ISSN 0976-7126

INTERNATIONAL JOURNAL OF PHARMACY & LIFE SCIENCES

Antimicrobial activity of terpenoid extracts from *Ganoderma* samples

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Abstract

Nine species of *Ganoderma,* collected from Western Ghats of Maharashtra (INDIA), were screened for antimicrobial activity using three different terpenoid (sesquiterpene, diterpene and triterpene) extracts. Seven standard antibiotics were also screened for their activity against the test organisms. The assays were performed using the well assay method. The sesquiterpenoid extracts were highly active against both gram positive, negative bacteria and *Candida albicans*, as compared to that of the standard antibiotics, where as triterpenes and diterpenes showed moderate to weak activity. *Candida albicans* showed resistance against diterpene and triterpene extract. From the different terpenoid extracts of *Ganoderma* samples, sesquiterpenoid extract showed antibacterial activity. *Ganoderma* samples especially *G. lucidum, G. chalceum*, and *G. stipitatum* might be potential source of antimicrobial agent

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

Introduction

Ganoderma is a medicinal mushroom contains large number of bioactive compounds posses medicinal properties like Anticancer, Antimalarial, and Antidiabetic etc. This mushroom is commonly used in the traditional Chinese medicine for various diseases. It is a well studied member of aphyllophorales and is known to possess various pharmacological properties and high commercial potential. The research has been restricted to few species like *G. lucidum, G. applanatum*, *G. tsugae*, *G. japonicum*, *G. capense*, *G. resinaceum G. annulare* and *G. pfeifferi* [1, 2, 3].

The major bioactive components of *Ganoderma* are recognized to be triterpenes and polysaccharides while minor as sterols, nucleic acid derivatives, alkaloids, tannins etc [4]. An array of bioactivities, using crude and/or pure compounds from *G. lucidum* has been reported [4, 5, 6, 7].

There are few reports of antimicrobial assay using various extracts from *Ganoderma*. The aqueous extracts of *G. lucidum* were screened against fifteen species of bacteria, *in vitro* [8]. Two farnesyl hydroquinones (Ganomycins A and B) were isolated and characterized from *Ganoderma pfeifferi* having antibacterial properties[9]. Methanolic extracts of seven *Ganoderma* species (unidentified) were screened against *B. subtilis* [www.kyotan.com/lectures/lectures/Lecture12.html]. Eight compounds including sterols and triterpenes from *Ganoderma annulare* were reported to possess antifungal activity [10].

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ISSN 0976-7126

In India *Ganoderma* is reported to be used against cataract, asthma, hydrocele by the tribal people from central India [11, 12]. It has been regularly used as anti inflammatory and to cure tumors, while it is regularly found as an adulterant in another folk medicine called Phansomba (*Phellinus* species) in the western Maharashtra, INDIA [13, 14]. The Indian species of *Ganoderma* have not been assessed for their bioactivities except a recent report on antibacterial activity of *Ganoderma lucidum*, *Navesporus floccosa*, *Phellinus rimosus* and prevention of nephrotoxicity, using crude extracts[15]. The present study, for the first time reports the antimicrobial activity of nine local species of *Ganoderma*.

Material and methods

Mushrooms

The basidiocarp of *Ganoderma stipitatum* (Murrill) Murrill (**GA - 7**); *G.* species -1 (**GA - 11**); *G. multiplicatum* (Mont.) Pat. (**GA - 12** & **GA - 27**); *G. lipsiense* (Batsch) Atk. (**GA - 19**); *G. multicornum* Ryvarden (**GA - 28**); *G. resinaceum* Boudier. (**GA - 36**); *G. praelongum* Murrill. (**GA - 37**); *G. lucidum* (Curtis: Fr.) P. Karst. (**GA - 38**); *G. chalceum* (Cooke) Steyaert (**GA - 39**) were collected from various localities in and around Pune city. The specimens were identified using morphological characters and submitted to the herbarium at department of Botany, University of Pune, INDIA.

Extraction

The samples were oven dried at 45- 50°C and powdered using a commercial mill with 100 mesh size, this powder was used further for extractions.

Total Triterpenes:

The powder (5gm) was defatted (with diethyl ether 100ml) this defatted powder was extracted in methanol (100ml × 2) with repeated heating or by refluxing [16, 4]. The method was modified, by adding ethyl acetate (1:1v/v) to the concentrated methanol extracts ($1/10^{th}$ of the volume, under vacuum). Further the residue was collected, weighed, dissolved in methanol : ethyl acetate (1:1v/v) so as to achieve 50µg/µl concentration for the assay (yield: 15.6 to 28%). The triterpenes were confirmed by TLC as mentioned by Harborne[16].

Sesquiterpenes:

In the present study, method mentioned by Harbone[16] was followed for extraction of sesquiterpenes. The sesquiterpenes were confirmed by TLC as mentioned by Harborne [16].

Diterpenes:

The method given by Harborne [16] was followed for extraction of diterpenes. The diterpenes were confirmed by TLC as mentioned by Harborne [16].

Bioassay

The different terpenoid extracts as given above were assessed for its anti microbial activity against seven human pathogenic microorganisms (Table 1). Seven standard antibiotics (Tetracyclin, Trimetaprim sulfumetoxazol, Nitrofurantoin, Gentamycin, Penicillin, Amikacin) and one antifungal compound, Flucanazole, were screened separately for the comparison with the different terpenoid extracts.

Preparation of Inocula. A loopful of freshly isolated colonies was suspended in 0.85% saline and/or sterile distilled water. The turbidity of the resultant suspension was adjusted by comparing with McFralands No. 1 standard. The well assay method was followed as mentioned by Barry[17].

Results and Conclusion

The activity for inhibition up to 10mm zone was considered weak, 11 to 20 mm was considered moderate, 21 to 30mm was considered strong and above 30 mm inhibition was considered extra strong (table 2). The control of all the compounds showed statistically insignificant activity (6-7 mm zone of inhibition) and hence not represented here.The sesquiterpenoid extracts of *G. lucidum, G. chalceum, G. stipitatum and G. lipsiense* showed strong activity against *Staphylococcus aureus*, comparable to that of tetracycline. Triterpene extracts of all the samples showed moderate activity, which was higher than that of Trimetaprim sulfumetoxazol, Nitrofurantoin and Penicillin.

ISSN 0976-7126

Similarly in case of *Acinetobacter calcoaceticus* the sesquiterpene extracts of *G. lucidum*, *G. chalceum* and *G. stipitatum* showed extra strong activity (31.6, 32.33 & 32.67 respectively). Diterpene and triterpene extracts showed moderate activity, the former in a range of 15 to 18 mm inhibition and the latter with 10 to 15 mm inhibition, comparable to that of Tetracyclin and Trimetaprim sulfumetoxazol, while Gentamycin, Penicillin and Amikacin showed weak activity against *Acinetobacter calcoaceticus*.

The sesquiterpenoid extracts of *G. chalceum* and *G. lucidum* exhibited extra strong activity against *Escherichia coli* and *Bacillus subtilis*, whereas *G. stipitatum*, *G. multicornum, G.* species-1 and *G. lipsiense* showed strong activity. All diterpene and some triterpene showed moderate activity while triterpene extracts showed weak activity. The tetracycline showed extra strong activity against *Escherichia coli*, comparable to the sesquiterpenoids, but failed against *Bacillus subtilis*. Trimetaprim sulfumetoxazol was highly active against both the organisms while others showed moderate to weak activity (table 3).

Klebsiella pneumoniae was found to be most resistant organism amongst the studied. The sesquiterpenoid extract from *G. chalceum* and *G. lucidum* only showed strong activity comparable to that of Nitrofurantoin and Amikacin respectively. The diterpene and triterpene extract showed weak to moderate activity.

It was found that the sesquiterpenoid extracts showed strong activity except for *G. lucidum* showed extra strong activity against *Proteus mirabilis*. The triterpene extracts of *G. lipsiense*, *G. multiplicatum* (GA -12 and GA -27) and of *G. multicornum* showed strong activity comparable to that of Nitrofurantoin and Amikacin, whereas the diterpene extracts of all the samples showed moderate activity.

The only pathogenic fungus assessed, *Candida albicans* showed some resistance to triterpene and diterpene extracts, while all the sesquiterpene extracts exhibited strong activity. The standard antifungal compound Flucanazol also exhibited strong activity (table 3).

The different terpenoid extracts of *Ganoderma lucidum* (GA- 38); *G. chalceum* (GA -39); *G. stipitatum* (GA-7) and *Ganoderma* species -1 (GA- 11) showed higher inhibition compared to other studied samples and even to the standard antibiotics.

The sesquiterpene extracts showed strong activity against all tested microorganism (table 2), which was higher or comparable to that of standard antibiotics (Table 5). Diterpene and triterpene extracts showed moderate activity (table 3, 4), the former in a range of 15 mm to 18 mm inhibition and the latter with 10 mm to 15 mm inhibition, comparable to that of Tetracyclin and Trimetaprim sulfumetoxazol, while Gentamycin, Penicillin and Amikacin showed weak activity against *Acinetobacter calcoaceticus*. Triterpene extracts (table 4) of all the samples showed moderate activity, which was higher than that of standard antibiotic-Trimetaprim sulfumetoxazol, Nitrofurantoin and Penicillin (Table 5).

The triterpenes from *Ganoderma* are known to possess various pharmacological activities, but were found moderate antimicrobial agents in the present study. There are no reports on diterpenes from *Ganoderma*, the present study reports moderate to strong activity of the crude diterpenes extracts. There is a single report of four Cadinene type sesquiterpenes from *Ganoderma mastoporum*[18] without any report on bioactivity. The sesquiterpenes extracts of *Ganoderma* samples in the present study were highly active against the gram positive as well as negative bacteria and hence is a broad spectrum antibiotic, while these also showed strong activities against *Candida albicans*.

The resistance of microorganisms to multiple antibiotics has compelled researchers to explore newer natural resources for competent antimicrobial agents. The sesquiterpenes from *Ganoderma* exhibits broad spectrum anti bacterial activity and anti fungal activity. In this event the local species of *Ganoderma*, especially *G. chalceum*, *G. stipitatum* and *G. lucidum* appears as a good source of antimicrobial agent possessing various other pharmacological properties.

ISSN 0976-7126

Table: 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)

ISSN 0976-7126

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)

ISSN 0976-7126

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)					

Acknowledgement

The authors are thankful to Department of Science and Technology, New Delhi, for the financial assistance.

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