, and its medicinal and economic possibilities have been extensively documented. Several species of Glossophyllum have been the focus of study thus far: G. lucidum, G. applanatum, G. tsugae, G. japonicum, G. capense, G. resinaceum, G. annulare, and G. pfeifferi
Triterpenes and polysaccharides, with smaller amounts of sterols, nucleic acid derivatives, alkaloids, tannins, etc., are known to constitute Ganoderma's primary bioactive components. 4. G. lucidum compounds, both in their crude and purified forms, have been found to have a wide variety of bioactivities.

The indigenous inhabitants of central India utilize ganoderma to treat conditions including cataracts, asthma, and hydroceles. 11, 12. In western Maharashtra, INDIA, it is often used as an anti-inflammatory and tumor treatment, and it is also commonly discovered as an adulterant in another folk medicine called Phansomba (Phellinus species). 13, 14. Except for a recent publication on the antibacterial activity of Ganoderma lucidum, Navesporus floccosa, and Phellinus rimosus, using crude extracts15, the bioactivities of the Indian species of Ganoderma have not been evaluated. This is the first research to report on the antibacterial activity of nine indigenous Ganoderma species.

Department of Quality Assurance, Astral Life Care, Ahmedabad,

Material and methods

Mushrooms

The basidiocarp of Ganoderma praelongum Murrill. (GA - 36); Ganoderma resinaceum Boudier. (GA - 36); Ganoderma praelongum Murrill. (GA - 37); Ganoderma lucidum (Curtis: Fr.) P. Karst. (GA - 38); and Ganoderma chalcedonicum (GA - 39). Using morphological features, the specimens were cataloged and submitted to the herbarium at the University of Pune's (India) Department of Botany.

Extraction

After being dried in an oven at 45–50 degrees Celsius, the samples were ground into a powder using a commercial mill set to a mesh size of 100.

Sum of Triterpenes:

Defatting the powder (using diethyl ether, 100 ml) and then extracting it in methanol (100 ml 2) by repeated heating or refluxing yielded the desired concentration of 16, 4. Concentrated methanol extracts (one tenth the volume, under vacuum) were changed by adding ethyl acetate (1:1v/v). The residue was then collected, weighed, and dissolved in methanol: ethyl acetate (1:1v/v) to a concentration of 50 g/l (yield: 15.6 to 28.2%) for the test. Harborne16 stated that triterpenes were verified by TLC; this was really the case.

Sesquiterpenes:

Sesquiterpenes were extracted using the same technique described by Harbone16. TLC verified the presence of the sesquiterpenes indicated in Harborne 16.

Diterpenes:

Diterpenes were extracted using the procedure described in Harborne 16. TLC verified the presence of the diterpenes, which are also described in Harborne 16.

Bioassay

Seven human pathogenic bacteria were tested for their antimicrobial efficacy against the various terpenoid extracts listed above (Table 1). The effectiveness of seven commonly used antibiotics (Tetracyclin, Trimetaprim sulfametoxol, Nitrofurantoin, Gentamycin, Penicillin, Amikacin) and one antifungal agent (Fluconazole) was tested against the various terpenoid extracts.Making an Inoculation. Newly separated colonies were floated in a mixture of 0.85% salt

water and/or distilled water to maintain sterility. McFralands No. 1 was used to calibrate the resulting suspension's turbidity. According to Barry17's description, we used the well assay technique.The Ends and the MeansWeak activity was found in the up to 10 mm zone, moderate activity was found between 11 and 20 mm, high activity was found between 21 and 30 mm, and extra strong activity was found above 30 mm (table 2). The inactive control compounds were not included since their action was statistically negligible (zone of inhibition, 6-7 mm).Strong antibacterial action against Staphylococcus aureus was shown by extracts of the sesquiterpenoids from G. lucidum, G. chalceum, G. stipitatum, and G. lipsiense. In comparison to the antibiotics trimetaprim sulfamethoxazole, nitrofurantoin, and penicillin, the moderate activity seen in the triterpene extracts of all the samples was impressive.

Sesquiterpene extracts from G. lucidum, G. chalceum, and G. stipitatum all shown very high activity against Acinetobacter calcoaceticus (31.6, 32.33, and 32.67, respectively). Both diterpene and triterpene extracts demonstrated moderate activity against Acinetobacter calcoaceticus, with the former exhibiting an inhibition zone of 15–18 mm and the latter displaying an inhibition zone of 10–15 mm; these results are comparable to those of Tetracyclin and Trimetaprim sulfamethoxazol.

Extra strong activity against Escherichia coli and Bacillus subtilis was shown by the sesquiterpenoid extracts of G. chalceum and G. lucidum, while strong activity was demonstrated by those of G. stipitatum, G. multicornum, G. species-1, and G. lipsiense. The activity of all diterpenes and most triterpenes was moderate, whereas that of triterpene extracts was low. Tetracycline, like the sesquiterpenoids, was very effective against Escherichia coli but ineffective against Bacillus subtilis. Among the antibiotics tested, only trimethoprim sulfametoxazol demonstrated strong action against both pathogens (table 3).

Among the bacteria and viruses tested, Klebsiella pneumoniae proved to be the most resilient. Extracts of the sesquiterpenoids from both G. chalceum and G. lucidum were as active as the antibiotics nitrofurantoin and amikacin. The activity of the diterpene and triterpene extract was low to moderate.

Extracts of sesquiterpenoids were shown to be very active against Proteus mirabilis, with the exception of G.

lucidum, which had extremely high activity. Strong activity equal to that of Nitrofurantoin and Amikacin was shown by the triterpene extracts of G. lipsiense, G. multiplicatum (GA -12 and GA -27), and G. multicornum, whereas moderate activity was demonstrated by the diterpene extracts of all the samples.

Candida albicans, the sole pathogenic fungus tested, showed modest resistance to triterpene and diterpene extracts but high activity to all sesquiterpene extracts. Table 3 shows that the common antifungal drug fluconazole also had significant efficacy.

Ganoderma lucidum (GA- 38), Ganoderma chalceum (GA- 39), Ganoderma stipitatum (GA-7), and Ganoderma species -1 (GA-11) terpenoid extracts all shown greater inhibition than the other samples tested, including conventional antibiotics.

The antimicrobial activity of the sesquiterpene extracts (Table 2) was either greater than or on par with that of the conventional antibiotics (Table 5). Tables 3 and 4 show that diterpene and triterpene extracts have moderate activity against Acinetobacter calcoaceticus, with diterpene extracts having an inhibition range of 15–18 mm and triterpene extracts having an inhibition range of 10–15 mm, respectively, similar to that of Tetracyclin and Trimetaprim sulfamethoxazol. Table 4 shows that all of the triterpene extracts tested had moderate action, which was greater than that of the usual antibiotics (Table 5: Trimethoprim sulfamethoxazole, Nitrofurantoin, and Penicillin).

Although the Ganoderma triterpenes have been linked to a wide range of pharmacological effects, this investigation only revealed them to be weak antimicrobials. Although Ganoderma diterpenes have not previously been reported, this study's crude diterpenes extracts show moderate to high activity. Four Cadinene-type sesquiterpenes were reported from Ganoderma mastoporum18, but their bioactivity was not mentioned. Sesquiterpenes extracts from Ganoderma samples in the current investigation exhibited substantial activity against both Candida albicans and gram-positive bacteria, making them a broad-spectrum antibiotic.

Microorganisms' growing resistance to existing medicines has prompted scientists to look elsewhere for effective antimicrobials. The Ganoderma sesquiterpenes have antibacterial and antifungal properties

that are effective against a wide variety of bacteria and fungi. As a result, the native Ganoderma species, particularly Ganoderma chalceum, Ganoderma stipitatum, and Ganoderma lucidum, stand out as a promising source of an antibacterial agent with additional pharmacological benefits.

able: 1 Different strains of test organisms used and their source and media used

Cultures (Strain; Source)	**Media used**
Acinetobacter calcoaceticus (NCIB 2886; NCL)	**Nutrient agar** (1gm beef extract, 2gm yeast extract, 5gm peptone, 5gm NaCl, 20gm agar; for 1L. pH 7 ± 0.5)
Bacillus subtilis (NCIM 2010; NCL)	**Nutrient agar**
Escherichia coli *(MTCC 724; IMTECH)*	**Nutrient agar**
Klebsiella pneumoniae (MTCC 432; IMTECH)	**Nutrient agar**
Proteus mirabilis (MTCC 1429; IMTECH)	**CM growth medium** (4gm yeast extract, 10gm malt extract, 4gm glucose, 20gm agar; for 1L, pH 7.2)
Staphylococcus aureus (HAL 2079; NCL)	**Nutrient agar**
Candida albicans (MTCC 1637; IMTECH)	**YEPD** (3gm yeast extract, 10gm peptone, 20gm dextrose, 20gm agar; for 1L)

IMTECH = Institute of Microbial Technology Chandigarh, India.
NCL= National Chemical Laboratory, Pune, India.

Table: 2 Antimicrobial activity of Sesquiterpene extracts from different *Ganoderma* samples

	Sa. 2079	**Ac. 2886**	**Ec. 724**	**Bs. 2010**	**Kp. 432**	**Pm. 1429**	**Ca. 1637**
GA 7	23.33 ± 1.15	32.67 ± 2.08	27.67 ± 0.58	28.67 ± 1.15	17.33 ± 1.15	22.67 ± 1.53	27.67 ± 1.53
GA 11	20.67 ± 0.58	26.00 ± 1.00	24.67 ± 0.58	28.33 ± 1.53	17.67 ± 1.15	25.00 ± 1.00	24.67 ± 1.15
GA 12	17.67 ± 0.58	24.33 ± 1.53	19.33 ± 1.15	19.00 ± 1.00	16.33 ± 1.53	19.67 ± 1.53	19.33 ± 1.15
GA 19	22.67 ± 1.15	26.67 ± 1.53	19.00 ± 1.00	21.67 ± 0.58	16.67 ± 1.15	25.33 ± 1.53	24.33 ± 0.58
GA 27	21.00 ± 1.00	23.33 ± 0.58	19.67 ± 0.58	20.33 ± 0.58	17.33 ± 0.58	24.33 ± 0.58	21.67 ± 0.58
GA 28	21.67 ± 0.58	25.67 ± 1.53	23.00 ± 1.00	22.67 ± 0.58	18.33 ± 1.53	23.33 ± 1.15	21.33 ± 0.58
GA 36	17.33 ± 0.58	24.33 ± 3.06	20.33 ± 0.58	20.67 ± 0.58	16.33 ± 1.53	21.33 ± 1.15	22.33 ± 0.58
GA 37	20.00 ± 1.00	26.33 ± 1.15	21.33 ± 0.58	22.33 ± 1.53	19.67 ± 1.53	26.67 ± 1.15	25.33 ± 1.15
GA 38	29.00 ± 1.00	32.33 ± 1.53	30.67 ± 1.53	32.67 ± 1.15	21.67 ± 0.58	31.33 ± 1.53	27.33 ± 0.58
GA 39	26.67 ± 0.58	31.67 ± 0.58	32.33 ± 1.53	32.67 ± 1.15	24.33 ± 0.58	23.67 ± 1.15	23.33 ± 0.58

(Values are mean ± SD)

Table: 3 Antimicrobial activity of Diterpene extracts from different *Ganoderma* samples

	Sa. 2079	Ac. 2886	Ec. 724	Bs. 2010	Kp. 432	Pm. 1429	Ca. 1637
GA 7	17.67 ± 0.58	18.33 ± 1.15	18.33 ± 0.58	18.33 ± 0.58	15.33 ± 1.53	19.67 ± 1.53	14.33 ± 0.58
GA 11	17.33± 1.15	17.67 ± 1.15	18.00 ± 1.00	19.00 ± 1.00	13.33 ± 1.15	19.33 ± 0.58	14.67 ± 1.53
GA 12	17.67 ± 1.53	17.67 ± 1.53	15.33 ± 0.58	19.67 ± 0.58	14.67 ± 0.58	21.67 ± 1.15	14.67 ± 0.58
GA 19	18.67 ± 1.15	17.67 ± 0.58	15.67 ± 1.53	20.33 ± 0.58	11.67 ± 0.58	23.67 ± 1.53	14.33 ± 1.15
GA 27	18.00 ± 1.00	18.67 ± 1.15	9.33 ± 0.58	17.67 ± 1.53	9.67 ± 0.58	25.00 ± 1.00	15.33 ± 1.53
GA 28	19.33 ± 1.15	18.00 ± 1.00	15.33 ± 1.53	18.67 ± 1.15	10.33 ± 0.58	20.67 ± 1.15	15.00 ± 1.00
GA 36	18.33 ± 1.15	18.67 ± 1.15	16.67 ± 1.53	17.67 ± 0.58	10.33 ± 0.58	18.33 ± 0.58	11.33 ± 0.58
GA 37	20.00 ± 1.00	18.33 ± 0.58	15.67 ± 1.15	18.67 ± 0.58	9.67 ± 1.15	19.00 ± 1.00	13.67 ± 1.15
GA 38	17.67 ± 1.15	15.67 ± 1.15	15.33 ± 0.58	17.33 ± 0.58	9.33 ± 0.58	19.33 ± 0.58	13.67 ± 1.53
GA 39	19.67 ± 0.58	16.0 ± 1.00	14.67 ± 0.58	19.67 ± 0.58	9.67 ± 0.58	18.67 ± 1.15	11.00 ± 1.00

(Values are mean ± SD)

Table: 4 Antimicrobial activity of Triterpene extracts from different *Ganoderma* samples

	Sa. 2079	Ac. 2886	Ec. 724	Bs. 2010	Kp. 432	Pm. 1429	Ca. 1637
GA 7	18.33 ± 0.58	11.00 ± 1.00	11.00 ± 1.00	10.33 ± 0.58	11.00 ± 1.00	18.33 ± 1.15	16.00 ± 1.00
GA 11	17.33 ± 0.58	12.33 ± 0.58	14.00 ± 1.00	13.33 ± 0.58	13.33 ± 0.58	14.67 ± 1.53	13.33 ± 0.58
GA 12	17.67 ± 1.15	11.33 ± 0.58	9.33 ± 0.58	14.33 ± 0.58	13.67 ± 0.58	17.67 ± 0.58	12.33 ± 0.58
GA 19	18.67 ± 1.15	10.00 ± 1.00	10.33 ± 1.15	9.33 ± 0.58	11.33 ± 1.15	15.33 ± 1.53	10.67 ± 0.58
GA 27	18.00 ± 1.00	15.33 ± 1.15	11.33 ± 1.53	9.67 ± 0.58	9.67 ± 0.58	14.33 ± 1.15	12.67 ± 1.15
GA 28	17.33 ± 0.58	13.33 ± 1.53	13.33 ± 0.58	9.33 ± 0.58	11.00 ± 1.00	17.67 ± 1.53	13.33 ± 1.15
GA 36	19.33 ± 0.58	11.33 ± 1.15	11.67 ± 0.58	10.00 ± 1.00	10.67 ± 0.58	17.00 ± 1.00	10.67 ± 0.58
GA 37	19.67 ± 0.58	11.00 ± 1.00	12.33 ± 0.58	12.33 ± 0.58	12.33 ± 0.58	15.33 ± 1.53	12.67 ± 0.58
GA 38	19.67 ± 1.53	11.33 ± 1.15	9.67 ± 0.58	9.33 ± 0.58	9.33 ± 0.58	14.67 ± 1.53	14.33 ± 1.15
GA 39	19.33 ± 1.15	15.33 ± 0.58	11.33 ± 1.53	11.00 ± 1.00	11.00 ± 1.00	13.67 ± 1.15	12.67 ± 0.58

(Values are mean ± SD)

Table: 5 Activity of standard antibiotics against the test organisms

Organisms	Tetracyclin	Trimetaprim sulfumetoxazol	Nitro furantoin	Gentamycin	Penicillin	Amikacin
Acinetobacter calcoaceticus	20	15	7	8	7	10
Bacillus subtilis	10	28	12	18	10	10
Escherichia coli	32	27	10	15	7	17
Klebsiella pneumoniae	7	7	23	12	7	20
Proteus mirabilis	32	7	25	12	7	14
Staphylococcus aureus	20	12	9	7	13	7
Candida albicans	30 (Fluconazol)					

References

Chang S. T. and Buswell J. A. (1999). *Ganoderma lucidum* (Curt.: Fr.) P. Karst. (Aphyllophoromycetideae) –A mushrooming medicinal mushroom. *Int. J. Med. Mush.* **1:** 139-146.

Mohammad-Fata Moradali, Hossein Mostafavi, Shirin Ghods and Ghorban-Ali Hejaroude (2008). Investigation of Antimicrobial Fatty Acids from Medicinal Artist Conk Mushroom *Ganoderma applanatum*(Pers.) Pat. (Aphyllophoromycetideae) by TLC and Spectroscopic Detection. *Int J of medicinal mushroom*. **10**: 149 -154.

Ofodile L. N., Uma N. U., Kokubun T., Grayer R. J., Ogundipe O. T. and Simmonds M. S. J. (2005). Antimicrobial activity of some *Ganoderma* species from Nigeria. *Phytotherapy research.* **19(4)**: 310-313.

Huie C. W. and Di X. J. (2004). Chromatographic and electrophoretic methods for Ling zhi pharmacologically active components. *Journal of Chromatography B.*, **812 (1-2)**: 241 –257.

Gao Y. and Zhou S. (2003). Cancer prevention and treatment by *Ganoderma*, a mushroom with medicinalproperties. *Food Reviews International.***19 (3)**: 275 –325.

Jong S. C. and Birmingham J. M. (1992). Medicinal benefits of the mushroom *Ganoderma*. *Advances in Applied Microbiology.* **37**: 101 –134.

Willard T. (1990). Reishi Mushroom: Herb of Spiritual Potency and Medical Wonder. *Sylvan Press. Issaquah, Washington, 168.*

Yoon S. Y., Eo S. K., Kim Y. S., Lee C. K. and Han S. S. (1994). Antimicrobial activity of *Ganodermalucidum* extract alone and in combination with some antibiotics. *Arch. Pharm. Res.* **17(6)**: 438 – 442.

Mothana R. A., Jansen R., Julich W. D. and Lindequist U. J. (2000). Ganomycins A and B, new farnesylhydorquinones from the basidiomycete *Ganoderma pfeifferi*. *J. Nat. Prod.* **63 (3)**: 416 – 418.

Samania E. F. A., Delle F., Monache A., Samania Jr., Yunes R. A. and Cuneo R. S. (2003). Antifungalactivity of sterols and triterpenes isolated from *Ganoderma annulare*. *Fitoterapia.* **(74)**:375 – 377.
Harsh N. S. K., Rai B. K. and Tiwari D. P. (1993). Use of Ganoderma lucidum in folk medicine. *Indian Trop. Biod.* **(1)**:324-326.
Harsh N. S. K., Tiwari C. K. and Rai B. K. (1996). Forest fungi in the aid of tribal woman of Madhya Pradesh. Sustainable Forestry; **(1)**:10-15.
Vaidya J. G. and Rabba A. S. (1993). Fungi in Folk Medicine. *The Mycologist.* **7(3)**: 131 -133.
Vaidya J. G. and Bhor G. L., (1991). Medicinal important wood rotting fungi with special emphasis on phansomba. *Deerghayu Int.* **7**:16-19.
Sheena N., Ajith, T. A.., Mathew Thomas A. and Janardhanan, K. K. (2003). Antibacterial Activity of Three Macrofungi, *Ganoderma lucidum*, *Navesporus floccosa* and *Phellinus rimosus* Occurring in South India, *Pharmaceutical Biology*. **41(8)**: 564-567.
Harborne J. B. (1984). Phytochemical Methods. Chapman and Hall, London, *2nd edn., 288.*
Barry A. L. (1986). Procedure for testing antimicrobial agents in agar media, Antibiotics in laboratory medicine. Second edition, *published by Williams and Wilkins, U.S.A,. 1-26.*
Hirotani M., Ino C., Hatano A., Takayanagi H. and Furuya T. (1995). Ganomastenols A, B, C and D, cadinene sesquiterpenes, from *Ganoderma mastoporum*. *Phytochemistry*. **40 (1)**:161 –165.