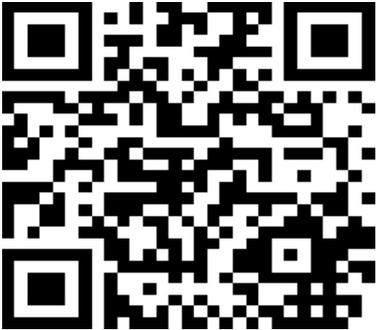


COMPARATIVE BIOCHEMICAL ESTIMATION OF GANODERMA LUCIDUM STRAINS DARL-4 AND MS-1

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<p>*For Correspondence: ¹Devsthali Vidyapeeth, College of Pharmacy, Rudrapur, Uttarakhand, India</p>	<p>ABSTRACT Ganoderma lucidum commonly known as Reishi, is a lignicolous high value medicinal mushroom belonging to family Ganodermataceae. The purpose of current research work is comparative estimation of carbohydrates, polysaccharides, proteins, reducing sugars, non-reducing sugars, fat, fiber, total ash, water soluble ash and acid insoluble ash present in G. lucidum strains DARL-4 and MS-1. DARL-4 is an indigenous strain and MS-1 is an exotic malayasian strain which in vitro cultivated under sterile condition. The amount of carbohydrates, polysaccharides, non-reducing sugars, proteins, fats and fibers were found higher in MS-1 than that of DARL-4. The amount of reducing sugars was observed higher in DARL-4. The study reveals the potential of DARL-4 and MS-1 as a rich source of good health supplement.</p> <p>KEY WORDS: Ganoderma lucidum, medicinal mushroom, Reishi, Nutraceutical.</p>
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INTRODUCTION

Mushrooms are higher fungi belonging to division Ascomycota and Basidiomycota (Takshak et al., 2014). Mushrooms are the major untapped source of miraculous potent pharmaceutical products. Of the approximately 10,000 known species of mushrooms, 2000 are safe for people's health and about 300 of them possess medicinal properties (Sheena et al., 2005). Medicinal mushrooms are widely used as traditional medicinal ingredients for the treatment of various diseases and related health problems (Quereshi and Pandey, 2010). *Ganoderma lucidum* (W.Curst.:Fr.) P.Karst. (Higher Basidiomycetes) is a very high valuable medicinal mushroom, which is well known for nutraceutical and pharmaceutical properties for promoting human health. The fruiting body of *G. lucidum* is popularly known as "Reishi" in Japan and "Lingzhi" in China. It is widely used as a high value medicinal mushroom and nutritional supplement in Traditional Chinese Medicine (TCM) (Mohsin et al., 2011). *G. lucidum* has been reported to possess antitumour, hypotensive, cytotoxicity, antioxidant, anti-allergic, antimicrobial, hepatoprotective, hypolipidemic, anti-diabetic and anti-inflammatory effects (Takshak et al., 2014).

G. lucidum mainly contains carbohydrate, protein, crude fat, crude fiber and bioactive components mainly triterpenoids, steroids, glycoproteins and polysaccharides (Ogbe et al., 2009, Kao et al., 2013, Rawat et al., 2012, Singh et al., 2013). These bioactive components play a role in maintaining a good health and fulfill the nutritional requirements. Wild *G. lucidum* is less abundant in nature and thus it is not available sufficiently for nutraceutical product development so its *in-vitro*

cultivation is developed for its easy availability for nutraceutical and pharmaceutical development. DARL-4 is an indigenous strain and MS-1 is an exotic malayasian strain which is *in vitro* cultivated under sterile condition. The aim of this study is to estimate the comparative biochemical parameters of *in vitro* cultivated *G. lucidum* strains DARL-4 and MS-1.

MATERIALS AND METHODS

Collection and authentication of *G. lucidum* strains DARL-4 and MS-1

In vitro cultivated samples of *Ganoderma lucidum* strains DARL-4 and MS-1 were collected from the polyhouse of DIBER, Pithoragarh between April-May 2015. The strains were authenticated by Mycology department, DIBER, field station, Pithoragarh. Samples were then air dried and grinded into powdered form. Powdered samples were then examined for biochemical contents.

Determination of Carbohydrates

Estimation of total carbohydrate content in *G. lucidum* strains DARL-4 and MS-1 was done by Anthrone method (Hedge and Hofreiter, 1962). 100 mg of sample was taken in a boiling tube and then hydrolyzed with 5ml of 2.5 N HCl by keeping it in boiling water bath for three hours and cooled to room temperature. It was neutralized with sodium carbonate until the effervescence ceased. Volume was then finally made to 100 ml and centrifuged. Different aliquots were taken from the supernatant for analysis. Standards were prepared by taking 0, 0.1, 0.2, 0.4, 0.6 and 0.8 ml of the working standard and '0' served as blank. Volume was made upto 1 ml with distilled water including the samples and standard test tubes. Then 4 ml of ice cooled anthrone reagent was added and heated for eight minutes in a boiling water bath. It was cooled rapidly and finally green to dark green colour was read at 630 nm. Standard graph was plotted between concentrations of the standard on the X-axis versus absorbance on Y-axis. From the graph the amount of carbohydrate present in the sample was calculated.

Determination of Polysaccharides

Polysaccharide content in *G. lucidum* strains DARL-4 and MS-1 was estimated by Phenol-sulphuric acid method (Varkhade and Pawar, 2013). In 1 ml of distilled water, 1 ml of 5% of phenol was added followed by 5 ml of concentrated H₂SO₄ served as blank. A stock solution of 100 µg/ml of glucose in distilled water was prepared. Different aliquots were taken from stock solution. 1 ml of 5% phenol solution was added to 1 ml of sugar solution followed by 5 ml of conc. H₂SO₄. Absorbance was measured after 10 min at 488 nm against blank. 10 mg of sample was dissolved in 100 ml of distilled water. From this 1ml was taken and 1ml of 5 % phenol was added to it followed by conc. H₂SO₄. Absorbance was measured after 10 min at 488 nm against blank.

Determination of Simple Carbohydrates

Simple carbohydrate was calculated by subtracting the amount of polysaccharides from the total amount of carbohydrates present in the sample.

Determination of Reducing Sugars

Estimation of reducing sugar in *G. lucidum* strains DARL-4 and MS-1 was done by using Dinitrosalicylic acid method (DNS) (Miller, 1972). 100 mg of the sample was weighed and the sugars were extracted with hot 80% ethanol twice (5 ml each time). Supernatant was collected and evaporated on a boiling water bath at 80°C. Then 10 ml water was added to dissolve the sugars. Pipetted out different aliquots of the test extract in test tubes and the volume was made to 3 ml with water in all the tubes. 3 ml of DNS reagent was added and then heated the contents of the tubes in a water bath for 5 min. When the contents of the tubes were still warm, 1 ml of 40 % Rochelle salt solution was added. The test tubes were cooled and finally the intensity of dark red color at 510 nm was read by UV-Vis Spectrophotometer. Also a series of standards was run using different concentration of glucose (0–500 µg) and finally plotted a graph. The amount of reducing sugars present in the sample was calculated by using the standard graph.

Determination of Non Reducing sugars

Non reducing sugars present in DARL-4 and MS-1 was calculated by subtracting the amount of reducing sugars from the amount of the simple carbohydrates present in the sample.

Determination of Crude Proteins

Estimation of total protein in DARL-4 and MS-1 was done by Lowry's method (Lowry et al., 1951). Weighed 500 mg of sample and grinded well with a pestle mortar in 5–10 ml of the extraction buffer, then centrifuged it and supernatant used for protein estimation. Pipetted out different aliquots of standard stock solution in the test tubes and also pipette out different aliquots of sample extract in other test tubes. Final volume was made to 5 ml in all test tubes. From these different aliquots, pipetted out 0.2 ml protein solution to different test tubes, then 2 ml of alkaline copper sulphate (analytical reagent) was added and mixed the solution well. The test tubes were incubated at room temperature for 10 mins. After that 0.2 ml of reagent Folin-Ciocalteu solution was added to each tube and incubated for 30 mins. The intensity of blue colour developed was read at 660 nm. A standard graph was plotted between the absorbance and protein concentration. The amount of protein present in the sample was calculated by using the standard graph.

Determination of Fat

Estimation of fat in DARL-4 and MS-1 was done by Soxhlet apparatus (Maynard, 1970). Oven dried sample (2gm) was taken in a thimble of Whatman filter paper no.1 and placed in the extractor. The extractor was connected with weighed round bottom flask below and the condenser above. Petroleum ether (60 ml) of was taken in weighed flask. Cold water was passed through the condenser during the extraction process. Extraction was carried out for 6 hours till the liquid was as clear as clean water. The flask was then disconnected and dried in a hot air oven at $100\pm 5^{\circ}\text{C}$ for 4 to 6 hours till the ether was completely evaporated. It was cooled in a desiccator and weighed to a constant weight. The fat present in the sample was calculated by the following formula:

$$\% \text{ Fat} = (W_2 - W_1 / W) \times 100$$

Where, W=wt. of dry sample taken, W_1 =wt. of an empty oil flask, W_2 =wt of oil flask after extraction

Determination of Crude fiber

The sample after ether extraction (from the above fat estimation) was transferred from the thimble to a 500 ml round bottom reflex condenser flask. 200 ml of 1.25 % Sulphuric acid solution was poured into the flask and was heated in a heating mantle. Cold water was allowed to flow through the condenser. After boiling for 30 minutes, the content of the flask was filtered through the muslin cloth. The residue on the cloth was washed with boiling water to remove the acid. The residue was transferred to the same flask and 200 ml of 1.25 % of sodium hydroxide solution was poured into the flask. The content was boiled for 30 minutes and filtered through the muslin cloth again. It was washed with 25 ml of boiling 1.25 % H_2SO_4 , three 50 ml portion of water and 25 ml of alcohol. The residue was transferred to a crucible and kept in a hot air oven for 2 hr at $130\pm 2^{\circ}\text{C}$ for drying. The crucible was cooled in a desiccator and weighed to a constant weight. The content of crucible was ashed in a muffle furnace at 600°C for 30 min, cooled in a desiccator and weighed (Maynard, 1970). The crude fiber was calculated as follows: % crude fiber = $[\text{Loss in weight on ignition } (W_2 - W_1) - (W_3 - W_1) / \text{wt of the sample}] \times 100$

Where, W_1 =wt of empty crucible, W_2 =wt of crucible with sample after drying, W_3 =wt of crucible with sample after ashing

Determination of Total Ash

The dried sample (500 mg) was taken in a weighed crucible. Then the crucible was kept in a muffle furnace for ashing at 500°C till the content was free of black particles. The crucible was cooled in a desiccator and weighed to a constant weight (Lavekar, 2010). The ash content was calculated by following formula:

$$\% \text{ Ash} = (W_2 - W_1 / W) \times 100$$

Where, W=wt. of dry sample taken, W_1 =wt. of an empty crucible, W_2 =wt. of crucible with ash

Determination of water soluble ash

The dried sample (500 mg) was taken in a weighed crucible. Then the crucible was kept in a muffle furnace for ashing at 500°C till the content was free of black particles. Boil the total ash for 5 min with 25ml of water. The insoluble matter was collected on an ashless filter paper. It was washed with hot water and ignited for 15 min at a temperature not exceeding 600°C. The water soluble ash percentage was calculated (Lavekar, 2010).

Determination of acid insoluble ash

The total ash was transferred in 250 ml beaker then adds 100 ml diluted Hydrochloric acid. Washed the crucible with 10 ml acid to remove all the content of ash from the crucible then beaker was heated till the liquid boils. The liquid was filtered and insoluble matter was collected on ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing the insoluble matter was kept in original weighed crucible. The crucible was dried and kept in muffle furnace at 600°C for ignition then crucible was cooled in a desiccator and weighed. The process was repeated until the constant weight was obtained. The percentage of acid insoluble ash was calculated (Lavekar, 2010).

STATISTICAL ANALYSIS

The results were expressed as mean±standard deviation of triplicate experiments. The data were subjected to one way ANOVA and significant differences between means were determined statistically using t test with In Stat software. P<0.05 was assumed as the level of significance for experimental results.

RESULTS AND DISCUSSION

Carbohydrates

Carbohydrates play a numerous roles in living organisms. MS-1 was found to contain slightly higher amount of carbohydrate was found 40.04±0.176 % in comparison to which DARL-4 contains 38.04±0.291 % (Fig 1).

Polysaccharides

G. lucidum have pharmacological active constituents such as polysaccharides and triterpenoids. Polysaccharides especially β -D glycans may possess antitumor effects. Polysaccharides also possess protective effect against free radicals. In the present study the percentage of polysaccharides was found high in MS-1 29.5±0.109 % as compared to DARL-4 was 27.62±0.191 %.

Simple carbohydrates

The content of simple carbohydrates was observed 10.49±0.067 % in MS-1 and 10.42±0.395 % in DARL-4.

Reducing sugars

The sample of DARL-4 had a greater amount of reducing sugar in DARL-4 was 2.33±0.115 % in comparison to which MS-1 contains 1.77±0.026 % reducing sugar.

Non- Reducing sugars

Non-reducing sugars were observed high in MS-1 8.72±0.041 % than 8.09±0.28% in DARL-4 respectively during the analysis.

Proteins

MS-1 was found to contain a greater amount of proteins 18.22±0.199 % as compared to DARL-4 17.12± 0.092 %. Proteins are the primary components of living things. Proteins are the basic nutrients for body building and body maintenance (Marichamy et al., 2011). Main protein in *G. lucidum* was found to be LZ-8 (lingzhi-8) (Wasser, 1996). The presence of higher protein content in the sample points towards increase in their food value.

Fat

Unsaturated fats are divided into monounsaturated fats and polyunsaturated fats both have beneficial effects on cholesterol levels. Monounsaturated fats help in lowering LDL (bad) cholesterol and

boosting HDL (good) cholesterol. Polyunsaturated fats also lower total and LDL cholesterol. The fat content was observed 3.48 ± 0.035 % in MS-1 and in DARL-4 3.2 ± 0.265 %. MS-1 was found to have slightly higher amount of fat (Table 1).

Table 1. Biochemical content in g/100 g (%) sample dry weight of DARL-4 and MS-1.

S.No	Biochemical Parameters	DARL-4 (%)	MS-1 (%)
1	Total Ash	3.27 ± 0.416	3.85 ± 0.115
2	Water Soluble Ash	1.73 ± 0.416	2.51 ± 0.26
3	Acid Insoluble Ash	2.11 ± 0.031	2.17 ± 0.026
4	Total Carbohydrates	38.04 ± 0.291	40.04 ± 0.176
5	Total Polysaccharides	27.62 ± 0.191	29.55 ± 0.109
6	Simple Carbohydrates	10.42 ± 0.395	10.49 ± 0.067
7	Reducing Sugars	2.33 ± 0.115	1.77 ± 0.026
8	Non-Reducing Sugars	8.09 ± 0.28	8.72 ± 0.041
9	Crude protein	17.12 ± 0.092	18.22 ± 0.199
10	Fat	3.2 ± 0.265	3.48 ± 0.035
11	Crude fiber	30.55 ± 0.309	32.49 ± 0.312

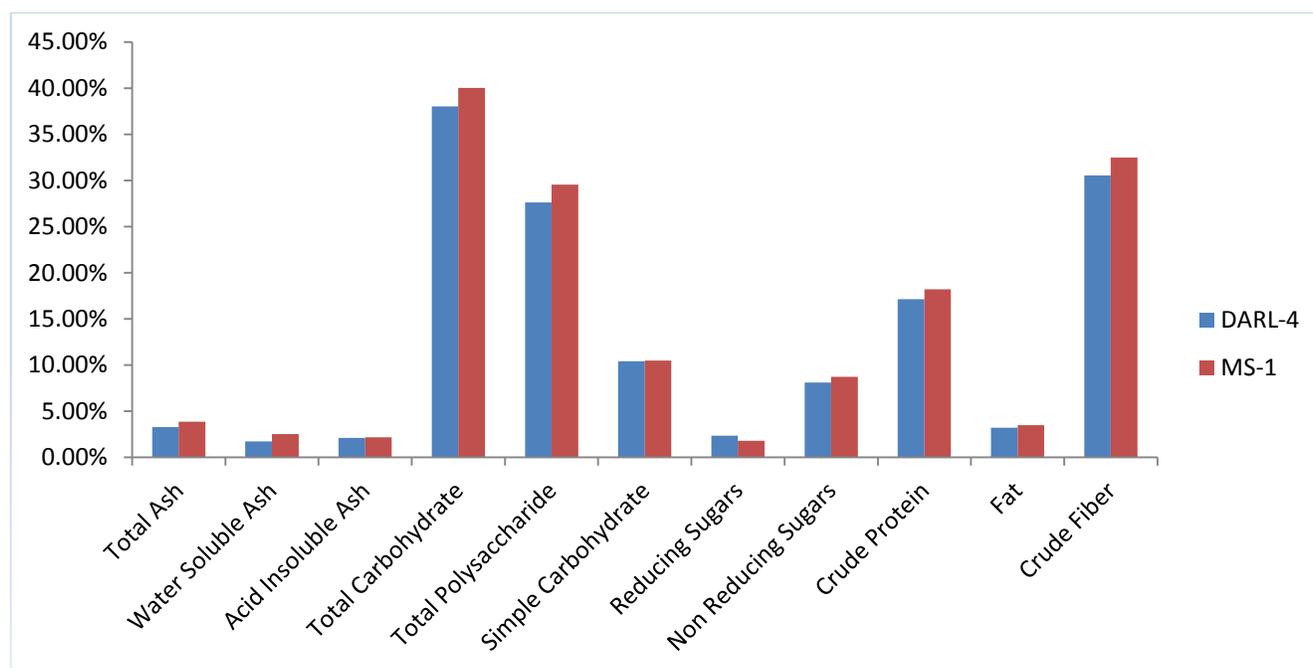


Fig 1: Biochemical content in g/100 g (%) sample dry weight of DARL-4 and MS-1.

Fiber

The fiber content were found higher 32.49 ± 0.312 % in MS-1 than 30.55 ± 0.309 % in DARL-4 respectively. Fibers are very important for digestive tract as they increase the surface area of food and make the digestive process easier.

Total ash

The ash represents the mineral content of any sample. The ash content was found 3.2 ± 0.416 % in DARL-4 and 3.85 ± 0.115 % in MS-1 which indicates the presence of significant amount of minerals in these samples (Sodde, 2011). MS-1 contains a higher amount of total ash.

Water soluble ash

The water soluble ash content in MS-1 was higher 2.51 ± 0.268 % as compared to DARL -4 1.73 ± 0.416 % respectively.

Acid insoluble ash

In DARL-4 the acid insoluble ash was showed 2.11 ± 0.031 %, similarly in MS-1 was showed 2.17 ± 0.026 % respectively. MS-1 was contains slightly high amount of acid insoluble ash.

CONCLUSION

Comparison of carbohydrates, polysaccharides, proteins, reducing sugars, non-reducing sugars, fat, fiber, total ash, water soluble ash and acid insoluble ash present in *G.lucidum* strains DARL-4 and MS-1 were done using the results obtained from the quantitatively estimated biochemical contents. The amount of carbohydrates and polysaccharides contents was higher in MS-1 than that of DARL-4. DARL-4 showing greater reducing sugars content than that of MS-1. MS-1 showed higher amount of proteins, fat and crude fiber contents in comparison to DARL-4. The amount of non-reducing sugars was slightly higher in MS-1. The total ash, water soluble ash and acid insoluble ash contents were observed greater in MS-1.

This study suggest that *in vitro* cultivated *G. lucidum* MS-1 which is an exotic Malaysian strain was found to have high biochemical contents when compared with DARL-4 which is an indigenous strain. This study reveals the potential of *G. lucidum* strains DARL-4 and MS-1 to be used as good diet supplement which is of the prime concern in the growing area of nutraceutical and pharmaceutical industries.

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