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# ESTIMATION OF TRI-TERPENOIDS FROM *GANODERMA LUCIDUM* THROUGH THIN LAYER CHROMATOGRAPHY

**Renu Thakur, Pooja Kapoor and B.M. Sharma**
Department of Plant Pathology, CSK HPKV, Palampur
Himachal Pradesh 176062 India
email. poojakapoor1237@gmail.com

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**Local strains of *Ganoderma lucidum* has been analyzed for the appearance of triterpenoids with cultivated *G. lucidum* as reference. Various strains of *G. lucidum* were isolated from different and far regions of Himachal Pradesh during 2010 and 2011. The fruiting bodies were obtained by growing them in sawdust after three months of incubation. Analysis of triterpenoids was done by silica gel thin layer chromatography with eleuntof chloroform: methanol (10: 1) for first extract and dichloromethane for the second extract. Detection of triterpenoids was done by spraying the plate with Carr-Price as well as Lieberman-Burchard reagents then observed under UV light (366 nm). Presence of more number of triterpenoids in local strains suggested that local strains of Himachal Pradesh can be exploited further for cultivation at commercial level.**

*Ganoderma lucidium* known to have some medicinal properties.Several biologically active compounds have been characterized from *Ganoderma lucidum* including adenosine, which is said to have an analgesic effect[1], R, S-ganodermic and ganasterone that have an antihepatoxic effect, and glucans which are responsible for the anti-inflammatory and antitumor properties. The most important pharmacologically active constituents are polysaccharides and triterpenoids[2]. Triterpenoids have been reported to possess hepatoprotective, hypocholesterolemic and anti-histaminic effects, anti-hypertensive, anti-tumor and anti-angiogenic activity.

Himachal Pradesh is rich in its mycoflora due to diversity of climatic conditions in this state. Lack of proper studies on locally available abundant basidiocarps of *G. lucidum* hinders us to exploit its potential. The aim of this study was to investigate the medicinal properties of local isolates of *G. lucidum*.Therefore, fruit bodies of *G. lucidum* were collected from natural habitat and analyzed for triterpenoids to see the difference in the bioactive components among local wild and cultivated strains.

## MATERIALS AND METHODS

**Samples:** Different isolates of *Ganoderma lucidum* were collected from the natural habitat during the surveys conducted in different localities of Himachal Pradesh in 2010 and 2011 from the first fortnight of July to second fortnight of September and some of the strains were procured from DMR, Solan. Ten most vigorously growing isolates/strains having highest average mycelial growth/day in their respective morphological and cultural subgroup (GL5, GL8, GL13, GL15, GL18, GL23, GL25, GL28, GL29 and strain OE53) were selected for Triterpenoids estimation.Thin layer chromatography was done on silica gel coated aluminum backed plates[3]

**Analysis for triterpenoids:** Extraction: A fresh fruiting body was washed, then sliced into small pieces and dried in an oven at 50?C before being blended to granules. Twenty five grams of dried granules were extracted with 250 ml wash benzene in soxhlet for 24 hours. Another extraction was done in the same manner by using 250 ml ethanol. Both extracts were concentrated by vacuum evaporation and heating evaporation at 70?C in a rotavapour for about two hours before running the Thin Layer Chromatography.

**Thin layer chromatography:** Detection was conducted by spotting 2 µl of the extract with a micropipette onto a silica gel plate as a stationary phase. The plates were developed in their respective solvent system. The developed plates were dried at room temperature and detection was done by spraying plates with Carr Price at 100 $^0$C for 10 minutes. Developed plates were also detected for desired spots by spraying with another detection reagent Lieberman Burchard at 85-95$^0$C for 15 minutes. The developed spots were then visualized under ultraviolet light (366 nm).

**Table-1. Rf value of non-polar triterpenoids of selected *Ganoderma lucidum* isolates/strain with Carr Price reagent**

| Spot no. | Rf Value | Colour | GL5 | GL8 | GL13 | GL15 | GL18 | GL23 | GL25 | GL28 | GL29 | OE53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.03 | Greenish violet | + | + | + | + | + | + | + | + | - | + |
| 2 | 0.05 | Yellow | - | + | - | + | + | + | + | + | + | + |
| 3 | 0.07 | Brown | + | + | + | + | + | + | + | - | - | + |
| 4 | 0.08 | Blue | + | + | + | + | + | + | + | + | - | + |
| 5 | 0.11 | Yellow | + | + | - | + | + | + | + | + | - | + |
| 6 | 0.15 | Brown | + | + | + | + | + | + | + | + | - | - |
| 7 | 0.26 | Blue | + | + | + | + | + | + | + | + | + | + |
| 8 | 0.30 | Violet | - | + | + | + | + | + | + | + | + | + |
| 9 | 0.34 | Pinkish brown | + | - | - | + | + | + | + | + | - | + |
| 10 | 0.42 | Brown | - | + | + | + | + | + | + | + | + | + |
| 11 | 0.46 | Brown | + | - | + | + | + | + | + | + | - | + |
| 12 | 0.50 | Brown | + | + | + | + | + | + | + | + | + | + |
| 13 | 0.58 | Brown | - | + | - | + | + | - | + | + | - | + |
| 14 | 0.69 | Brown | + | + | - | + | + | - | - | - | + | + |
| 15 | 0.77 | Brown | - | - | - | + | + | + | + | + | + | + |
| 16 | 0.88 | Pinkish brown | + | + | + | + | + | + | + | + | + | + |
| 17 | 0.95 | Violet | + | + | + | + | + | + | + | + | + | + |
| Total no. of spots | | | 12 | 14 | 11 | 17 | 17 | 15 | 16 | 15 | 9 | 16 |

**Table-2. Rf value of non-polar triterpenoids of selected *Ganoderma lucidum* isolates/strain with Lieberman Burchard reagent**

| Spot No. | Rf Value | Colour | GL5 | GL8 | GL13 | GL15 | GL18 | GL23 | GL25 | GL28 | GL29 | OE53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.03 | Pink | + | + | + | + | + | + | + | + | + | + |
| 2 | 0.05 | Pink | - | + | + | - | + | + | - | - | + | + |
| 3 | 0.09 | Pink | - | + | - | + | + | + | + | + | + | + |
| 4 | 0.15 | Pink | + | + | + | + | + | - | + | + | - | + |
| 5 | 0.22 | Pink | + | + | - | + | + | - | + | + | + | + |
| 6 | 0.30 | Pink | + | - | + | + | + | + | + | + | - | + |
| 7 | 0.33 | Pink | - | + | + | - | + | + | + | + | - | - |
| 8 | 1.04 | Pink | + | + | + | + | + | + | + | + | + | + |
| Total no. of spots | | | 5 | 7 | 6 | 7 | 8 | 6 | 7 | 7 | 5 | 7 |

[+ denotes presence of spot, - denotes absence of spot]

**Table-3. Rf value of polar triterpenoids of selected *Ganoderma lucidum* isolates/strain with Carr Price reagent**

| Spot no. | Rf Value | Colour | GL5 | GL8 | GL13 | GL15 | GL18 | GL23 | GL25 | GL28 | GL29 | OE53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.02 | Brown | + | + | + | + | + | + | + | + | + | + |
| 2 | 0.06 | Brown | + | + | + | + | + | + | + | + | + | + |
| 3 | 0.11 | Brown | + | + | + | + | + | + | + | + | + | + |
| 4 | 0.20 | Brown | - | - | - | - | + | + | + | + | + | - |
| 5 | 0.30 | Brown | + | - | - | + | + | - | - | - | - | + |
| 6 | 0.43 | Pink | + | + | + | - | + | - | + | + | + | + |
| 7 | 0.47 | Pink | - | + | - | + | + | + | - | - | + | + |
| 8 | 0.53 | Pink | - | + | - | - | + | + | - | - | - | + |
| 9 | 0.60 | Brown | - | - | - | + | + | + | + | + | + | + |
| 10 | 0.83 | Pink | + | + | + | + | + | + | + | + | + | + |
| Total no. of spots | | | 6 | 7 | 5 | 7 | 10 | 8 | 7 | 7 | 8 | 9 |

[+ denotes presence of spot, - denotes absence of spot]

**Table- 4. Rf value of polar triterpenoids of selected *Ganoderma lucidum* isolates/strain with Lieberman Burchard reagent**

| Spot No. | Rf Value | Colour | GL5 | GL8 | GL13 | GL15 | GL18 | GL23 | GL25 | GL28 | GL29 | OE53 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.20 | Pink | + | + | - | - | + | + | + | + | + | + |
| 2 | 0.31 | Pink | + | + | - | + | + | - | + | + | - | + |
| 3 | 0.34 | Pink | + | + | + | + | + | + | + | + | + | + |
| 4 | 0.42 | Pink | - | + | + | + | + | + | + | - | + | + |
| 5 | 0.58 | Pink | + | + | + | + | + | - | + | + | + | + |
| 6 | 0.60 | Pink | + | + | + | + | + | + | - | - | + | - |
| 7 | 0.83 | Pink | + | + | + | + | + | + | + | + | + | + |
| Total no. of spots | | | 6 | 7 | 5 | 6 | 7 | 6 | 6 | 5 | 6 | 6 |

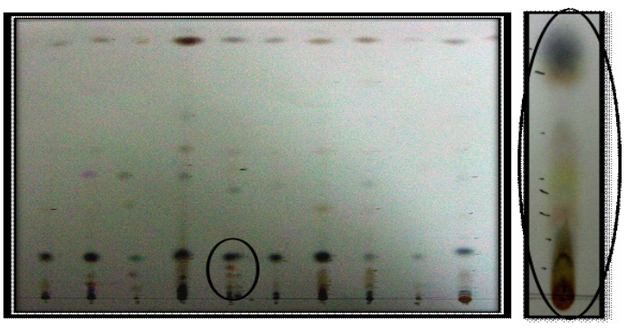
[+ denotes presence of spot, - denotes absence of spot]

**Table-5. Comparative composition of different biochemical constituents in selected *Ganoderma lucidum* isolates/strain**

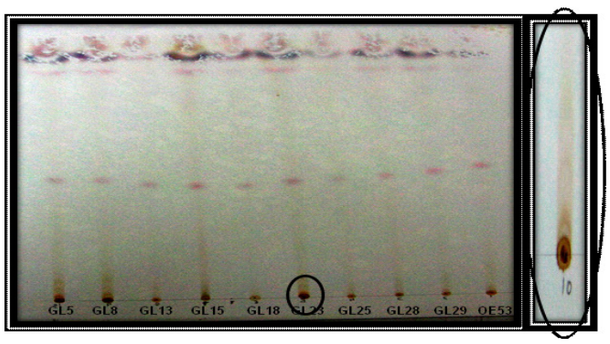
| Isolate | Polar triterpenoids (g/10g)* | Non-polar triterpenoids (g/10g)* | Total triterpenoids (g/10g)* |
|---|---|---|---|
| GL5 | 0.17 | 0.31 | 0.48 |
| GL8 | 0.27 | 0.51 | 0.78 |
| GL13 | 0.11 | 0.39 | 0.50 |
| GL15 | 0.33 | 0.51 | 0.84 |
| GL18 | 0.30 | 0.55 | 0.85 |
| GL23 | 0.12 | 0.42 | 0.54 |
| GL25 | 0.36 | 0.49 | 0.85 |
| GL28 | 0.24 | 0.45 | 0.69 |
| GL29 | 0.15 | 0.29 | 0.44 |
| OE53 | 0.23 | 0.42 | 0.65 |
| CD (5%) | 0.01 | 0.04 | 0.05 |

[*Average of three replications]

**Plate 1. Thin layer chromatogram of triterpenoidsof selected *Ganoderma lucidum* isolates/strain**



**A. Non-polar triterpenoids with Carr Price reagent**




**B. Polar triterpenoids with Carr Price reagent**

**Estimation of triterpenoids:** The dark brown precipitates collected in a weighed round bottom flask were dried in an oven at 50$^0$C for 20 minutes and final weight was taken. The difference between empty pre-weighed beaker and the final dry weighed beaker represented the weight of triterpenoids.

## RESULTS AND DISCUSSION

**Quanlitative estimationof triterpenoids:** The dried powder of fruiting bodies was evaluated by chromatographic technique i.e. thin layer chromatography. First extraction by wash benzene, a non-polar solvent, was able to extract non-polar triterpenoids. Thin layer chromatogram of wash benzene extract sprayed with Carr Price reagent depicted 17 spots (Plate1) representing non-polar triterpenoids having Rf value ranging from 0.03-0.99. Maximum number of non polar triterpenoids were observed in case of isolate GL18 followed by isolate GL15, GL25 and strain OE53 (Table-1).

However, after applying Lieberman Burchard reagent, only 8 non-polar triterpenoids were detected with Rf value varying from 0.03-1.04 (Table-2). Maximum number of non-polar triterpenoids was recorded in isolate GL23 (8 spots) followed by isolates viz. GL8, GL15, GL18, Gl25, GL28 and strain OE53 (7 spots each).

Ethanol extraction separated the polar compounds including glycosidic triterpenoids. Thin layer chromatogram plates of ethanol extract after spraying with Carr Price reagent revealed the presence of 10 polar triterpenoids(Plate 1B) while, Lieberman Burchard reagent detected only 7 polar triterpenoids, having Rf ranging from 0.02-0.83. Maximum number of polar triterpenoids was detected from the fruiting bodies of isolate

GL18 followed by strain OE53 and isolate GL8 (Table -3 and Table -4).

The isolates/strain did not depict similar TLC pattern of triterpenoids which suggested that all the isolates are not the same in triterpenoids composition. It is evident from the data obtained that fruiting bodies of *Ganoderma lucidum* contain more non polar triterpenoids (17 spots) than polar triterpenoids (10 spots), because more number of spots suggests the presence of more number of triterpenoids. Most of the spots had same value of retention factor (Rf) and colour indicating the presence of same triterpenoid. Both reagents reacted with triterpenoids producing various colours ranging from red, yellow, purple and blue with different colour intensity suggesting difference in quantity. Darker spot indicated the presence of more quantity of triterpenoids. Most intense spots were seen with isolate GL15 and GL18 (Plate 1A). Although both Carr Price and Lieberman Burchard reagent react with triterpenoids producing various colours ranging from blue, purple and red, yet Carr Price was better in detecting triterpenoids diversity[4]. The presence of 8 triterpenoids (Rf 0.05-0.90) was detected using thin layer chromatography[3]. TLC was applied for the differentiation of *G. lucidum* species[5] and unique triterpene patterns were reported. The presence of triterpenoidswas detected in the mycelial extract of *G. lucidum* and it was found that all the separation spots of the isolates had an Rf value below 0.92[6].

**Quantitative estimationof triterpenoids:** Quantitative estimation of polar and non-polar triterpenoids from the dried fruiting bodies of *G. lucidum* isolates was done and results showed significant differences in the triterpenoids content among isolates/strain (Table-5). The total triterpenoids content of 0.85 g/10g dried powder estimated in isolate GL18 was highest and statistically at par with the content estimated in isolate GL15 and GL25, whereas, the lowest content was found in isolate GL5 (0.48 g/10g dried powder). The difference in triterpenoids content of various isolates might be due to the difference in natural substratum from which they were collected or due to difference in their ability to produce metabolite. The metabolite production was influenced by the way; culture is maintained[7]. The dried fruit bodies of *G. lucidum* isolates was analysedfor their triterpenoids content and was found to vary from 0.29-0.61g/10g dried powder[8].

Qualitative analysis through thin layer chromatography revealed that Carr Price reagent found to be better for detecting triterpenoids as compared to Lieberman Burchard reagent. Seventeen non-polar and ten polar triterpenoids were detected with Carr Price reagent. Quantitative estimation revealed that the triterpenoid of selected isolates varied from 0.44-0.85g dried powder. Among selected isolates/strain, GL18 possessed the highest amount of total triterpenoids, indicating the better medicinal properties as compared to others.

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