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An Antioxidant and Bioactive Compound Studies of *Parmelia Perlata, Ganoderma Lucidum And Phellinus Igniarius*-Supplimentory Drug

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ABSTRACT

Medicinal mushrooms and lichens have been a great demand for their medicinal property and for food supplements. For this study, three extracts have been taken such as *Parmelia perlata, Ganoderma lucidum* and *Phellinus igniarius*; the solvents used for the extraction is aqueous and ethanol. The objective of the work is to qualitative and quantitative analysis of mechanical and nutritional analysis. In vitro analysis of antioxidants and free radical scavenging assay also were done. All the extracts show virtuous amount of bioactive compounds, appreciable amounts of antioxidant compounds and also good free radical scavenging effect against different radicals. This shows a good source for developing a medicinal drug.

Key-words: *Parmelia perlata, Ganoderma lucidum, Phellinus igniarius*, antioxidants, bioactive compounds, free radicals, nutritional values.

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INTRODUCTION:

Nowadays there are lots of diseases arising due to imbalance between intracellular antioxidants and intracellular reactive oxygen species (ROS) or the so-called state of oxidative stress. The synthetic antioxidants may prevent oxidative damage of biomolecules and cells, ROS-induced diseases by reacting among free radicals, scavenging free radicals and gelatin free catalytic metals but exerts a toxic and carcinogenic effect [1]. Hence, strong restrictions have been placed on their application and tend to substitute with naturally occurring antioxidants from Mushrooms and lichen. The positive health effect of our mushrooms and lichen bioactive extract in the management of disease associated with oxidative stress [2].

In this our particular interest lay on *Parmelia Perlata* (lichen), *Ganoderma Lucidum* and *Phellinus Igniarius* (mushroom). Lichen is in symbiotic association's form of fungi and algae or cyanobacterial components as primary partners. Lichenes, separate from fungi, mosses, and algae [3]. *Parmelia perlata* belongs to family Parmeliaceae and commonly known as Charila in India [4]. It's acts as expectorant, antibiotic, anti-inflammation and astringent. It assists in avoiding the calculi and it helps in maintaining the normal body temperature. It also used as anti-fungal, anti-bacterial, anti-viral agent not only that it helps against baldness, period aches, worms and lice [5].

Ganoderma Lucidum (GL) belongs to the family ganodermataceae, Ganoderma derives from the Greek meaning ganos "brightness" and Derma "skin", The specific epithet Lucidum is Latin for "shining"[6] Reishi or Lingzhi is known among Chinese and Japanese as a "magic Herb" as it plays a pivotal role in curing different kinds of diseases and also applied for longevity. The bioactive compounds found in GL can be easily absorbed by the dermal layer skin. This can be attributed to improve the immunity level of our body, thus providing us the ability to fight against dreadful diseases and can also reduce the adverse effects caused by insomnia, high blood pressure, chronic bronchitis, asthma, diabetes (DM) and has an effect on anti-inflammation and hyper susceptibility [7]. Currently GL are also used widely in the development of cosmetics such as creams, lotions etc.

The fruiting body of the fungus *Phellinus Igniarius* belong to family Polyporaceae[8]. It's also one of most expensive mushroom and shown to improve heart health, reduce the risk of cancer, promote immune system, can be used as anti- viruses, bacteria, and fungi; reduce inflammation, allergies and help to control sugar levels and also in the removal of toxic compounds [9]. These naturally occurring bioactive compounds such as Mycochemical, vitamins and minerals, which extracted from mushrooms and lichen shows strong antioxidant property, therefore help to protect the cell constituents against destructive oxidative damage, inhibition of hydrolytic and oxidative enzymes, including lipid peroxidation, thus limiting the risk of various vulnerable diseases associated with oxidative stress [10].

MATERIALS AND METHODS:

Mushrooms and lichen sample shown in the inset of fig1 obtained from around the college campus and Ooty was washed several times with deionised water, dried in the sun and then pulverized with electric blender.

EXTRACTION OF SAMPLES: The ethanol extract of the samples was carried out using methods previously described [11]. A fifty gram (50 g) sample of dried Mushroom and lichen material was extracted with 200 ml of solvent (in the ratio of 9:1 ml ethanol: water respectively). The sample was completely immersed in the solvent and then wrapped with aluminium foil, it was kept for 48 h. The extract was transferred and the solvent removed by evaporation at room temperature (28 + 30°C) to obtain the extract. The air dried sample was stored for 48 h in sterile bottles at room temperature. The sterility of the extract was tested before use. The water extract of the samples were done by taking 20gms of the sample in 100ml water of double distilled water boil for 30 min. Then filter it by whatmann's filter paper and the filtrate is used for the various studies.

MYCOCHEMICAL SCREENING: The mycochemical components of the medicinal plants were screened using the methods[12-14].

Fig 1: G-showing G. lucidum- showing P. igniarius, L- showing P. perlata

QUALITATIVE ANALYSIS:

Test for terpenoids (Salkowski test): To 0.5 g of the sample was added 2 ml of chloroform. 3 ml of concentrated sulphuric acid (H_2SO_4) was carefully added to form a layer. A reddish brown colouration between the interfaces indicates the presence of terpenoids.

Test for anthraquinones: About 0.5 g of the sample was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered using Whatmann's filter paper No.1.The filtrate was vigorously shaken with 5 ml of chloroform. The chloroform layer was pipetted out into another test tube and 1 ml of 10% of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for flavonoids: Two methods were used to test for flavonoids. First, 10% of dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow colouration will disappear on standing indicates the presence of flavonoids. Second, a few drops of 1% aluminum solution were added to a portion of the filtrate. A yellow colouration indicates the presence of flavonoids.

Test for saponins: To 0.5 g of sample was added to 5 ml of distilled water in a test tube. The solution was shaken vigorously and experimental for a stable persistent froth. It was mixed with 3 drops of olive oil; shaken vigorously after which it was observed in the formation of an emulsion.

Test for tannins: About 0.5 g of the sample was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride were added and observed for brownish-green or a blue-black colouration.

Test for Phenol (Ferric chloride test): Take 1ml of extract in a test tube, add 1ml of solution to test tube and vortex it. Note for appearance of colour

NUTRITIONAL STUDIES:

Test for Amino Acid (Ninhydrin): To 1ml of extract taken at test tube, add a few drops of Ninhydrin reagent and vortex it. Place the test tube in a water bath for 5 min and the latest cool it, note for colour change.

Test for Proteins (Biuret test): To 2ml of the protein solution add a few drops of alkali and vortex. Add copper reagent drop wise with continuously vortexing till the purple colour appears.

Test for Carbohydrate (Molisch test): To 1ml of extract taken in a test tube, add a few drops of α-napthol reagents and vortex the contents. Carefully, add Conc. Sulphuric acid along the sides of the test tube, keeping the tube in inclined position.
Test for Reducing sugar (Benedict's test): To 2ml of Benedict solution taken in a test tube, add a few drops of extract and place in water bath for 5-10 min.
Test for Glucose: To 3ml of extract taken in a test tube, add 0.5gms of phenylhydrazine reagents and a few drops of glacial acetic acid. The contents kept for 15min in boiling water bath. Cool the solution and observe in the microscope.
Test for Lipids: Take small amount of solution and mixed with water, test for solubility.
Test for Cholesterol (salkowski test): To 2ml of extract and chloroform in a test tube, add a few drops Conc. Sulphuric acid along the sides of the test tube. Observe colour in the acid and chloroform phase.

QUANTITATIVE ANALYSIS:

ESTIMATION OF PHENOL BY FOLIN –CIOCALTEAU REAGENT (15): Weigh 0.5 to 1.0g of the sample and grind it in a mortar and pestle at ten times the volume of 80% ethanol, Centrifuge it at 10,000 RPM for 20 min. Save the supernatant re-extract the residue the five times with the volume of 80% ethanol, centrifuge and collect supernatant. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of Distilled water. Pipette out different aliquots (0.2-1ml) into test tubes. Make up the volume in each tube 103ml with water. Add 0.5ml of the folin-ciocalteau reagent after 3min; add 2ml of 20% of sodium carbonate solution to each tube. Mix thoroughly. Place the tube in a boiling water for exactly 1min, cool and measure the absorbance at 650nm against blank. Prepare std curve using different conc. of phenol.
ESTIMATION OF TANNIN BY FOLIN DENNIS REAGENT [16]: weigh 0.5g of powder materials and transfer to 250ml 0f flasks, add 75 ml of water .Heat the flask for about 30min.Centrifuge at 200 RPM for 20min and collect the supernatant in 100ml of std flask and make up the volume. Transfer 1ml of the sample extract to 100ml of std flask containing 75ml of water. Add 5ml of Folin-Denis reagent, 10ml of sodium carbonate and dilute to 100ml with water. Shake well. Read the absorbance at 700nm after 30minPrepare std graph by using 0-100 µg tannic acid.
ESTIMATION OF FLAVOINDS BY ALUMINUM CHLORIDE [17]: 0.5ml of extract was separately mixed with 1.5ml of methanol. After that 0.1ml of aluminium chloride, 0.1 of potassium acetate and 2.8ml of water. It was left at room temperature for 30min.After the absorbance of the reaction mixture is taken at 415 nm. The std graph prepared by quercetin.
ESTIMATION OF TOTAL GLUCOSE BY ORTHO-TOLUIDENE REAGENT [18]: To1ml of the std and test solution add 4ml of ortho-toluidene reagent rapidly into test tube and vortex it. Heat the tube in a boiling water bath for 10 min. Cool the tubes to room temperature. Read colour developed against at 620nm.Construct a calibration curve with std value on graph paper. Compute the concentration of the sugar in the sample.
ESTIMATION OF PROTIEN BY LOWRY METHOD [19]:
To 1ml of std protein and test solution, add 4ml of copper reagent and mix it. After 10min incubation at room temperature, add 0.4ml of Folin's reagent and vortex the contents immediately. Run a reagent blank with 1ml of distilled water along the standard and sample. After 30min of incubation at room temperature, read the blue colour developed at 720nm.Construct a calibration curve on a graph paper with std value. Compute the conc. of sample with the graph

ANTIOXIDANT & FREE RADICAL SCAVENGING:

ESTIMATION OF VITAMIN C BY DYE2, 6 DICHLOROPHENOL [20]:
1ml of test solution was pipetted out into a conical flask. To this 10ml of 4%oxalic acid was added and titrated against the dye in the burette. The end point was the appearance of pale pink colour. The

procedure was repeated for concordant values. The amount of dye consumed was equal to the amount of ascorbic acid present. The experiment is repeated with stock solution in different concentration. The unit of vitamin c in the unknown sample was calculated using standard graph.

ESTIMATION OF VITAMIN A BY CARR-PRICE REAGENT:

0.5ml of the test solution was pipetted out in a clean test tube. To the above solution 0.5ml of chloroform was added to make the volume up to 1ml, 4ml of carr-price reagent was added to all test tube. Similar to the stock solution of different concentration chloroform was added to make the final volume of 1ml and 4ml of carr-Price reagent. The absorbance was taken at 620nm.

REDUCING POWER DETERMINATION [21]:

Different amounts of each extract (25-800 µg/ ml) in water were mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10%) was added to the mixture to stop the reaction, then centrifugation was done at 3000 RPM for 10 min. Then the supernatant was taken and the absorbance was measured at 700 nm. The increased value of absorbance indicates higher reducing power. Vitamin C was used as standard drug.

ASSAY OF NITRIC OXIDE-SCAVENGING ACTIVITY [22]:

The procedure is based on the principle that, sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. For the experiment, sodium nitroprusside (10 mm), in phosphate-buffered saline, was mixed with different concentrations of each extract and incubated at room temperature for 150 min. After the period of incubation, 0.5 ml of Griess reagent was added and it was read at 546 nm. Quercetin was used as standard drug.

SCAVENGING OF HYDROGEN PEROXIDE [23]:

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (0.1-1 mg/ ml) in distilled water were added to a hydrogen peroxide solution (0.6ml, 40 mM). The absorbance of hydrogen peroxide was read at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging by the extracts and standard compound were calculated as follows:

% Scavenged [H_2O_2] = [(Ao − A1) /Ao] × 100

Where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample of extract and standard.

RESULTS & DISCUSSIONS:

STUDY OF BIOACTIVE COMPONENTS IN MUSHROOMS AND LICHEN:

In recent years, Interest towards the herbal medicines has been rising than the allopathic medicine in day today market. In addition to different parts of higher plants, mushroom also have raised in the field of folk medicine, Especially Chinese and Japanese traditional medicines, mushroom and lichen play a vital role. In vitro chemical and biological properties of fruiting body are numerous and well documented [24].

Lingzhi is commonly used mushroom in therapeutically field, constituting of wide variety of polyphenolic, polysaccharides compounds have been identified [25].Phellinus Sps were reputed as oriented and Russian folklore, Especially *Phellinus igniarius* has been intensively studied due to their immunological and anticancer properties, as the bioactive/polyphenolic compounds may be responsible for all these properties[26,27].Lichen-*Parmelia perlata* one of the orgin of India, it has been

widely used in the food industry and supplementary drug[28,29], not much studies has been done still now.

QUALITATIVE ANALYSIS:
MYCOCHEMICAL STUDIES:

CONSTITUENTS	G. *lucidum*		*P.iginiarius*		P.sulcata	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Tannin	++	+++	+++	+++	++	+++
Saponin	+	+	+	++	+	++
Flavonoid	++	+++	++	+++	++	+++
Phenol	+	+++	+	++	+	++
Terpernoid	++	+++	+	+++	++	+++
Anthroquoine	+		+		+	

TABLE 1: Phytochemical components.
+-slightly, ++-moderately,+++-strongly

NUTRITIONAL VALUE:

COMPOUNDS	G. *lucidum*	*P.iginiarius*	P.perlata
Amino acid	+	+	-
Imino acid	-	-	+
Protein	+	+	+
Carbohydrate	+	+	+
Lipid	+	+	+
Reducing sugar	+	+	+
Glucose	+	+	+
Cholesterol	-	+	+

TABLE 2: Nutritional components.

The preliminary investigation of Mycochemical analysis and Nutritional analysis (table 1 & 2) revealed the presence of tannin, saponins, flavonoids, phenol, terpenoids, anthroquoine, Glucose, Protein, Carbohydrate, lipid, Amino acid, Reducing sugar and cholesterol, all the three samples gave the best results in water extract but in ethanol extract the colour formation of each sample was more prominent. Ethanol has the power to rupture the cell wall of the given sample, thus help in the release of mycophenolic compounds from the sample than the water extract.

QUANTITATIVE ANALYSIS:

CONSTITUENTS	G. *lucidum*(µg/ml)	*P.iginiarius*(µg/ml)	*P.perlata*(µg/ml)
Phenol	37±1	50±0.8	55±0.6
Tannin	42±2	16±0.6	60±0.2
Flavonoid	13.75±0.3	10±0.5	7.5±0.3
Protein	60±0.5	80±2	100±1.2
Glucose	3.75±0.5	5±0.2	5.25±1

Table 3: Quantitative amount of components.

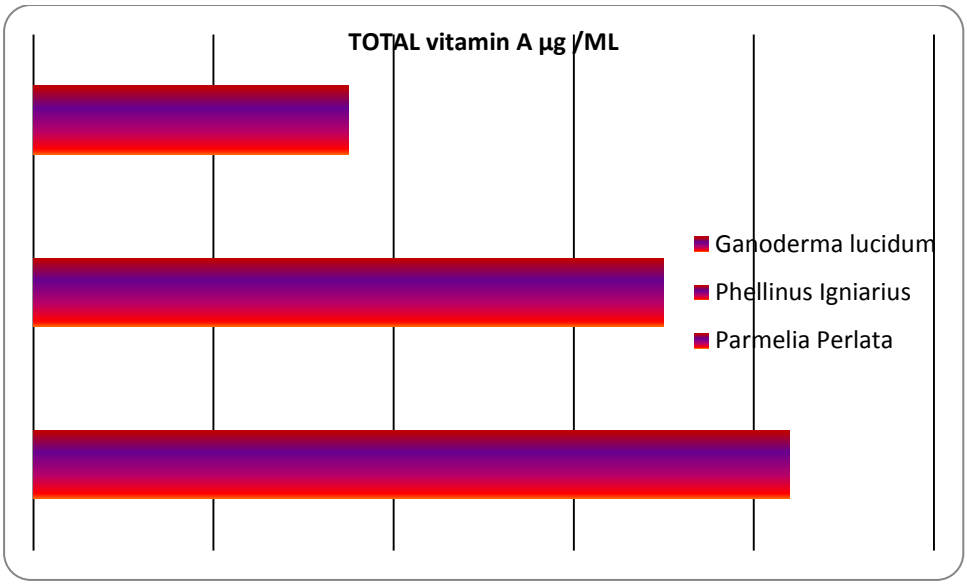
Medicinal mushrooms have been very good dietary supplementary drug helping in healing various ailments in the body [23]. The secondary metabolites present in the mushroom show increase amount of therapeutic application in various fields. The results (table 3) obtained from this quantitative analysis shows *P. perlata* gave the best results for (phenols-55±0.6µg/ml, tannin-60±0.2µg/ml, protein-100±1.2µg/ml, glucose-5.25µg/ml), *G. lucidum* gave the best results for (flavonoids-13.75µg/ml) and *P. igniarius* gave average results for all the compounds. Hence this mushroom and lichen can be used as best food supplements and spices.

ANTIOXIDANT ACTIVITY: VITAMIN A & C:

ESTIMATION OF VITAMIN A:

SAMPLE	TOTAL vitamin A (µg /ML)
Ganoderma lucidum	84±1
Phellinus Igniarius	70±5
Parmelia Perlata	35±1

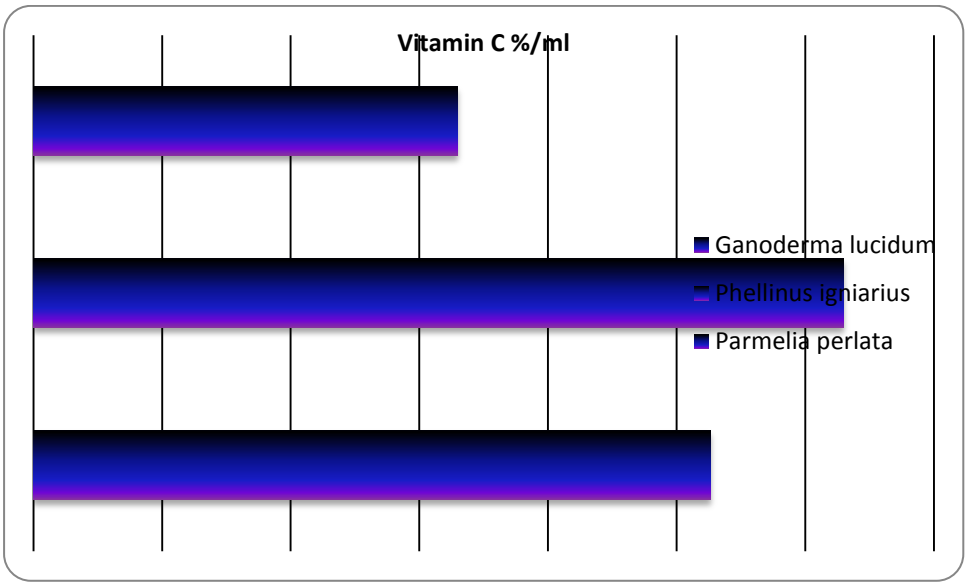
TABLE 8: The amount of Vitamin A present in the samples



GRAPH 1: Estimation of Vitamin A.

SAMPLES	Vitamin C %/ml
Ganoderma lucidum	26.3±2
Phellinus igniarius	31.5±3
Parmelia perlata	16.5±1

TABLE 9: The amount of Vitamin C present in the samples



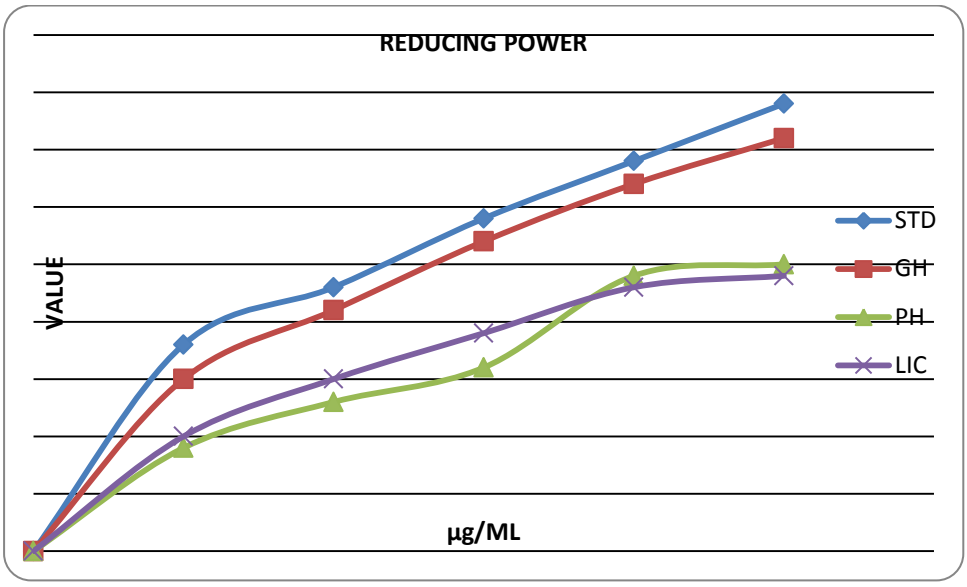
GRAPH 2: Estimation of Vitamin C

The vitamins are the main source of antioxidant compounds that plays vital role in our health system, in that vitamin A and C are best supplementary compounds. The results obtained from studies shows *G. lucidum* (G) have higher amount of vitamin A than other two samples. As per it can be used as better supplementary for vitamin A.(Table 8 & Graph 1) The results obtained from studies shows *P. igniarius*

(P) have higher amount of vitamin C than other two samples. As per it can be used as better supplementary for vitamin C. (Table 9 & Graph 2)

FREE RADICAL SCAVENGING ASSAY:

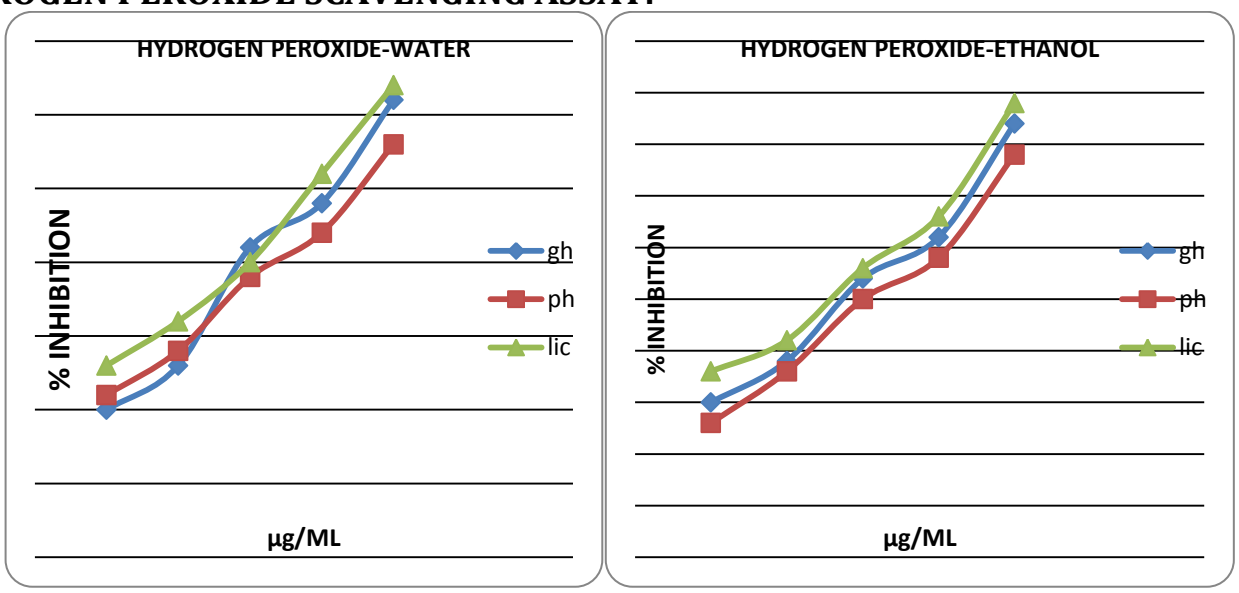
REDUCING POWER ASSAY:



GRAPH 3: Reducing power of the samples.

Fe (III) reduction used as an indicator of electron donating activity, which shows an important mechanism of phenolic antioxidant action. In the reducing power assay, the presence of antioxidants in the samples resulted in the reducing of Ferric iron lll to Ferric iron ll by donating an electron. Amount of Ferric iron ll complex can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. An increase in the absorbance at 700 nm indicates an increase in reductive ability. Graph 3 shows dose-response curves for the reducing powers of the extract. It was found that increase in the concentration of the sample increase the reducing powers. All extracts showed good reducing power, in that *G. lucidum* was more comparable with Ascorbic acid. (Graph 3)

HYDROGEN PEROXIDE SCAVENGING ASSAY:

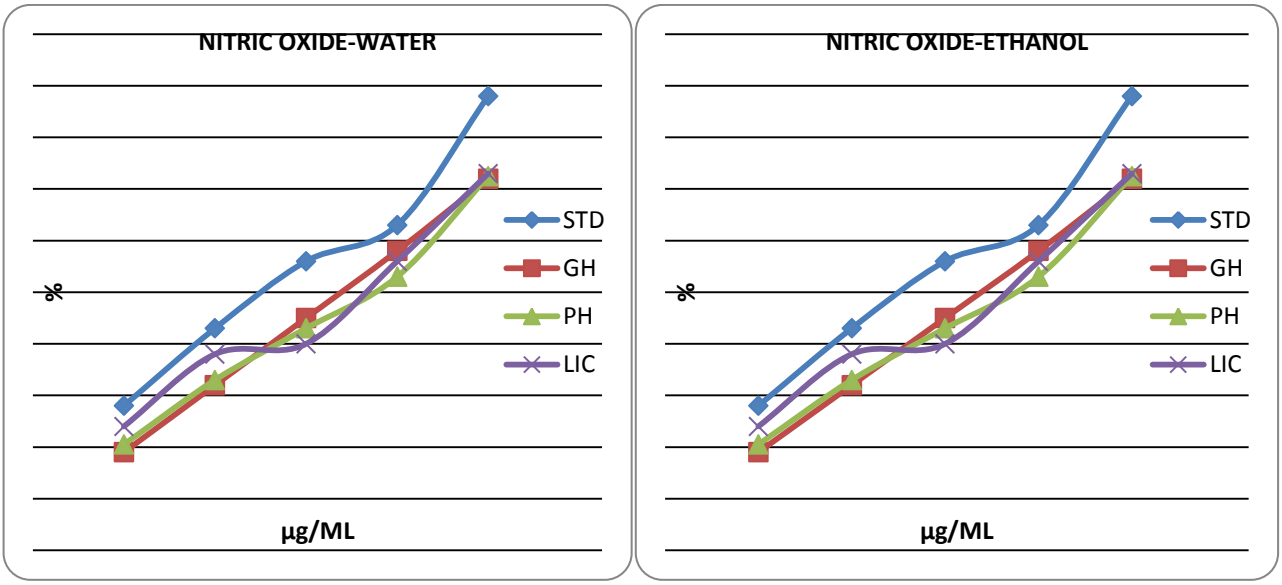


GRAPH 4: Hydrogen peroxide scavenging of the water samples
GRAPH 5: Hydrogen peroxide scavenging of the ethanol samples

Scavenging of H_2O_2 by mushroom and lichen extracts may be endorsed to their phenolics that helping in donating electrons to H_2O_2, thus deactivate it to water. The ability of the extracts to effectively

scavenge hydrogen peroxide was determined and compared with that of vitamin C as standard [26]. The extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. IC50 for scavenging of H_2O_2in water samples were 127±10.2 µg/ ml for *G. lucidum*, 110 ± 6.95 µg/ ml for *P. igniarius*, 111 ± 11.67 µg/ ml for *P. perlata*. IC50 for scavenging of H_2O_2in ethanol samples were 130±1.67 µg/ ml for *G. lucidum*, 115 ± 5 µg/ ml for *P. igniarius*, 118 ± 6.2 µg/ ml for *P. perlata*. The IC50 values for Ascorbic acid are 21.4 ± 0.12 µg/ ml, respectively, but it was not reactive as it gives rise to hydroxyl radicals and it might cause Cytotoxicity. (Graph 4&5).

NITRIC OXIDE SCAVENGING ASSAYS:



GRAPH 6: Nitric oxide scavenging of the water samples
GRAPH 7: Nitric oxide scavenging of the ethanol samples.

The extracts showed good nitric oxide-scavenging activity between0.05 to 0.25 µg/ml. The % inhibition was increased with increasing concentration of the extract. The ethanol extract of samples had shown better scavenging activity when compared with ascorbic acid. Nitric oxide is also implicated in cancer, inflammation and other pathological conditions [24&25]. All extracts exhibited different levels of antioxidant activities and gives better results as compared to standard ascorbic acid. (Graph 6&7)

CONCLUSION:

The results revealed the presence of important biochemical compounds present in the mushroom and lichen samples can be very useful in the treatment of various diseases. Therefore, *G. lucidum*, *P. igniarius* and *P. perlata* could be seen as a good source of food and useful drug. All the extracts exhibited different levels of antioxidant and free radical scavenging assay. Further studies can be done using *in vivo* animal model to study the underlying mechanism of each compound.

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CONFLICT-OF-INTEREST NOTIFICATION:

We authors don't have any conflict in publishing articles.

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