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ELUCIDATION OF ANTIOXIDANT COMPETENCE IN MACROFUNGAL SOLVENT EXTRACTS VIA REDUCING POWER ASSAY

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ABSTRACT

Macrofungi are increasingly recognized as reservoirs of nutraceutical compounds with potent antioxidant attributes, largely due to their abundance of polyphenols, flavonoids, and polysaccharides. The present investigation evaluated the antioxidant potential of four macrofungal species—*Geoporasumneriana*, *Ganoderma leucocontextum*, *Pleurotuspulmonarius*, and *Trametes versicolor*—using the reducing power assay (RPA). Fruiting bodies collected from distinct locations of the Kashmir valley were subjected to freeze-drying, pulverization, and ultrasound-assisted extraction with methanol and water as solvents. Extracts were assessed at 300, 600, 900, and 1200 ppm, while ascorbic acid served as the reference standard. Absorbance values at 700 nm indicated a concentration-dependent increase in reducing activity, reflecting electron-donating efficiency. Among aqueous and methanolic fractions, methanol consistently yielded higher antioxidant activity. The comparative order of reducing power was ascorbic acid > *G. sumneriana* > *G. leucocontextum* > *P. pulmonarius* > *T. versicolor*. Based on regression analysis of absorbance versus concentration, approximate IC_{50} values (ppm) were lowest for *G. sumneriana* (≈420 methanolic; 510 aqueous), followed by *G. leucocontextum* (≈480 methanolic; 560 aqueous), *P. pulmonarius* (≈640 methanolic; 720 aqueous), and *T. versicolor* (≈890 methanolic; 950 aqueous), while ascorbic acid exhibited markedly lower IC_{50} (≈250 ppm), confirming its superior potency. These findings highlight species-specific and solvent-dependent differences, with *G. sumneriana* emerging as the most promising antioxidant source.

***Key words*:** Antioxidant activity, IC_{50} values, Macrofungi, Reducing power assay, Solvent

Introduction

Macrofungi are an underexplored source of essential nutrients, including high-quality proteins, vitamins, dietary fibers, minerals and trace elements. In addition to their nutritional value, macrofungi exhibit a range of pharmacological and therapeutic properties, such as antioxidant, anticancer and anti-inflammatory activities (Fernando *et al.*, 2015). In many Asian countries, wild edible macrofungi are widely consumed as flavorful and nutritious food sources (Vishwakarma *et al.*, 2017). Their utilization as functional foods, dietary supplements and components of traditional medicine has been steadily increasing, owing to their abundant nutrients and multiple health-promoting effects (Lu *et al.*, 2020). Bioactive

Table 1: Details of macrofungal specimens used in the study.

Common name	Scientific name	Family	Higher Taxonomy*	Location	Morphology
Earthcup fungus	*Geoporas-umneriana*	Pyronem-ataceae	Phylum: Ascomycota Class: Pezizomycetes Order: Pezizales	Local market, Baramulla	Forms large subterranean ascocarps, often emerging partially above ground. Fruiting bodies are cup-shaped, pale to brownish, with a brittle texture.
Lingzhi mushroom (Tibetan Ganoderma)	*Ganoderma-leucocon-textum*	Ganoderm-ataceae	Phylum: Basidiomycota Class: Agaricomycetes Order: Polyporales	Dachigam National Park, Srinagar	Perennial woody bracket fungus with a sessile, fan-shaped basidiocarp. The upper surface is zonate and shiny (laccate), while the pore surface is white to cream with fine pores.
Turkey tail	*Trametes versicolor*	Polypo-raceae	Phylum: Basidiomycota Class: Agaricomycetes Order: Polyporales	Dachigam National Park, Srinagar	Thin, leathery basidiocarps with multicolored concentric zones resembling a turkey's tail. Underside has small white pores. Grows in overlapping rosettes on decaying wood.
Oyster mushroom	*Pleurotusp-ulmonarius*	Pleuro-taceae	Phylum: Basidiomycota Class: Agaricomycetes Order: Agaricales	Srigufwara, Anantnag	A fast-growing edible mushroom. Fruiting bodies are shell- or fan-shaped with decurrent gills. The stipe is short or absent and the pileus is whitish to pale brown.

compounds present in edible mushrooms, including phenolics, flavonoids and polysaccharides, contribute to their antioxidant potential. Due to these properties, mushrooms are considered an important natural source of antioxidants and may serve as viable candidates for natural antioxidant additives in food and pharmaceutical applications (Boonsong *et al.*, 2016).

In the present study, the antioxidant activity of selected macrofungal species—*Ganoderma leucocontextum*, *Geoporasumneriana*, *Pleurotuspulmonarius* and *Trametes versicolor*was evaluated to explore their potential as sources of natural antioxidants using reducing power assay (RPA). Reducing power assay is one of the most widely applied spectrophotometric methods to evaluate the antioxidant potential of plant extracts, mushroom extracts and bioactive compounds. It is based on the principle that antioxidants can act as reductants by donating electrons to convert ferric (Fe^{3+}) into ferrous (Fe^{2+}), thereby reflecting their ability to counter oxidative processes (Oyaizu, 1986). In the classical method, test samples in phosphate buffer are incubated with potassium ferricyanide, leading to reduction of ferricyanide (Fe^{3+}) to ferrocyanide (Fe^{2+}). The addition of ferric chloride thereafter results in the formation of a Prussian blue–colored ferric–ferrocyanide complex, whose absorbance is measured at 700 nm and correlates with the sample's reducing power (Oyaizu, 1986; Jayaprakasha *et al.*, 2001). Because this assay relies on electron transfer, it differs mechanistically from free radical scavenging assays such as DPPH and ABTS, thus providing complementary insight into the antioxidant capacity of samples (Duh, 1998). Plant or macrofungal specimen-based phenolics, flavonoids and other phytochemicals rich in hydroxyl groups often display high reducing capacity, which has been linked to their protective roles against lipid peroxidation and oxidative stress in biological and food systems (Yildirim *et al.*, 2001). Therefore, the reducing power assay forms an integral part of antioxidant profiling strategies in phytochemical and pharmacological research.

Materials and Methods

Collection of macrofungal specimens

During the course of this study, four different macrofungal species were collected from diverse locations of the Kashmir valley in the year 2023. *Geoporasumneriana* was obtained from the local market of Baramulla on 28 March, while *Ganoderma leucocontextum* was collected from Dachigam National Park on 30 August. Similarly, *Pleurotuspulmonarius* was collected from Srigufwara, Anantnag on 9 August and *Trametes versicolor* was collected from Dachigam National Park, Srinagar, on 23 September. All the specimens were harvested at the fully mature stage with complete fruit body development.

Sample Preparation and Drying

Fresh fruiting bodies of *Ganoderma leucocontextum*, *Geoporasumneriana*, *Trametes*

Table 2: Reducing Power Assay for antioxidant potential evaluation of Macro-fungi extracts and Ascorbic Acid.

Aqueous extracts								Conc. (ppm)	Methanolic extracts								Ascorbic Acid	
per cent increase				Absorbance					Absorbance				per cent increase					
GS	TV	PP	GL	GS	TV	PP	GL		GS	TV	PP	GL	GS	TV	PP	GL	Abs	PI
560	100	280	440	0.33	0.1	0.19	0.27	300	0.36	0.13	0.23	0.31	620	160	360	520	0.75	1400
680	240	460	660	0.39	0.17	0.28	0.38	600	0.43	0.19	0.31	0.4	760	280	520	700	0.83	1560
1040	360	580	920	0.57	0.23	0.34	0.51	900	0.63	0.26	0.39	0.56	1160	420	680	1020	0.91	1720
1360	460	700	1180	0.73	0.28	0.4	0.64	1200	0.79	0.31	0.47	0.69	1480	520	840	1280	0.99	1880

GS= *Geopara sumneriana;* GL=*Ganoderma leucocontextum;* PP= *Pleurotus pulmunarius;* TV=*Trametes versicolor*, abs. = absorbance; PI: per cent increase

versicolor and *Pleurotuspulmonarius* were collected and immediately frozen at –80 °C. The frozen samples were lyophilized in the Division of Post-Harvest Management, SKUAST-Kashmir, Shalimar. The resulting dry specimens were ground into a fine powder using a laboratory mill/grinder.

Ultrasound-Assisted Extraction (UAE)

The powdered macrofungal samples were subjected to ultrasound-assisted extraction at the Islamic University of Science and Technology (IUST), Awantipora, Jammu and Kashmir. Based on the established protocol by Valu *et al.*, (2020) for *Hericiumerinaceus*, which effectively isolates polyphenols and antioxidants using ultrasonication, the following extraction conditions were adopted (slight modifications of the established protocol):

- **Extraction Device:** Probe-type ultrasonicator (Ultrasonicator Coleparmer 04711-35), selected for its superior efficiency in disrupting the chitin-rich fungal cell wall (facilitating higher extraction yields compared to bath-type sonication).
- **Solvents used:** Methanol and water.
- **Solute to Solvent Ratio:** 5:30 (g/mL).
- **Extraction Time:** 40 minutes.
- **Temperature Control:** Samples were kept in an ice bath with continuous magnetic stirring to maintain low temperatures and prevent thermal degradation of heat-sensitive compounds.

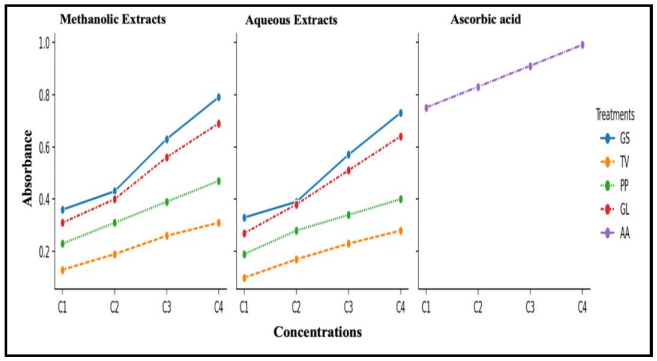

Fig. 1: Reducing Power Assay for antioxidant potential evaluation of methanolic and aqueous.

After ultrasonication, the extracts were filtered and centrifuged at ~2500 rpm for 5 minutes to remove particulates. The supernatants underwent solvent removal via rotary evaporation to yield dry extract powders suitable for downstream analyses.

Reducing Power Assay for antioxidant Evaluation

The reducing potential of the extracts was determined by the method of Oyaizu (1986) with slight modifications. Briefly, 1 ml of extract solution at different concentrations (300, 600, 900 and 1200 ppm) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1% w/v). The mixture was incubated at 50 °C for 20 minutes, followed by the addition of 2.5 ml of trichloroacetic acid (10% w/v). The solution was then centrifuged at 3000 rpm for 10 minutes and 2.5 ml of the upper layer was collected. This fraction was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance of the reaction mixture was recorded at 700 nm. Ascorbic acid, at the same concentrations, served as the reference standard.

The antioxidant potential was expressed as a percentage increase in absorbance, which was calculated using the following formula:

$$Per\ cent\ increase\ in\ absorbance = \frac{A\ sample - A\ control}{A\ control} \times 100$$

Where,

A_{sample}: Absorbance of the extract/standard

$A_{control}$: Absorbance of the control mixture without extract or standard

Ahigher percentage increase in absorbance means the extract is better at donating electrons, i.e., stronger antioxidant/reducing power.

Results and Discussion

The antioxidant potential of aqueous and methanolic extracts of four macrofungal specimens, namely *Geoporasumneriana*, *Ganoderma leucocontextum*, *Pleurotuspulmonarius* and *Trametes versicolor* was evaluated using the reducing power assay at concentrations of 300, 600, 900 and 1200 ppm. Ascorbic

acid was chosen as the positive control and was also evaluated at the same concentrations. Absorbance values were recorded at 700 nm using a spectrophotometer and blank readings were used to calculate the per cent increase in absorbance over the control. The results of the study are presented in Table 2 and illustrated in Fig. 1.

For aqueous extracts, *G. sumneriana* showed the strongest reducing activity, with absorbance values of 0.33, 0.39, 0.57 and 0.73 corresponding to 560, 680, 1040 and 1360 per cent increase in absorbance over control at 300, 600, 900 and 1200 ppm, respectively. *G. leucocontextum* followed, with absorbance values of 0.27, 0.38, 0.51 and 0.64 translating into 440, 660, 920 and 1180 per cent increase in absorbance over control. *P. pulmonarius* exhibited moderate activity with absorbance values of 0.19, 0.28, 0.34 and 0.40 corresponding to 280, 460, 580 and 700 per cent increase in absorbance over control. *T. versicolor* consistently recorded the lowest activity with absorbance values of 0.10, 0.17, 0.23 and 0.28, which equated to 100, 240, 360 and 460 per cent increase in absorbance over control. For methanolic extracts, *G. sumneriana* again exhibited the highest activity, with absorbance values of 0.36, 0.43, 0.63 and 0.79 corresponding to 620, 760, 1160 and 1480 per cent increase in absorbance over control. *G. leucocontextum showed* slightly lower activity, with absorbance values of 0.31, 0.40, 0.56 and 0.69, translating into 520, 700, 1020 and 1280 per cent increase in absorbance over control. *P. pulmonarius* recorded 0.23, 0.31, 0.39 and 0.47 absorbance values, corresponding to 360, 520, 680 and 840 per cent increase in absorbance over control. *T. versicolor* was lowest, with absorbance values of 0.13, 0.19, 0.26 and 0.31 which translated to 160, 280, 420 and 520 per cent increase in absorbance over the control. The standard antioxidant, ascorbic acid, evaluated at the same concentrations (300–1200 ppm), exhibited markedly higher activity than both aqueous and methanolic extracts. Absorbance values were 0.75, 0.83, 0.91 and 0.99 at 300, 600, 900 and 1200 ppm respectively, corresponding to 1400, 1560, 1720 and 1880 per cent increase in absorbance over control.

Overall, both aqueous and methanolic extracts of all four macrofungi demonstrated concentration-dependent antioxidant potential, with methanolic extracts being slightly more potent than aqueous ones. Among the tested species, *Geoporasumneriana* consistently showed the strongest reducing power, followed by *Ganoderma leucocontextum*, *Pleurotuspulmonarius* and *Trametes versicolor*. However, all extracts were lower in activity compared to the standard ascorbic acid.

The antioxidant activities observed in aqueous and methanolic extracts of *Geoporasumneriana*, *Ganoderma leucocontextum*, *Pleurotuspulmonarius* and *Trametes versicolor* are consistent with studies demonstrating significant redox potential among edible and medicinal macrofungi (Mwangi *et al*., 2022; Abdullah *et al*., 2011). The concentration-dependent increase in absorbance values, particularly for *G. sumneriana*, agrees with reports that mushroom extracts exhibit higher reducing power at elevated concentrations due to increased electron-donating antioxidants (Kosanic *et al*., 2012). Methanolic extracts outperformed aqueous extracts, corroborating that organic solvents improve the extraction efficiency of phenolic and flavonoid compounds, which strongly correlate with antioxidant activity in mushrooms (Giraldo *et al*., 2023; Michalska *et al*., 2025). Ascorbic acid, the positive control, exhibited markedly higher reducing power than fungal extracts, consistent with its established potent electron-donating ability and common use as a standard in reducing power assays (Abdullah *et al*., 2011; Giraldo *et al*., 2023). Mushroom extracts, while less potent than ascorbic acid, have been shown to confer health benefits, especially when rich in phenolics and polysaccharides due to antioxidant properties: Kosanic *et al*., 2012. The reducing power order found (*G. sumneriana* > *G. leucocontextum* > *P. pulmonarius* > *T. versicolor*) aligns with observations on species-specific antioxidant variation driven by differing profiles of polyphenols, flavonoids and carotenoids (Mwangi *et al*., 2022; Abdullah *et al*., 2011; Giraldo*et al*., 2023). These metabolites are crucial determinants of mushrooms' biological oxidative stress defense (Mwangi *et al*., 2022).

In summary, this study reinforces the value of macrofungi as natural antioxidant sources and highlights the importance of solvent and species factors in antioxidant extraction. These results are substantiated by extensive recent research using diverse macrofungal species and standardized antioxidant assays.

Conclusion

In conclusion, the current findings not only confirm macrofungi as valuable sources of natural antioxidants but also highlight that solvent choice significantly influences extract activity. Methanolic extracts exhibited higher antioxidant activity than the aqueous extracts. Overall trend of antioxidant potential in selected macrofungal extracts was Ascorbic acid >*Geoporasumneriana*> *Ganoderma leucocontextum*> *Pleurotuspulmunarius*> *Trametes versicolor* across all the concentrations.

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