Medicinally important terpenoids of *Ganoderma lucidum* from Uttarakhand, India

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Since ancient times, *Ganoderma lucidum* has been acclaimed to be a divine herb in China and Japan that could bestow youth, vigor and longevity (Hirotani *et al.,* 1987). The fruiting bodies of this fungus possess a wide spectrum of medicinal properties and are in use since ages in Traditional Chinese Medicine (Wasser and Weis, 1999). The medicinal properties are attributed to the presence of various polysaccharides and terpenoids in the fungus (Zhou and Gao, 2002). About 80 terpenoids including ganoderic acid derivatives have been isolated from the fruitbodies and the mycelia of *G. lucidum* (Hirotani *et al*., 1985; Sato *et al*., 1986; Nishitoba *et al*., 1986; Hirotani *et al*., 1987; Lin *et al*., 1988; Shiao *et al*., 1988; Nishitoba *et al*., 1989; Tokuyama *et al*., 1990; Hirotani and Furuya, 1990; Min *et al*., 2000). Some of them have shown cytotoxicity against hepatoma cells *in vitro* (ganoderic acids U, V, W, X and Y), anti-histamine releasing activity in rat mast cells (ganoderic acids C and D), inhibitory activity against angiotensin converting enzyme (ganoderic acid F), hepatoprotective activity (ganoderic acid A) and an inhibitory effect on farnesyl protein transferase (ganoderic acid A and methyl ganoderate A) (Min *et al.*, 2000). The present investigation is aimed to identify the presence of the ganoderic acids A, C2 and H from the methanolic extract of dried fruiting bodies of indigenous *Ganoderma lucidum*, performing High Performance Liquid Chromatography.

The fruiting bodies of *Ganoderma lucidum* collected from Pantnagar (Distt. Udham Singh Nagar), Haldwani (Distt. Nainital) and Ranikhet (Distt. Almora) of Uttrakhand were identified and designated as Gl-1w, Gl-2w and Gl-3w. The fungus was isolated using malt extract agar medium. The pure culture of each isolate was used to prepare master and commercial spawn (Garcha, 1984). Commercial spawn was further used for cultivation of *Ganoderma lucidum* using different agro-wastes (Mishra and Singh, 2006). Fruiting bodies obtained were designated as Gl-1c, Gl-2c and Gl-3c.

Fruiting bodies were shade-dried and grounded into powder by a mechanical grinder. The materials were soaked into methanol for 96 h by replacing the solvent at regular interval of 24 h. The extract was filtered through a muslin cloth. All the filtrates were pooled and vacuumed dried at 40±1°C. Residue was partitioned between chloroform and water. This process was repeated 3- 4 times to ensure maximum extraction. Chloroform fraction was made alkaline using aqueous sodium bicarbonate, whereas, water fraction was acidified to pH 3-4 with 6M hydrochloric acid (HCl). The precipitate was dissolved in alkaline chloroform fraction and was dried under vacuum at 45±1°C. Residue was re-dissolved in mobile phase (methanol) and subjected to HPLC. For comparison, standard Ganoderic acids A, C2 and H were also taken. The extracts and standards were filtered through 0.22 µ PTFE filter prior to injection.

Spectrum was taken at $\ddot{e}_{max}$=254nm and the retention time were noted for all the three Ganoderic acids. The results showed that retention time for standard Ganoderic acids A, C2 and H were 4.2, 3.9 and 4.4 minutes, respectively (Fig.1). The patterns obtained on HPLC analysis of the methanolic extracts of the fruiting bodies of wild and cultivated isolates of *Ganoderma lucidum* revealed the presence of all the three medicinally important terpenoids, namely, Ganoderic acid A, C2 and H (Fig. 2 & 3) comparing with their respective standards. These observations are in accordance with Hirotani and Furuya (1990) who reported the presence of Ganoderic acids A, B and H among the fruit bodies of *Ganoderma lucidum*. Min *et al.* (2008) identified the presence of Ganoderic acid C2 from methanolic extract of the spores of *Ganoderma lucidum*. The presence of Ganoderic acids C and H were also identified by Nishitoba *et al.* (1987) from the fruiting body of *Ganoderma lucidum* using spectroscopic and chemical methods. Spectra also revealed other small and large peaks of other biological compounds, which could not be identified and needs further study for identification. Since Ganoderic acids were present in all the isolates studied, these isolates can be exploited for the formulation of commercial health products.

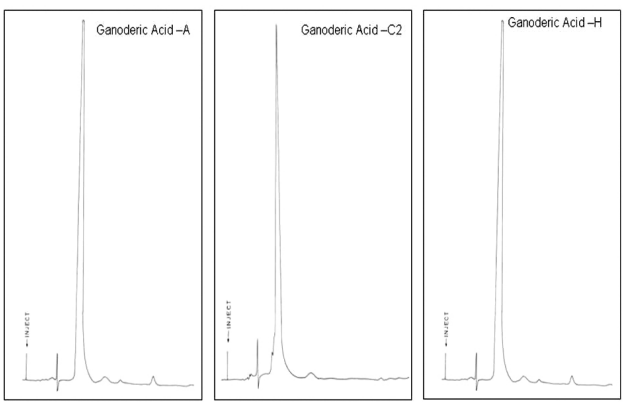

Fig. 1. HPLC Spectra of standard Ganoderic Acids

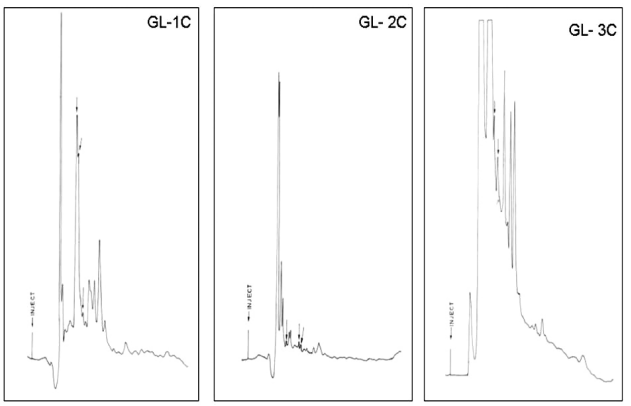

Fig. 2. HPLC Spectra of cultivated isolates of *Ganoderma lucidum*

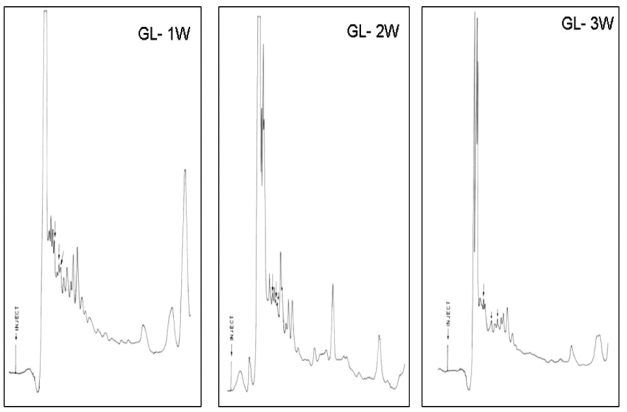

Fig. 3. HPLC Spectra of wild isolates of *Ganoderma lucidum*

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