



Original Article

In Vitro Antibacterial Activity, Extraction Yield and Qualitative Phytochemical Profile of *Ganoderma lucidum* from the Melghat Region, Maharashtra, India

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Abstract:

The present study evaluated the extraction yield, qualitative phytochemical profile and antibacterial activity of *Ganoderma lucidum* collected from the Melghat region of Amravati District, Maharashtra, India. Dried basidiocarps were extracted using acetone, ethanol, ethyl acetate, methanol and distilled water, and the resulting extracts were evaluated for their extraction yield and major phytochemical groups. Antibacterial activity was assessed against the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus aureus* by the disc diffusion method, with ciprofloxacin used as the reference antibiotic. Distilled water produced the highest extraction yield ($26.84 \pm 0.21\%$), followed by methanol ($19.73 \pm 0.14\%$), ethanol ($15.42 \pm 0.11\%$), acetone ($7.26 \pm 0.06\%$) and ethyl acetate ($5.89 \pm 0.05\%$). Qualitative screening revealed variable occurrence of flavonoids, phenols, saponins, steroids, tannins and terpenoids, whereas alkaloids were not detected in any extract. The ethanol, distilled water and methanol extracts exhibited antibacterial activity against both test bacteria. The methanol extract showed the highest activity against *E. coli* (17.5 mm), whereas the distilled water extract showed the highest activity against *S. aureus* (18.5 mm). The results indicate that *G. lucidum* possesses solvent-dependent antibacterial potential and may represent a promising source of bioactive fungal metabolites.

Keywords: *Ganoderma Lucidum*; Antibacterial Activity; Phytochemical Screening; Extraction Yield; *Escherichia Coli*; *Staphylococcus Aureus*.

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Introduction:

Macrofungi constitute an important source of biologically active metabolites and have attracted considerable attention because of their nutritional, medicinal and pharmaceutical properties. Among medicinal macrofungi, *Ganoderma lucidum* is one of the most extensively studied species and has a long history of use in traditional medicine. Its basidiocarps and other tissues contain diverse groups of biologically active constituents, including polysaccharides, triterpenoids, phenolic compounds, sterols and proteins (Bisen et al., 2010; Cör et al., 2018).

The biological properties of *G. lucidum* are strongly influenced by the chemical composition of its extracts. Solvent polarity plays an important role in determining the amount and nature of compounds recovered from fungal material. Studies have demonstrated that extraction conditions and solvent composition can substantially influence the recovery of triterpenoids and phenolic constituents from *G. lucidum* (Taofiq et al., 2018). Therefore, comparative extraction using solvents of different polarities is useful for preliminary assessment of the chemical diversity and biological potential of the species.

Antibacterial activity of *G. lucidum* has been reported against both Gram-positive and Gram-negative bacteria, although the magnitude of activity varies according to extraction solvent, fungal material, concentration and test organism. The antimicrobial properties of *Ganoderma* have been associated with its diverse groups of secondary metabolites, although the precise mechanisms responsible for the activity of crude extracts are not always established (Cör et al., 2018; Ahmad et al., 2024).

Despite considerable research on *G. lucidum*, studies based on material collected from particular geographical regions remain useful because environmental conditions, substrate and extraction procedures may influence the chemical profile and biological activity of fungal material. The present investigation was therefore undertaken to evaluate the extraction yield, qualitative phytochemical profile and antibacterial activity of *G. lucidum* collected from the Melghat region of Amravati District, Maharashtra, India, against *Escherichia coli* and *Staphylococcus aureus*.

Materials and Methods:

Collection and Identification of Fungal Material:

Basidiocarps of *Ganoderma lucidum* were collected from the Melghat region of Amravati District, Maharashtra, India, during the course of the present mycological investigation. The collected material was documented on the basis of macromorphological and microscopic characters and identified as *Ganoderma lucidum*. Representative specimens were preserved for reference.

Preparation of Fungal Material:

The collected basidiocarps were cleaned to remove adhering debris and woody material and were dried prior to extraction. The dried fungal material was powdered and used for preparation of solvent extracts.

Preparation of Extracts:

Extracts were prepared using five solvents representing different solvent polarities, namely acetone, ethanol, ethyl acetate, methanol and distilled water. The fungal material was extracted according to the



experimental extraction procedure used in the present study. The obtained extracts were concentrated, and the dried extract mass was recorded. Extraction yield was calculated as the percentage of dried extract recovered relative to the initial dry fungal material.

Determination of Extraction Yield:

Extraction yield was calculated using the following formula:

$$\text{Extraction yield (\%)} = \left(\frac{\text{Weight of dried extract}}{\text{Weight of dried fungal material}} \right) \times 100$$

The yield of each solvent extract was recorded and expressed as percentage.

Qualitative Phytochemical Screening:

The different extracts of *G. lucidum* were subjected to preliminary qualitative phytochemical screening for alkaloids, flavonoids, phenols, saponins, steroids, tannins and terpenoids. The tests were based on standard qualitative procedures. The intensity of the reactions was recorded as strongly present (++), present (+), weakly present (±) or absent (–).

Test Organisms:

The antibacterial activity of the fungal extracts was evaluated against *Escherichia coli* and *Staphylococcus aureus*. These organisms

represent Gram-negative and Gram-positive bacteria, respectively.

Antibacterial Assay:

The antibacterial activity of the extracts was evaluated by the disc diffusion method. Extract-impregnated discs were tested against the selected bacterial cultures under the experimental conditions followed in the present study. Ciprofloxacin was used as the positive control. After incubation, antibacterial activity was determined by measuring the diameter of the zone of inhibition around the discs. The results were recorded in millimetres (mm).

Results:

Extraction Yield:

The extraction yield of *G. lucidum* varied markedly among the five solvents (**Table 1**). Distilled water produced the highest yield of $26.84 \pm 0.21\%$, followed by methanol with $19.73 \pm 0.14\%$. Ethanol yielded $15.42 \pm 0.11\%$, whereas acetone and ethyl acetate produced comparatively lower yields of $7.26 \pm 0.06\%$ and $5.89 \pm 0.05\%$, respectively.

The highest mass of dried extract was obtained from the distilled-water extract (13.42 ± 0.11 g), while the lowest was obtained from the ethyl acetate extract (2.94 ± 0.03 g).

Table 1. Extraction yield (%) of *Ganoderma lucidum* using different solvents

Sr. No.	Solvent extract	Mass of dried extract (g)	Percentage yield (%)
1	Acetone	3.63 ± 0.03	7.26 ± 0.06
2	Ethanol	7.71 ± 0.05	15.42 ± 0.11
3	Ethyl acetate	2.94 ± 0.03	5.89 ± 0.05
4	Methanol	9.86 ± 0.07	19.73 ± 0.14
5	Distilled water	13.42 ± 0.11	26.84 ± 0.21



Qualitative Phytochemical Profile:

The qualitative phytochemical profile of *G. lucidum* extracts is presented in **Table 2**. Alkaloids were not detected in any of the five extracts. Flavonoids were weakly detected in acetone, ethyl acetate and distilled-water extracts, while they were present in ethanol and methanol extracts. Phenols were detected in all extracts, with strong reactions in ethanol and methanol.

Saponins were weakly present in acetone and distilled-water extracts and present

in ethanol and methanol extracts; they were absent from the ethyl acetate extract. Steroids were weakly detected in acetone, present in ethanol and ethyl acetate, strongly present in methanol and absent in distilled water. Tannins showed weak reactions in acetone and ethyl acetate and were present in ethanol, methanol and distilled water. Terpenoids were present in acetone, ethanol, ethyl acetate, and methanol, while they showed a weak reaction in distilled water.

Table 2. Qualitative phytochemical profile of *Ganoderma lucidum* extracts

Sr. No.	Phytochemical	Acetone	Ethanol	Ethyl acetate	Methanol	Distilled water
1	Alkaloids	—	—	—	—	—
2	Flavonoids	±	+	±	+	±
3	Phenols	+	++	+	++	+
4	Saponins	±	+	—	+	±
5	Steroids	±	+	+	++	—
6	Tannins	±	+	±	+	+
7	Terpenoids	+	++	+	+	±

Legend: ++ strongly present; + present; ± weakly present; — absent.

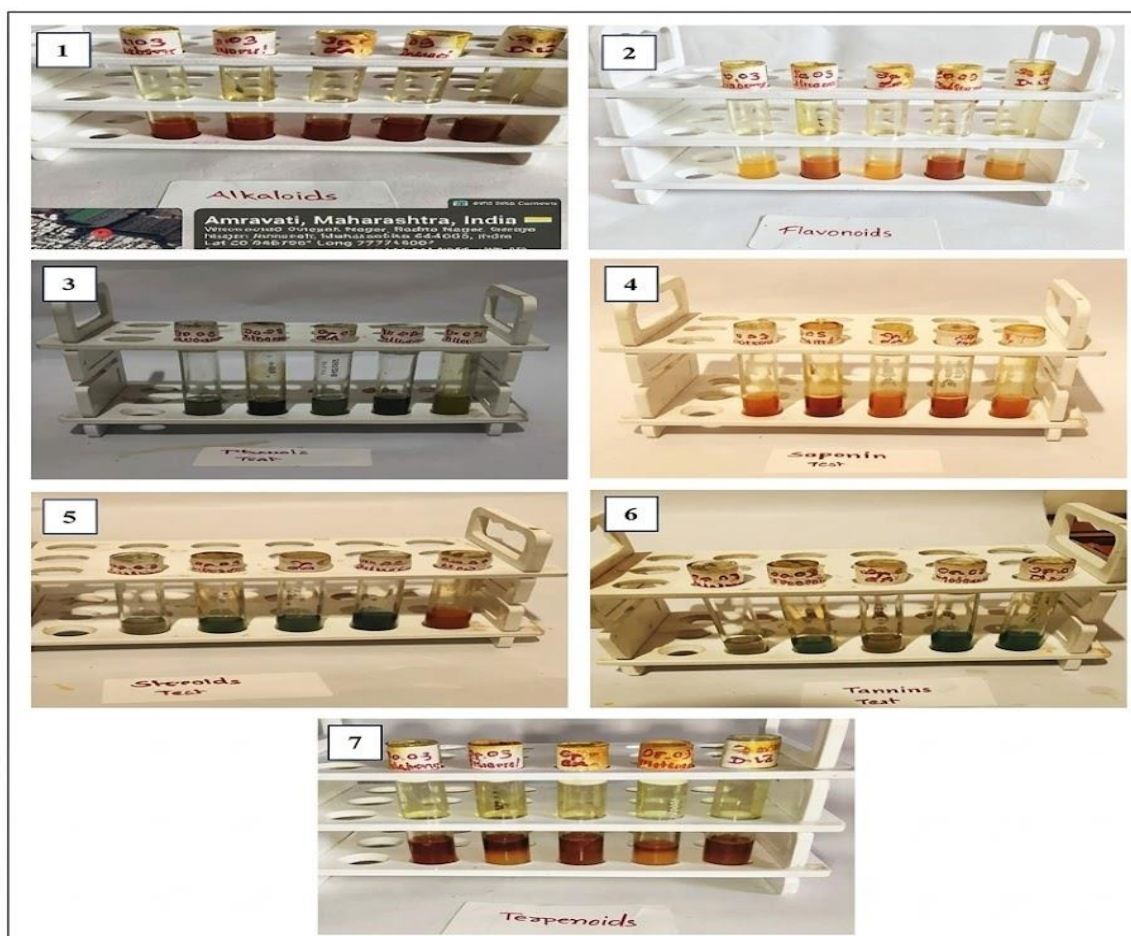


Fig. 1 Phytochemical analysis of *Ganoderma lucidum*

1. Test for Alkaloid 2. Test for Flavonoid 3. Test for Phenols
 4. Test for Saponins 5. Test for Steroids 6. Test for Tannins 7. Test for Terpenoids

Antibacterial activity against *Escherichia coli*:

The antibacterial activity of *G. lucidum* extracts against *E. coli* is presented in **Table 3**. No inhibition was recorded for the acetone and ethyl acetate extracts. The ethanol extract

produced an inhibition zone of 16.0 mm, while the distilled-water extract produced a zone of 12.5 mm. The methanol extract exhibited the largest inhibition zone among the fungal extracts, measuring 17.5 mm. Ciprofloxacin produced an inhibition zone of 21.0 mm.

Table 3. Antibacterial activity of *Ganoderma lucidum* extracts against *Escherichia coli*

Fungal species	Acetone	Ethanol	Distilled water	Ethyl acetate	Methanol	Ciprofloxacin
<i>Ganoderma lucidum</i>	–	16.0	12.5	–	17.5	21.0

Values represent inhibition-zone diameter in mm; – = no detectable inhibition.

Antibacterial activity against *Staphylococcus aureus*:

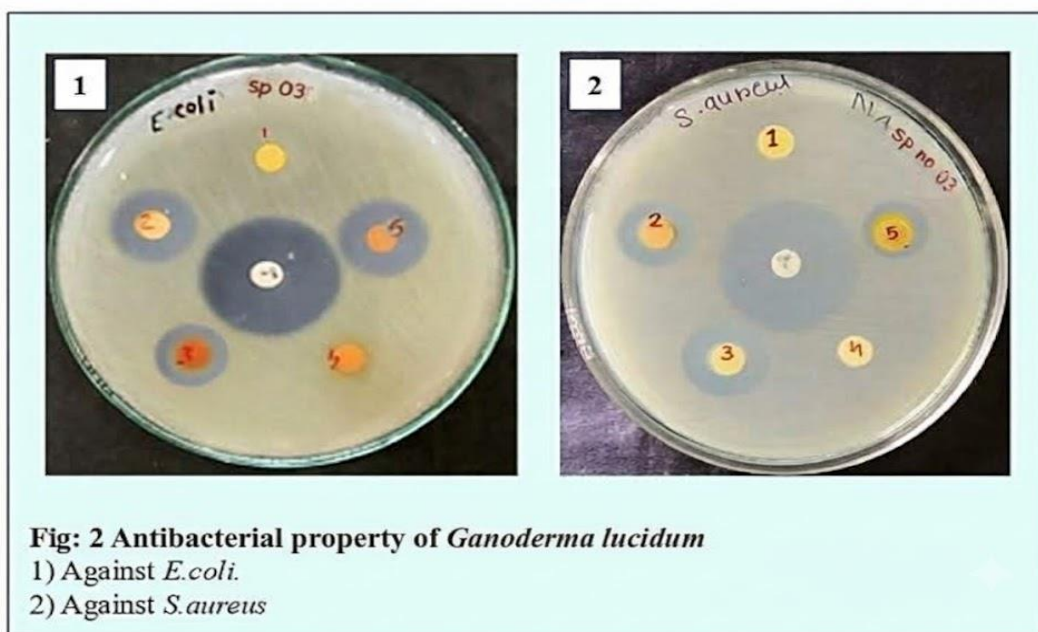
Table 4 presents the antibacterial activity against *S. aureus*. The acetone and ethyl acetate extracts showed no detectable inhibition.

Ethanol and distilled-water extracts produced inhibition zones of 18.0 and 18.5 mm, respectively. The methanol extract produced an inhibition zone of 15.8 mm. Ciprofloxacin showed a zone of 21.0 mm.

Table 4. Antibacterial activity of *Ganoderma lucidum* extracts against *Staphylococcus aureus*

Fungal species	Acetone	Ethanol	Distilled water	Ethyl acetate	Methanol	Ciprofloxacin
<i>Ganoderma lucidum</i>	–	18.0	18.5	–	15.8	21.0

Values represent inhibition-zone diameter in mm; – = no detectable inhibition.



Discussion:

The present study demonstrated considerable variation in extraction yield among the solvents used for *Ganoderma lucidum*. Distilled water produced the highest yield (26.84%), followed by methanol (19.73%) and ethanol (15.42%), whereas ethyl acetate produced the lowest yield (5.89%). The higher aqueous yield may be associated with the recovery of water-soluble constituents, particularly polysaccharides, which are important components of *G. lucidum* (Bisen et

al., 2010; Cör et al., 2018). Solvent polarity and extraction conditions can influence the recovery of different groups of fungal metabolites (Taofiq et al., 2018).

Qualitative phytochemical screening showed variation among the extracts. Phenols were detected in all extracts, with strong reactions in ethanol and methanol, while terpenoids were detected in all extracts except that only a weak reaction was observed in distilled water. Flavonoids, saponins, steroids and tannins showed variable occurrence among



the solvents. Alkaloids were not detected in any extract. These findings are consistent with the reported chemical diversity of *G. lucidum*, which includes phenolic compounds, terpenoids, steroids and other bioactive constituents (Bisen et al., 2010; Cör et al., 2018).

The antibacterial assay revealed solvent- and organism-dependent activity. Against *Escherichia coli*, the methanol extract showed the highest inhibition zone (17.5 mm), followed by ethanol (16.0 mm) and distilled water (12.5 mm), while acetone and ethyl acetate showed no detectable activity. Against *Staphylococcus aureus*, the distilled-water extract showed the highest inhibition zone (18.5 mm), followed by ethanol (18.0 mm) and methanol (15.8 mm). Ciprofloxacin produced inhibition zones of 21.0 mm against both bacteria.

The observed antibacterial activity agrees with previous reports describing antibacterial properties of *G. lucidum* extracts against Gram-positive and Gram-negative bacteria. Differences in activity among extracts may be related to solvent polarity, fungal origin, extraction conditions and the composition of the recovered metabolites. Importantly, the highest extraction yield did not correspond to the highest antibacterial activity, indicating that extraction yield alone cannot predict biological activity.

The antibacterial effects observed in the present study may result from the combined action of different groups of fungal metabolites. However, the qualitative phytochemical tests used here do not establish the identity or contribution of individual compounds. Further studies involving minimum inhibitory concentration, minimum bactericidal concentration and chromatographic

characterization are required to identify the active constituents and confirm the antibacterial potential of *G. lucidum*.

Conclusion:

The present investigation demonstrated that *Ganoderma lucidum* from the Melghat region possesses solvent-dependent extraction characteristics, diverse qualitative phytochemical profiles and measurable antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Distilled water produced the highest extraction yield, whereas methanol exhibited the greatest activity against *E. coli*. Against *S. aureus*, distilled water produced the largest inhibition zone. Acetone and ethyl acetate extracts showed no detectable antibacterial activity against either test organism under the conditions employed. The results suggest that *G. lucidum* represents a promising source of antibacterial fungal extracts. Further studies involving quantitative phytochemical estimation, MIC/MBC determination and chemical characterization of active extracts are required to establish the constituents responsible for the observed activity.

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