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Effect of Storage Temperature on Bio-efficacy of Aqueous Extract of *Ganoderma lucidum*

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ABSTRACT

Stability testing is key requirement in the drug development process along with storage temperature which may deteriorate bio-constituents and efficacy of natural products. Aqueous extract of *Ganoderma lucidum* has revealed pharmacological effects against high altitude stressors and has potential for mitigating high altitude maladies. In the present study, the extract of *Ganoderma lucidum* was stored at different storage conditions such as room temperature, 4 °C and -20 °C for two year and qualitative and quantitative analysis of bio-constituents and bio-efficacy was carried out. No significant change was observed in any extract kept in different temperature conditions in terms of its polysaccharide, phenolic and flavonoids content. The extract kept at room temperature absorbed slight moisture in few samples but no change in overall polysaccharide, phenolic and flavonoids content was recorded. The moisture absorption problem was not observed in extracts stored at 4 °C and -20 °C. The bio-efficacy of the extract at room temperature, 4 °C or -20 °C were comparable to the freshly prepared extract and the data from the studies suggest that extract has good shelf life up to two year without loss of bio-efficacy. Overall, the extract retained its bio-efficacy for two years at different temperature storage conditions.

Keywords: Ganoderma lucidum; Shelf life; Stability; Phytochemical assays; Cell viability

1. INTRODUCTION

Exposure to adverse climatic conditions or stressors in organisms may cause pathological changes and beyond threshold stress limits, alterations in physiological functions appear. At high altitudes soldiers are exposed to adverse climatic conditions such as hypobaric hypoxia and suffer from symptoms of acute mountain sickness resulting in decreased physical and mental performance. A number of biologically active extracts derived from herbal plants growing at high altitudes have been reported to enhance stress tolerance and helps in faster adaptation in experimental animals[1-2]. However, in lack of specific drugs to alleviate high altitude pathologies, there is requirement for development of herbal agents to induce faster adaptation in high altitude conditions.

Mushrooms have been part of the normal human diet and reported to be effective in promoting quality life and prevention of age related and life style disorders[3-5]. Many bio-active compounds of mushrooms such as gallic acid, caffeic acid and its derivatives, quercetin, vanillin etc. can act as anti-oxidants[6-8]. *Ganoderma lucidum* (Fr.) Karst is one of such medicinal mushroom having distribution in tropical and temperate geographical regions[9]. In India, *Ganoderma lucidum* grows naturally at an altitude of over 4000 m of the North-West Himalayas at different locations of Uttarkhand, India. Further, in Southern parts of India, the presence of *Ganoderma lucidum* also has been recorded[9]. *Ganoderma lucidum* biosynthesizes phytochemicals including polysaccharides, adenosine, alkaloids, coumarin, ergosterols, ganoderic acids, lactones, mannitol, organic germanium, triterpenoids and rare minerals[10-11] that are linked to its numerous medicinal properties such as improving blood circulation, eliminates fatigue, strengthen immune system, enhance energy, discard toxins, anti-cancer property, effective in immunological disorders, heart disease and infection[12-20]. Our previous studies has demonstrated that aqueous extract of *Ganoderma lucidum* possess anti-stress, anti-inflammatory and endurance enhancing potential and further, prevent transvascular leakage in rat lung and has cardio-protective effects[21-22]. The findings from these provide rational for development of prophylactics/therapeutics agent based on aqueous extract of *Ganoderma lucidum* to offset high altitude maladies. However, drug stability is key issue that needs to be addressed in any drug development process and hence present investigations were carried out. The effect of storage temperature, if any, on qualitative and quantitative analysis of bio-constituent and efficacy of extract was studied up to two year.

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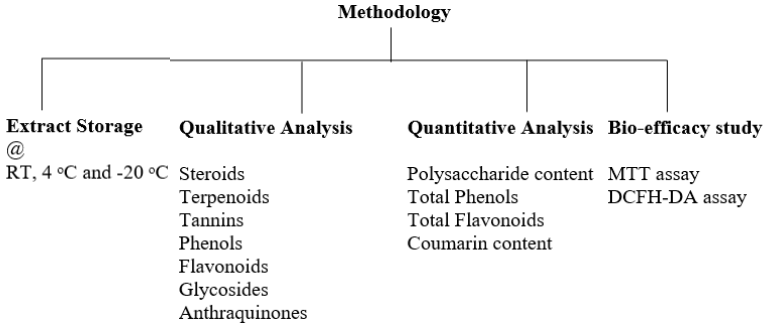
2. METHODS AND MATERIALS



2.1 Collection of Plant Material

Reddish brown matured fruiting bodies of *Ganoderma lucidum* was collected from Pithoragarh, Uttrakhand, India (North-West Himalayas, ~ 4000 m altitude) from wood logs and tree stumps in rainy season. An Ethano-botanist, Dr. Mousin, Defence Institute of Bio Energy Research (DIBER), Haldwani, India characterised collected study material (voucher specimen DIP-GL/2014). The mature fruiting bodies of *Ganoderma lucidum* was washed with nanopure water and milled into powder after drying.

2.2 Extraction Procedure

Aqueous extract of *Ganoderma lucidum* was prepared using Accelerated Solvent System ASE 350 equipped with a solvent controller unit (Dionex Corporation, CA, USA). A 15 min cycle was used for every extraction performed at room temperature. A rinse cycle was performed between extraction cycles to clear any previous extraction leftover. Finally, the supernatant solution was lyophilised (Allied frost, India) and the dried extract was stored at 4 ºC.

2.3 Qualitative Analysis

As described elsewhere[23] phytochemical analysis was performed on extract of Ganoderma as follows:

2.3.1 Steroids

2 ml acetic anhydride was added to 500 mg aqueous extract of *Ganoderma lucidum* and incubated on ice for 15 min and gradually sulphuric acid was added and color change was recorded. A change in colour to blue-green from violet shows presence of steroid in extract.

2.3.2 Terpenoids

The aqueous extract of *Ganoderma lucidum* 0.5 g was added 2 ml chloroform and then sulphuric acid was later added to form a lower layer. The presence of terpenoids was confirmed by reddish- brown colour developed at the interface.

2.3.3 Tannins

The aqueous extract of *Ganoderma lucidum* 5 g was stirred in 10 ml distilled water and filtered with Whattman's filter paper and then ferric chloride reagent was then added to this filtrate and formation of precipitate of blue-black or blue-green color show presence of tannin.

2.3.4 Phenols

The aqueous extract of *Ganoderma lucidum* 50 mg was dissolved in 5 ml distilled water and then 2-5 drops of neutral 5 per cent ferric chloride solution was added. The appearance of dark green coloration confirmed presence of phenol.

2.3.5 Flavonoids

A solution of aqueous extract of *Ganoderma lucidum* (2.5 %) was prepared in distilled water and then 2-5 drops of 10 N sodium hydroxide solution were added. Flavonoid presence can be detected with development of yellow colour.

2.3.6 Glycosides

The aqueous extract of *Ganoderma lucidum* 5 ml (10 mg/ml) was mixed with 5 ml of 25 per cent H_2SO_4 and was boiled in water bath for 15 mins. After 15 min of boiling, 5 ml Fehling's solution (5 ml) was added to this boiled mixture. Presence of steroidal ring of glycosides was confirmed with development of reddish brown colour.

2.3.7 Anthraquionones

The aqueous extract of *Ganoderma lucidum* 5 g was mixed with 5 ml benzene and shaken to properly dissolve the extract. After this 5 ml of 10 per cent ammonia solution was added to the mixture and shaken. Development of pink, red, or violet coloration in lower ammonical layer indicates the presence of free hydroxyl anthroquionones.

2.4 Quantitative Analysis

Quantification of total polysaccharide, phenol, flavonoid and coumarin was carried out in extract of Ganoderma.

2.4.1 Determination of Total Polysaccharides Content

The total polysaccharide content of aqueous extract was estimated using phenol-sulphuric acid method using D-glucose as standard as mentioned elsewhere[24]. Briefly, 1 ml of extract (10 mg/ml) solution was mixed with 1 ml of 5 per cent phenol solution and 5 ml of concentrated sulphuric acid and mixture was kept for 30 min with shaking and finally was read at 490 nm and sugar content was measured using glucose standard curve. All experiments were repeated three time and data has been expressed in percentage.

2.4.2 Determination of Total Phenol Content

The total phenolic content in extract was estimated using 20 µl of stock solution (1 mg/ml) of the extract, 80 µl of water and 500 µl of Folin–Ciocalteu reagent as described elsewhere[25]. After 5 min incubation in the dark at room temperature, 400 µl of 7.5 per cent sodium carbonate solution was added and mixture incubated for 30 min at room temperature (in dark) and finally absorbance was recorded at 765 nm. Total phenols (mg/g) in the extract were expressed as gallic acid equivalent (GAE), estimated from standard curve prepared from gallic acid (0.1 mg/ml) solution.

2.4.3 Determination of Total Flavonoid Content

Aluminium chloride method was used for estimation of total flavonoid content as described elsewhere[26] using rutin as a standard. 1 ml extract was added to 4 ml distilled water and subsequently 0.3 ml of 5 per cent $NaNO_2$ solution was added. After 5 min, 0.3 ml of 10 per cent $AlCl_3$ solution was added and allowed to stand for 5 min, then 0.2 ml of 4 per cent NaOH solution was added to this mixture and finally distilled water was used to make volume to 10 ml and absorbance at 510 nm in spectrophotometer. The flavonoid content was expressed as mg/g of extract it terms of rutin equivalent.

2.4.4 Determination of Coumarin

Coumarin was quantified in aqueous extract of *Ganoderma lucidum* using HPLC analysis (Waters Corporation, USA) equipped with Waters 515 HPLC pump, Waters 717 plus autosampler, and Waters 2487 UV detector. Separation was performed in a symmetry C18 250 mm × 4.7 mm ID, 5 μm column (Waters, USA) by maintaining the isocratic flow rate (1 mL/min) of the mobile phase A. 0.01 M KH2PO4 pH 3.7 and B. Methanol 90A:10B.

2.5. Biological Assays

The retention of bio-efficacy of extract was established using cell viability and reactive species levels *in vitro*.

2.5.1 Mitochondrial Integrity MTT Assay

Cell viability was measured using MTT assay through reduction of mitochondrial enzyme succinate dehydrogenase as described elsewhere[27]. Briefly, MTT was dissolved in culture medium and filtered to remove a small amount of insoluble residue. Cell culture medium containing MTT at a concentration of 0.5 mg/ml was added to each well having adherent HT22 cells in a total 200 µl medium and then incubated for 3 h. After incubation culture supernatant was aspirated and 100 µl of DMSO was added to dissolve formazan. Finally, absorbance was read at 570 nm following 30 min of incubation at room temperature (Power Wave XS2, BioTek, USA).

2.5.2 Assay for Redox State

A fluorescent dye, H2-DCFH-DA was used to evaluate intracellular redox state. Briefly, cells medium was replaced with HBSS medium containing 5 µg of DCFH-DA and incubated at 37 °C for 30 min and fluorescence was recorded as 485 nm excitation and 530 nm emission (Cary Eclipse, Varian, USA).

2.5.3 Statistical analysis

The data obtained from the study is presented as mean ± standard error. The data was subjected to one-way ANOVA with corrections for multiple comparisons using Dunnett's test (Graph Pad Prism 2.01). Statistical significance was estimated at the level of $p < 0.05$.

3. RESULTS AND DISCUSSIONS

The products based on the extracts of *Ganoderma lucidum* are available widely as health care products[28] and our previous studies provide rational for use of aqueous extract of *Ganoderma lucidum* as prophylactic or therapeutic agents for accelerating acclimatisation process at high altitude. In present study, stability of extract in terms of its bio-efficacy after its storage at different temperature has been addressed which has not been addressed previously.

Table 1. Qualitative phytochemical screening of aqueous extract of *Ganoderma lucidum*

Class of compounds	Analysis conducted on freshly prepared and stored extract		
	Freshly prepared	After one year of storage	After two year of storage
Anthraquinones	Absent	Absent	Absent
Glycosides	Present (+++)	Present (+++)	Present (+++)
Flavonoids	Present (++)	Present (++)	Present (++)
Phenols	Present (+++)	Present (+++)	Present (+++)
Polysaccharides	Present (++)	Present (++)	Present (++)
Steroids	Absent	Absent	Absent
Terpenoids	Present (+)	Present (+)	Present (+)
Tannins	Present (+)	Present (+)	Present (+)

Representative data of extract stored at room temperature for two years. Similar findings were obtained for extract stored at 4 °C or -20 °C.

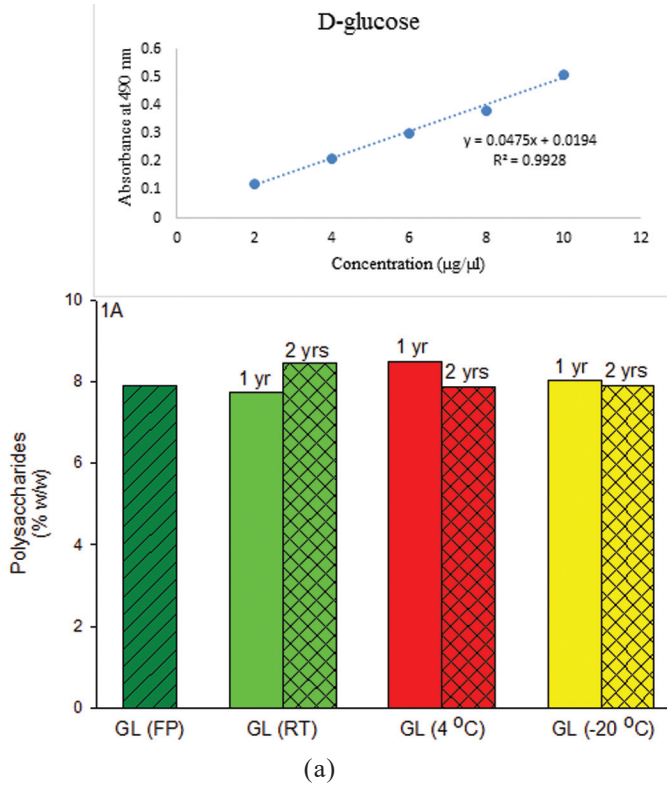
+ = fairly present; ++ = moderately present; +++ = strongly present

3.1 Qualitative Analysis: Phytochemical Screening of Aqueous Extract of *Ganoderma lucidum*

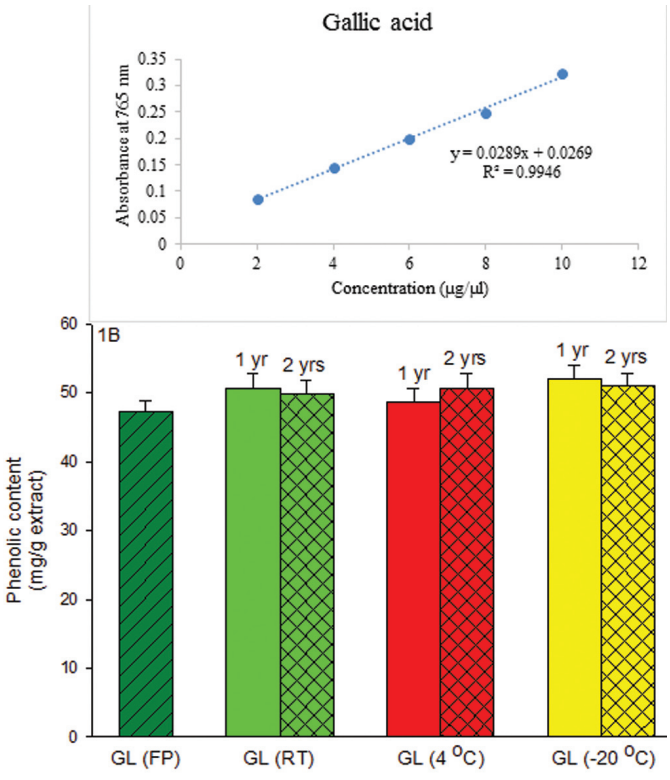
The physical characteristic of the extract was recorded. The freshly prepared extract had light brown color which did not change following two years of storage at cool conditions i.e. 4 °C or -20 °C. However, light brown colour changed to dark brown color in extract stored at room temperature due to absorption of moisture nevertheless the problem was noticed in few samples. The problem was resolved when the extract was stored at room temperature in gelatin capsules.

The qualitative analysis of *Ganoderma lucidum* extract was carried out by estimating following metabolites: glycosides, flavonoids, phenols, polysaccharides, terpenoids, tannins and steroids. Orole[23] extracted fruiting body of *Ganoderma lucidum* with two solvent system ethyl acetate and n-hexane and reported presence of phenol, flavonoid, saponins, terpenoids, glycosides and sterols. In another study, authors prepared aqueous and solvent extracts using ethyl acetate, chloroform, hexane and dichloromethane from two Ganoderma species from Tamil Nadu, Southern region of India and showed presence of saponins, terpenoids and tannins[29]. However, in all studies available on qualitative screening on *Ganoderma lucidum* extracts using water and solvents were conducted on freshly prepared extracts and no time dependent

analysis was conducted. In the present study, extract revealed presence of glycosides, flavonoids, phenols, polysaccharides, terpenoids and tannins but negative results for steroids and anthraquinones was recorded. The study was carried out over two year of storage and no qualitative change in extract was observed after one or two year of storage (Table 1).



(a)



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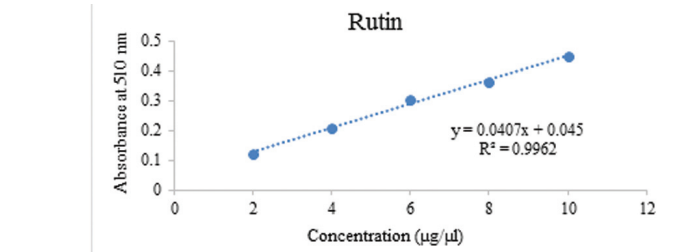

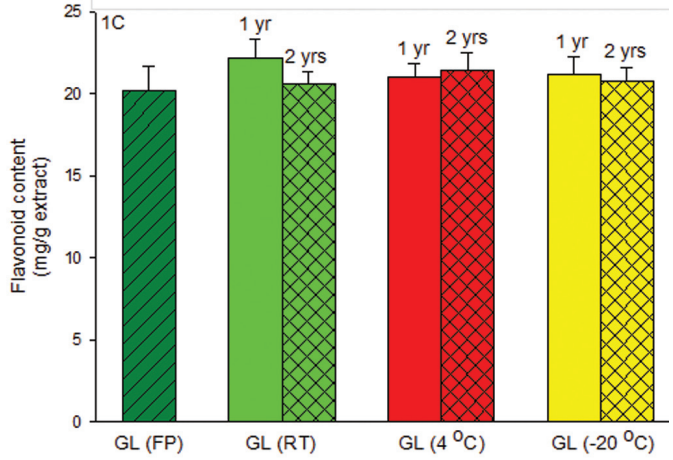

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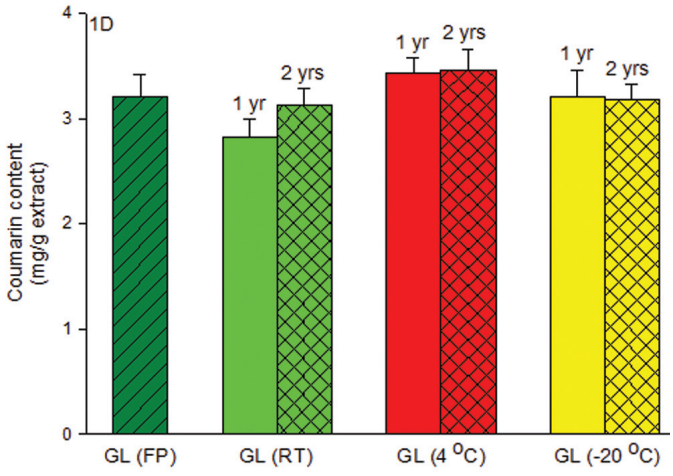
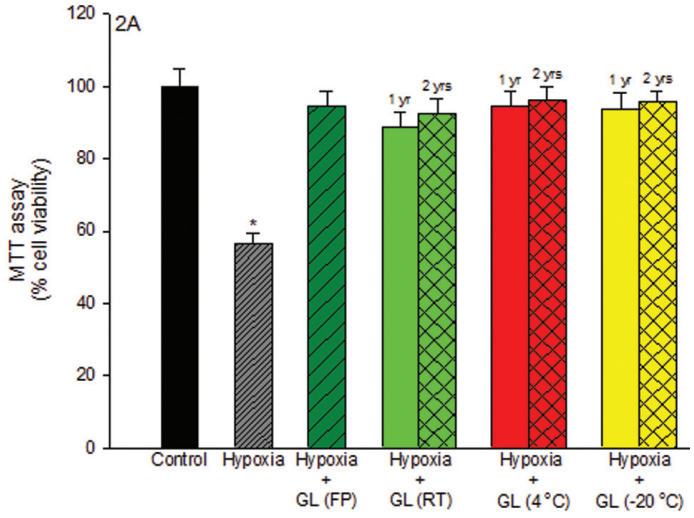


(d)

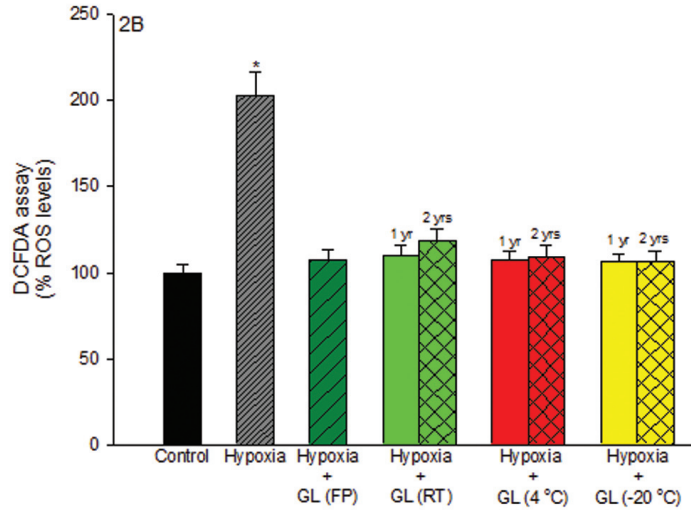
Figure 1. Total polysaccharides (a), phenolic content (b), flavonoid content (c) and coumarin content (d) of aqueous extract of *Ganoderma lucidum* freshly prepared [GL (FP), bar 1] or stored at room temperature [GL (RT)], 4 °C [GL (4 °C] and -20 °C [GL (-20 °C] for one year (1 yr, bars 2, 4 and 6) and two years (2 yrs, bars 3, 5 and 7), respectively. Mean values n =10 and data is expressed as percentage or mg/g extract.

3.2 Quantitative Analysis: Studies on Effect of Storage Temperature on Aqueous Extract of *Ganoderma lucidum* using Chemical Test System

The aqueous extract was further subjected to quantitative analysis. It is reported that numerous pharmacological effects exhibited by Ganoderma are linked to its polysaccharide content and anti-oxidative activity[30-32]. Therefore, the content of polysaccharide, phenols and flavonoids were determined in aqueous extract of *Ganoderma lucidum*. In the present



(a)



(b)

Figure 2. Cell viability (a) and generation of reactive oxygen species (b) under hypoxia microenvironment in absence or presence of aqueous extract of *Ganoderma lucidum* freshly prepared [GL (FP), bar 3] or stored at room temperature [GL (RT)], 4 °C [GL (4 °C] and -20 °C [GL (-20 °C] for one year (1 yr, bars 5, 6 and 7) and two years (2 yrs, bars 5, 7 and 9), respectively. Mean values n =10 (*p<0.05) and data is expressed as percentage.

study, polysaccharides content did not show any significant deviations in the extract stored at various temperature conditions such as ambient room temperature, 4 °C and -20 °C for two years (Fig. 1 (a)). This study is in agreement to earlier report that main characteristic band of polysaccharide remain unchanged at different storage durations using FTIR and 2DIR spectroscopy[33]. Further, it is documented that plant polyphenols has anti-oxidative role in human health and diseases[34-35]. However, there is lack of studies on different storage conditions and duration on phenolic and flavonoid content of aqueous extract of *Ganoderma lucidum*. In this study, no significant change was observed in any extract kept in different temperature conditions (ambient room temperature, 4 °C and -20 °C) in terms of its phenolic and flavonoid up to two years of storage (Figs. 1(b) & (c)). The extract contains mixture of compounds and it is not possible to target all molecules hence coumarin which has been reported as active constituent in *Ganoderma lucidum* was determined in the extract. Further, major constituents in the *Ganoderma lucidum* are believed to be ganoderic acids however commercially these chemicals are not widely available. The extract content of coumarin which has been reported in *Ganoderma lucidum* also did not show any change in its content (Fig. 1(d)).

3.3 Bio-efficacy study: Effect of Storage Conditions on Bio-Efficacy of Extract of *Ganoderma lucidum*

Numerous studies are available on pharmacological studies on aqueous extract of *Ganoderma lucidum* but still there is lack of reports on effects of different storage conditions and duration on bio-effects which is essential part for drug discovery particularly natural products. Therefore, effects of storage conditions and time dependent studies were carried out for the first time. The bio-efficacy of *Ganoderma lucidum* aqueous extract stored at ambient room temperature, 4 °C and -20 °C for two years was evaluated using HT22 cell model against hypoxia stress. Cells exposed to hypoxia (0.5 % O_2) for 24 h showed cell death and elevation of reactive species. The cells treated with extract prevented cell death and generation of reactive oxygen species (Figs. 2(a) & (b)). The storage temperature did not show any significant adverse impact on bio-efficacy of aqueous extract up to two years of storage. The aqueous extract of *Ganoderma lucidum* retained biological effects even after two years of storage at different temperature conditions i.e. ambient room temperature, 4 °C and -20 °C.

4. CONCLUSIONS

The major inquest was to examine retention of bio-efficacy preferably in extract stored at room temperature or cooler conditions. In this study, no deterioration was observed in bio-efficacy of extract in cells exposed to hypoxia even after storage at room temperature for two years. Overall, data obtained from the studies reveal that extract has good shelf life up to two years and extract stored at room temperature, 4 °C and -20 °C did not affect its bio-efficacy. Further, the extract is devoid of steroid content and hence suitable for the development of products for performance enhancement of soldiers at high altitude.

REFERENCES

1. Kumar, R.; Grover, S.K.; Shyam, R.; Divekar, H.M. & Srivastava, K.K. Enhanced thermogenesis in rats by a composite Indian herbal preparation-I and its mechanism of action. *J. Altern. Complement. Med.,* 1999, **5**, 245–251.
doi: 10.1089/acm.1999.5.245.
2. Grover, S.K.; Divekar, H.M.; Kumar, R.; Pahwa, M.L.; Bhardwaj, S.K.; Gupta, A.K. & Srivastava, K.K. Experimental evaluation of a Composite Indian Herbal Preparation II (CIHP II) as an adaptogen and its mechanism of action. *Int. J. Pharmacogn.,* 1995, **33**, 148–154.

doi: 10.3109/13880209509055216.
3. Qin, D. & Han, C. Medicinal and edible fungi as an alternative medicine for treating age-related disease. Evidence-Based Complementary and Alternative Medicine*,* 2014, article id 638561, 2 p. doi: 10.1155/2014/638561.
4. Valverde, M.E.; Hernández-Pérez, T. & Paredes-López, O. Edible Mushrooms: Improving human health and promoting quality life. *Int. J. Microbiol.,* 2015, article id 376387, 14 p. doi: 10.1155/2015/376387.
5. Sirisidthi, K.; Kosai, P. & Jiraungkoorskul, W. Antidiabetic activity of the lingzhi or reishi medicinal mushroom Ganoderma lucidum: A review. *S. Afr. Pharm. J.,* 2016, **83**(8), 45-47.
6. Ferreira, I.C.; Barros, L., & Abreu, R.M. Antioxidants in wild mushrooms. *Curr. Med. Chem.,* 2009, **16**(12), 1543-1560.
7. Kozarski, M.; Klaus, A.; Jakovljevic, D.; Todorovic, N.; Vunduk, J.; Petrović, P.; Niksic, M.; Vrvic, M.M. & Griensven, L.V. Review antioxidants of edible mushrooms. *Molecules,* 2015, **20**, 19489-19525. doi: 10.3390/molecules201019489.
8. Islam, M.S.; Rahi, M.S.; Koli, H.K.; Jerin, I.; Sajib, S.A.; Hoque, K.M.F. & Reza, M.A. Evaluation of phytochemical, antioxidant, cytotoxicity and in vitro antibacterial activity of aqueous extract of Ganoderma lucidum cultivated in Bangladeshi habitat. *Malaya J. Biosci.*, 2018, **5**(1), 1-13.
9. Jones, S. & Janardhanan, K.K. Antioxidant and antitumor activities of Ganoderma lucidum (Curt.: Fr) P. Krast. Reshi (Aphyllophoromycedideae) from South India. *Int. J. Med. Mushrooms,* 2000, **2**, 195-200. doi: 10.1615/IntJMedMushr.v2.i3.20.
10. Min, B.S.; Nakamura, N.; Miyashiro, H.; Bae, K.W. & Hattori, M. Triterpenes from the spores of Ganoderma lucidum and their inhibitory against HIV-1 protease. *Chem. Pharmaceut.,* 1998, **46**, 1607–1612. doi: 10.1248/cpb.46.1607.
11. Kim, H.W. & Kim, B.K. Biomedicinal triterperoids of Ganoderma lucidum (Curt.:Fr.) P. Karst. (Aphyllophoromycetideae). *Int. J. Med. Mushrooms,* 1999, **1**, 121–138. doi: 10.1615/IntJMedMushrooms.v1.i2.20.
12. Klupp, N.L.; Chang, D.; Hawke, F.; Kiat, H.; Cao, H.; Grant, S.J. & Bensoussan, A. Ganoderma lucidum mushroom for the treatment of cardiovascular risk factors. *Cochrane Database Syst. Rev.,* 2015, **2**. doi: 10.1002/14651858.CD007259.pub2.
13. Wang, G.H.; Wang, L.H.; Wang, C. & Qin, L.H. Spore powder of Ganoderma lucidum for the treatment of Alzheimer disease. A pilot study. *Medicine (Baltimore),* 2018, **97**(19), e0636. doi: 10.1097/MD.0000000000010636.
14. Tüzün, F.; Tüzün, B.S. & Konyalıoğlu, S. Effects of *Ganoderma lucidum* in some neurological diseases. *Int. J. Nature Life Sci. (IJNLS),* 2018, **2**(1), pp. 1-9.
15. Bensky, D.; Gamble, A. & Stoger, E. Chinese herbal medicine: Materia medica. Eastland press, Seattle. 2004.
16. Gao, H.; Chan, E. & Zhou, F. Immunomodulating activities of Ganoderma, a mushroom with medicinal properties. *Food Rev. Int.,* 2004, **20**, 123−161. doi: 10.1081/FRI-120037158.
17. Sliva, D. Ganoderma lucidum in cancer research. *Leukemia Res.*, 2006, **30**, 767–768. doi: 10.1016/j.leukres.2005.12.015.
18. Boh, B.; Berovic, M.; Zhang, J.S. & Lin, Z.B. Ganoderma lucidum and its pharmaceutically active compounds. *Biotechnol. Ann. Rev.,* 2007, **13**, 265–301. doi: 10.1016/S1387-2656(07)13010-6.
19. Liu, Y.W.; Gao, J.L.; Guan, J.; Qian, Z.M.; Feng, K. & Li, S.P. Evaluation of antiproliferative activities and action mechanisms of extracts from two species of Ganoderma on tumor cell lines. *J. Agric. Food Chem.,* 2009, **57**, 3087–3093. doi: 10.1021/jf900011f.
20. Welti, S.; Moreau, P-A, Azaroual, N.; Lemoine, A.; Duhal, N.; Kouach, M.; Millet, R. & Courtecuisse, R. Antiproliferative activities of methanolic extracts from a neotropical ganoderma species (Aphyllophoromycetideae): Identification and characterisatiion of a Novel Ganoderic Acid. *Int. J. Med. Mushrooms,* 2010, **12**(1), 17-31. doi: 10.1615/IntJMedMushr.v12.i1.20.
21. Tulsawani, R.K.; Manickam, M.; Suryakumar, G. & Kaur, N. Aqueous extract of Ganoderma lucidum prevent svascular leakage in rat lung and hypothermia following hypobaric hypoxia exposure. *Indian J. Pharmacol.,* 2015, **47**(1), S13-S57.
22. Tulsawani, R.K.; Singh, M. & Manimaran, M. Aqueous extract of *Ganoderma Lucidum* facilitates tolerance in rat heart by inducing nuclear factor (Erythroid-Derived 2)-Like 2 and Hypoxia inducible factor 1 dependent signaling. *Indian J. Pharmacol.* 2014, **46**(7), 3-6.
23. Orole, O.O. GC-MS Evaluation, Phytochemical and antinutritional screening of ganoderma lucidum. *J. Adv. Biol. Biotech.,* 2016, **5**(4), 1-10. doi: 10.9734/JABB/2016/24261.
24. Skalicka-Woźniak, K.; Szypowski, J.; Łoś, R.; Siwulski, M.; Sobieralski, K.; Głowniak, K. & Malm, A. Evaluation of polysaccharides content in fruit bodies and their antimicrobial activity of four Ganoderma lucidum (W Curt.: Fr.) P. Karst. strains cultivated on different wood type substrates. *Acta. Soc. Bot. Pol.,* 2012, **81**(1), 17-21. doi: 10.5586/asbp.2012.001.
25. Singleton, V.L. & Rossi, J.A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am. J. Enol. Vitic.,* 1965, **16**, 144-158.
26. Zhishen, J.; Mengcheng, T. & Jianming, W. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. *Food Chem.,* 1999, **64**, 555-559. doi: 10.1016/S0308-8146(98)00102-2.
27. Mosman, T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J. Immnunol. Meth.,* 1983, **65**, 55-63.
28. Hapuarachchi, K.K.; Elkhateeb, W.A.; Karunarathna, S.C.; Cheng, C.R.; Bandara, A.R.; Kakumyan, P.; Hyde,

K.D.; Daba, G.M. & Wen, T.C. Current status of global Ganoderma cultivation, products, industry and market. *Mycosphere,* 2018, **9**(5), 1025–1052. doi: 10.5943/mycosphere/9/5/6.

29. Rajesh, K. & Dhanasekaran, D. Phytochemical screening and biological activity of medicinal mushroom Ganoderma Species. *Malaya J. Biosci.,* 2014, **1**(2), 67–75.
30. Zeng, P.; Chen, Y.; Zhang, L. & Xing, M. Ganoderma lucidum polysaccharide used for treating physical frailty in China. *Prog. Mol. Biol. Transl. Sci.,* 2019, **163**, 179-219. doi: 10.1016/bs.pmbts.2019.02.009.
31. Liang, Z.; Yuan, Z.; Guo, J.; Wu, J.; Yi, J.; Deng, J. & Shan, Y. *Ganoderma lucidum* polysaccharides prevent palmitic acid-evoked apoptosis and autophagy in intestinal porcine epithelial cell line via restoration of mitochondrial function and regulation of MAPK and AMPK/Akt/mTOR signaling pathway. *Int. J. Mol. Sci.,* 2019, **20**(3), 478. doi: 10.3390/ijms20030478.
32. Rathor, R.; Tulsawani, R. & Misra, K. Hydro-ethanolic extract of Ganoderma lucidum (HEGL) shows anti-inflammatory activity on THP1 cytokines and NF-κB P65 response. *Int. J. Pharmaceut. Sci. Res.* 2014, **5**(6), 2337-2348. doi: 10.13040/IJPSR.0975-8232.5(6).2337-2248.
33. Choong, Y.K.; Sun, S.Q.; Zhou, Q.; Ismail, Z.; Rashid, B.A.A. & Tao, J.X. Determination of storage stability of the crude extracts of Ganoderma lucidum using FTIR and 2DIR spectroscopy. *Vib. Spectrosc.,* 2011, **57**(1), 87-96. doi: 10.1016/j.vibspec.2011.05.008.
34. Pandey, K.B. & Rizv, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell Longev.,* 2009, **2**(5), 270–278. doi: 10.4161/oxim.2.5.9498.
35. Renaud, J. & Martinoli, M.G. Review considerations for the use of polyphenols as therapies in neurodegenerative diseases. *Int. J. Mol. Sci.,* 2019, **20**, 1883. doi: 10.3390/ijms20081883.

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