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Phytochemical Profiling, *In-Vitro* Antioxidant, and Antidiabetic Evaluation of *Morchella esculenta*: a Comprehensive Investigation

Keshav Anand [1], Debojyoti Mandal [2], Pranav Kumar Parbhakar [3,*]

[1] Department of Medical Laboratory Sciences, School of Allied Medical Sciences, Lovely Professional University, Phagwara Punjab, India-144411; keshavanand836@gmail.com (K.A.);
[2] Department of Botany, School of Bioengineering and Biosciences, Lovely Professional University, Phagwara Punjab, India-144411; debojyoti2108@gmail.com (D.M.);
[3] Parul Institute of Applied Sciences, and Research and Development Cell, Parul University, Post Limda, Waghodia, Gujarat, 391760 India; prabhakar.iitm@gmail.com (P.K.P.);
* Correspondence: prabhakar.iitm@gmail.com;

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Abstract: The morel, *Morchella esculenta*, is a treasured culinary fungus that is also a rich source of bioactive chemicals with an extensive spectrum of medicinal benefits. Recent studies have demonstrated that morel mushrooms have antioxidant, anti-inflammatory, and antidiabetic qualities that have already been employed in the context of the conventional medical system for many years. Locals utilize *Morchella esculenta* to treat diabetes and other illnesses, although the medicinal components have not been thoroughly characterized. The purpose of this study was to do a qualitative phytochemical analysis of *Morchella esculenta* extract in order to determine its contents. In order to separate the metabolites, solvents like methanol, chloroform, and ethanol were used. Different phytochemical methods were used to analyze the extracted metabolites, revealing that they included phenol, saponin, alkaloid, tannin, steroids, terpenoid, flavonoid, and phenolic compounds. Furthermore, the scavenging activity of the plant *Morchella esculenta* was measured using ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid)), DPPH (1,1-diphenyl-2-picrilhydrazil), Lipid peroxidation, Nitric oxide, and Hydrogen peroxide. The antioxidant efficacy of chloroform as a major solvent was established during scavenging. Additionally, the α-glucosidase inhibitory activity assay revealed an antidiabetic effect. The investigation validates the occurrence of alkaloids, flavonoids, phenols, saponins, and tannins. Free radical scavenging and antioxidant properties have also been confirmed by the experiment.

Keywords: *Morchella esculenta*; phenolics; antioxidant; free radical scavenging; anti-inflammatory; diabetes.

1. Introduction

Diabetes mellitus, also known as simply diabetes, is among the most common types of systemic disease in the world. Diabetes mellitus develops when the body either develops insulin resistance or is unable to produce enough insulin [1]. The incidence of diabetes mellitus in all of its forms, according to data from the WHO (World Health Organization), has soared over the course of the previous few decades worldwide. According to the estimates provided by the International Diabetes Federation (IDF) for 2019, the number of adults aged 20–79 years

old who are living with diabetes mellitus (DM) in the world is approximately 463 million. It is anticipated that this number will reach 578.4 million by 2030 and 700.2 million by 2045 [2]. Rates of diabetes mellitus (DM) could rise in emerging countries, particularly in comparison to rates in developed countries. There are currently no safe preventative medicines available despite the alarmingly high rates of diabetes mellitus. Rising rates of diabetes mellitus (DM) are possible in developing countries, especially when compared to rates in developed countries. There are currently no safe preventative medicines available despite the alarmingly high rates of diabetes mellitus [3].

For centuries, people have turned to plants, fungi, bacteria, and other forms of life for their medicinal needs, harvesting their own natural products and compounds to create a wide variety of preparations made from natural ingredients that can be used to treat a wide range of medical issues [4].

Mushrooms are relatively high fungi that have been used by people for a long time. Since prehistoric times, people from many different cultures have eaten mushrooms and used them as medicine [5]. Antioxidants, anti-inflammatory, antimutagenic, and antidiabetic bioactive components have been found in mushrooms [6]. Biophysical and biochemical approaches have provided a detailed description of the polysaccharides in many mushrooms whose bioactivities hold promise for the therapeutic application of various illnesses [7].

Any chemical that delays or prevents oxidative stress to a specific molecule is termed an antioxidant [8]. The potential to neutralize potentially harmful free radicals is an essential quality of an antioxidant. Antioxidant substances, e.g., phenolic compounds and flavonoids, neutralize free radicals like peroxide, hydroperoxide, or lipid peroxyl, preventing them from triggering the oxidative pathways that cause degenerative illnesses [9]. Since earlier civilizations, both herbal plants and fungi have been thought of as having beneficial antioxidant properties.

Morchella esculenta (Guchi or sponge mushroom) belongs to the family Morchellaceae. It is one of the wild species of mushroom that has the most potential for economic benefit and importance in the field of medicine [10]. Fruiting bodies of *Morchella esculenta* are packed with diverse bioactive components, including vitamin supplements, peptides, stimulants, mineral resources, carbohydrates, natural polymers, and polysaccharides [11]. Substantial anticarcinogenic, neuroprotective, anti-parasitic, cardioprotective, antimicrobial, antiallergic, and antidiabetic properties are exhibited by *Morchella esculenta* as well as its bioactive constituents [12].

Nature provides a plethora of resources with potential medical applications. *Morchella esculenta* extracts have been linked to positive effects on glucose metabolism, but their role in diabetes remains poorly understood. This research was conducted to find out how well three different solvents could preserve the antidiabetic and antioxidant effects of an extract of *Morchella esculenta*.

2. Materials and Methods

2.1. Sample collection and processing.

Morchella esculenta had been gathered from their natural environment in the mountainous region of Jammu (J&K), India. An in-house mycologist from the Jammu and Kashmir Department of Forestry was tasked with carrying out the investigation. After being

washed in distilled water to eliminate any remaining soil particles, the mushrooms were air-dried in the shade before being crushed in a grinder.

2.2. Identification.

Identification is done from Govt. Forest Dept. of Jammu & Kashmir.

2.3. Preparation of Extract.

Organic solvent extraction was used to separate the phytochemicals. Soxhlet extraction technique was used for the organic material [13]. Extractions were made by placing 25 g of dried plant powder in a glass thimble and then independently extracting them with 250 ml of various solvents like chloroform, ethanol, and methanol [14]. The extraction process is finished when the solvent in the siphon tube of the Soxhlet device has become colorless. After that, the extract was evaporated at 35° C in a rotatory vacuum evaporator. The dried plant's crude extract was kept in the fridge at a temperature of 2 to 8° C until usage [15].

2.4. Qualitative photochemical analysis.

The extract solution was made by dissolving 200 mg of the extract in 3 ml of the solvent and made up to 20 ml with distilled water. We followed the same procedure for three solvents, i.e., methanol, ethanol, and chloroform [16].

Photochemical Tests [15-17]:

Detection of Saponins (Foam test) - The mixture was shaken vigorously after simply adding distilled water and 2 ml of extract. The existence of saponins was demonstrated by the development of foam in the experiment.

Detection of Tannin (Ferric chloride test) -The 1 ml extract was combined with 2 ml of FeCl3. The development of a greenish-black coloration demonstrates the existence of tannins.

Detection of Terpenoids, steroids (Salkowski test) -1 ml of sample was combined with chloroform and concentrated H2SO4. The presence of terpenoids is indicated by a ruddy-brown hue at the interface. A red hue appears in the lower chloroform layer, indicating the existence of steroids.

Test for Glycosides (Keller-Kilani test) - 2 ml of H_2SO_4 was combined with the sample. The creation of a brown color suggested the existence of glycoside.

Test for Flavonoid (Alkaline reagent test) - 2 ml sample was combined with sodium hydroxide and diluted hydrochloric acid. The development and fading of yellow signify the presence of flavonoids in the extracted sample.

Test of Alkaloid (Mayer's test) – 2 ml of sample extract and concentrated hydrochloric acid had been mixed in addition to a few drops of Mayer's reagent. The presence of alkaloids is denoted by a green or white precipitate.

Test of Quinones (Borntrager's test) - Formation of red color appeared when 1 ml of extract, H2SO4 (conc.) was mixed, which confirmed the quinines existence.

Test of Phenols (Ferric chloride test) - 1 ml of the extract was dissolved in 2 ml filtered water, then 3 to 4 drops of 10% ferric chloride. The appearance of a blue, green, or bluish-black hue confirms the inclusion of phenols.

Test of Coumarins - 1ml of sodium hydroxide was combined to extract the sample. The formation of a yellowish color confirms the existence of coumarins.

Test of Carbohydrates (Molisch test) - 1 ml of Molisch reagent was mixed with extract. 1 ml of concentrated H_2SO_4 was carefully added. The mixed sample was left alone for around three minutes. If a purple, crimson, or drab violet color develops between the two layers, carbohydrates are present.

2.5. In vitro analysis.

2.5.1 1,1-diphenyl-2-picrilhydrazil (DPPH) assay.

To evaluate the antioxidant efficacy of the extracts, we adopted the DPPH free radical scavenging experiment published by Nithianantham *et al*. [17] and Zuraini *et al*. [18]. The original protocol was changed in a few ways. To summarise, 50 microliter of *Morchella esculenta* extracts concentration ranging from 0.2 mg/ml to 1 mg/ml were placed inside a glass beaker, and then 5 ml of DPPH solution [0.004% (w/v)] had also been added to the beaker. The resulting mixture was vortexed, and absorbance at 517 nm was obtained using a spectrophotometer after 30 minutes of incubation at room temperature in a moderately dark atmosphere. Ascorbic acid was used to make a comparison [19]. Triplicate readings were taken for each parameter. The equation below was used to determine the DPPH scavenging efficiency.

$$DPPH\ scavenging\ assay\ (\%se) = \left[\frac{(A_{C(0)} - A_{A(t)}}{A_{C(0)}}\right] X100$$

A is the absorbance with the extract present, while A0 is the absorbance of the negative control (a 0.004% DPPH solution).

By graphing the percentage of DPPH scavenging efficacy against the extract's various concentrates, we could calculate the concentration at which DPPH color was reduced by 50% (IC50).

2.5.2. 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS) assay.

We measured the samples' ability to quench free radicals with an ABTS radical cation decolorization assay, following a procedure based on that described by RE *et al*.,1999 [20]. The ABTS was diluted to 7 mM by dissolving it in water. The ABTS cationic radicals (ABTS*+) were synthesized by mixing ABTS standard solution containing 2.45 mM potassium persulphate (highest concentration) [21]. Before utilizing the mixture, it was given time to settle in the dark and at room temperature for between 12 and 16 hours so that ABTS radical cation could be formed. When kept at room temperature and dark, the free radical was able to maintain its stability for more than two days. For the purpose of analyzing the test samples, the ABTS*+ solutions were adjusted to have an absorbance of 0.700 (0.02) at 734 nm after being diluted with 100% ethanol and equilibrated at 30°C. Blank reading was taken (A0). After adding of 2ml of diluted ABTS*+ solution (A734 nm = 0.700(0.02) to 50 µL of test material (0.2 to 1 mg/mL), the absorbance reading was measured at 30°C precisely six minutes after the first mixing (At). Each test included a solvent blank to ensure accurate results. A minimum of three separate assessments were performed. Using the above method and the decline in absorbance between A0 and At, we were able to determine the inhibition percentage of absorbance at 734 nm.

$$Inhibition\ of\ ABTS\ radical\ (\%se) = \left[\frac{(A_{C(0)} - A_{A(t)}}{A_{C(0)}}\right] X100$$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidant at t = 6 min.

The graph was plotted with the inhibition percentage versus the concentrations, and the values for IC50 were determined from that graph.

2.5.3. Nitric oxide inhibitory assay (NO inhibitory assay).

The Griess reaction was used to quantify nitric oxide (NO) produced from sodium nitroprusside in an aqueous solution at a physiological pH. Sodium nitroprusside (10 mM) in phosphate-buffered saline and the test extract (0.2 mg/ml to 1 mg/ml) were combined in a 3 ml reaction mixture and incubated at 25°C for 150 minutes [22]. After waiting for the incubation period to end, 1.5 ml of the reaction mixture was discarded, and 1.5 ml of Griess reagent (1% sulphanilamide, 2% orthophosphoric acid, and 0.1% Napthylethyline diamine hydrochloride) had been applied [23]. A chromophore was generated, and its absorbance was measured at 546 nm. Inhibition of nitric oxide scavenging as a percentage was determined using the given formula.

$$Nitric\ oxide\ scavenging\ assay\ (\%se) = \left[\frac{(A_C - A_A}{A_C}\right] X100$$

2.5.4. Hydrogen peroxide scavenging activity.

We applied the technique described by Ruch *et al.*, 1989 [24] to determine the plant extract's ability to remove hydrogen peroxide. A 4 ml mixture of plant extract prepared in distilled water (at varying concentrations) and a 0.6 ml mixture of 4 mM H_2O_2 solutions made in phosphate buffer (0.1 M pH 7.4) was incubated for 10 minutes [25]. At 230 nm, the absorbance was taken off the solution compared to a blank solution made up of the plant extract, excluding H_2O_2. The control consisted of the reaction mixture, including H_2O_2 without plant extract. The following equation was used to determine the percentage of hydrogen peroxide radical that was suppressed by the extract.

$$H_2O_2\ scavenging\ assay\ (\%se) = \left[\frac{(A_C - A_T}{A_C}\right] X100$$

where Abs control is the absorbance of H_2O_2 radical; Abs sample is the absorbance of H_2O_2 radical + sample extract.

2.5.5. Lipid peroxidation.

The amount of lipid peroxidation caused by the $FeSO_4$-ascorbate system in sheep liver homogenates was calculated using the method developed by Ohkawa *et al.*,1979 [26] with reference to thiobarbituric acid reactive substances (TBARS). The reaction mixture was comprised of 0.1 ml of sheep liver homogenate (at a concentration of 25 %) in Tris-HCl buffer (20 mM, pH 7.0); KCl (30 mM); $FeSO_4$ (NH_4) $SO_4.7H_2O$ (0.06 mM) and different concentrations of synthesized peptides in a total volume of 0.5 milliliters. The mixture was incubated at 37 °C. A volume of 0.4 ml is withdrawn after incubation and treated with 0.2 ml of sodium dodecyl sulfate (8.1%), 1.5 ml of thiobarbituric acid (TBA) (0.8%), and 1.5 ml of trichloroacetic acid (20%). Add enough distilled water to bring the total volume to 4.0 ml, and then the sample was kept at 95 °C for 1 hour. The reaction mixture was cooled, then 1.0 ml of distilled water and 5.0 ml of n-butanol and pyridine mixture (15:1) were added, and the whole

thing was shaken and centrifuged at 4000g for 10 minutes. The absorbances were taken at 532 nm after removing the butanol pyridine layer. A phosphate buffer was used instead of peptide in a similar control experiment. By comparing the test sample's OD to the OD of the control, we calculated the extent to which lipid peroxidation has been inhibited [27].

$$Lipid\ Peroxidation\ assay\ (\%se) = \left[\frac{(A_{C\ at\ 532\ nm} - A_{T\ at\ 532\ nm}}{A_{C\ at\ 532\ nm}}\right] X100$$

2.5.6. Anti-diabetic activity(α- glucosidase inhibitory activity).

The purpose of this experiment was to compare the antidiabetic efficacy of various extracts. Watanabe *et al.* (1997)'s antidiabetic test protocol was adopted in this study [28]. For the enzyme, we used 0.7 U of yeast α-glucosidase into 100 mM phosphate buffer (pH 7.0) containing 2 g/l of bovine serum albumin; for the substrate, we used 0.2 g/l of sodium nitrite and 5 mM of pnitrophenyl—D-glucopyranoside (used as an enzyme) in the same buffer (pH 7.0). When the enzyme solution (1000 µl) and the test sample (100 µl) of varying quantities were combined, the absorbance of the mixture(at different concentrations) at 405 nm was determined by using a spectrophotometer (UV1800 Shimadzu, Japan)[29]. Following an initial incubation period of 5 minutes, 50 microliters of the substrate solution were added, and the resulting mixture was incubated for another 5 minutes. We quantified the amount by which the absorbance had increased beginning at time zero, and we calculated the inhibitory activity as a percentage of the blank control. The following formula was used to determine the percentage of inhibition.

$$Inhibition\ of\ \alpha - glucosidase\ assay\ (\%se)\ \left[\frac{(A_{C\ at\ 405\ nm} - A_{T\ at\ 405\ nm}}{A_{C\ at\ 405\ nm}}\right] X100$$

3. Results and Discussion

3.1. Phytochemical extraction.

In the phytochemical study of *Morchella esculenta*, which was performed using the Soxhlet extraction method that was described before, many pharmacologically active components were shown to be present. These constituents are given below [Table 1].

It has been revealed the inclusion of saponins, tannins, flavonoids, alkaloids, phenols, and carbohydrates with methanolic extraction. Terpenoids, glycosides, quinones, coumarins, and carbohydrates were all detected with ethanolic extraction [30]. The prevalence of saponins, terpenoids, glycosides, alkaloids, quinones, and carbohydrates was finally revealed by chloroformic extraction in the experiment [31].

Table 1. Phytochemical analysis for methanolic, ethanolic, and chloroform extract of *Morchella esculenta.*

Sl No	Phytochemical test	Compounds for detection	Solvents		
			Methanol	Ethanol	Chloroform
1.	Foam test	Saponins	+	-	+
2.	Ferric chloride test	Tannin	+	-	-
3.	Salkowski test	Terpenoids	-	+	+
		Steroids	-	-	-
4.	Keller-Kilani test	Glycosides	-	+	+
5.	Alkaline reagent test	Flavonoids	+	+	-
6.	Mayer's test	Alkaloids	+	-	+
7.	Borntrager's test	Quinones	-	+	+
8.	Ferric chloride test	Phenols	+	-	-
9.	Alkaline reagent test	Coumarins	-	+	-

Sl No	Phytochemical test	Compounds for detection	Solvents		
			Methanol	Ethanol	Chloroform
10.	Molisch test	Carbohydrates	+	+	+

3.2. Free radical inhibition of extracts.

3.2.1 DPPH assay.

The free radical-scavenging capabilities of bioactive substances are, in most instances, the source of these compounds' antioxidant capabilities. When it comes to determining the level of radical scavenging activity that a sample possesses, the DPPH assay is one of the most commonly used procedures. DPPH is a nitrogen-centered powerful oxidizing agent that is known for its stability and generates a violet color when dissolved in methanol. The color loss in the solution caused by the reaction between DPPH radicals and a suitable reducing agent acting as antioxidants are proportional to the number of electrons absorbed. The figure displays the samples' DPPH radical scavenging activities (Figure 1).

Different extractions had shown comparable activity in a range between 20 to 65%. Our results showed that ethanolic extract has a maximum inhibition rate of 65.59%. Chloroform had shown inhibition of free radical DPPH (16.51 ± 0.45, 22.66 ± 0.71, 30.35 ± 0.44, 38.58 ± 0.58, 57.66 ± 0.29) with increasing extraction concentration (0.2 mg/ml to 1 mg/ml). On the other hand, the methanolic extract showed the lowest inhibition rate, i.e., 1.85, 5.2, 8.64, 14.45, and 19.24 % with increased concentration. The results showed that *Morchella esculenta* exhibits significant free radical scavenging potential, especially its ethanol extract (IC 50:0.783mg/mL), while IC50 values of chloroform extract & methanolic extract were 0.93mg/mL and 2.62mg/mL, respectively (Figure 1).

The hydroxyl groups found in plant polyphenols can donate hydrogen, enabling them to function as reductants and anti-oxidants [32]. The DPPH activities of the extracts are comparable to those of ascorbic acid and are quite significant. As a result, we can conclude that the demonstrated antioxidant properties in this investigation are due to the presence of these polyphenols, capable of snuffing out free radicals within the human body and preventing oxidative damage caused by free radicals.

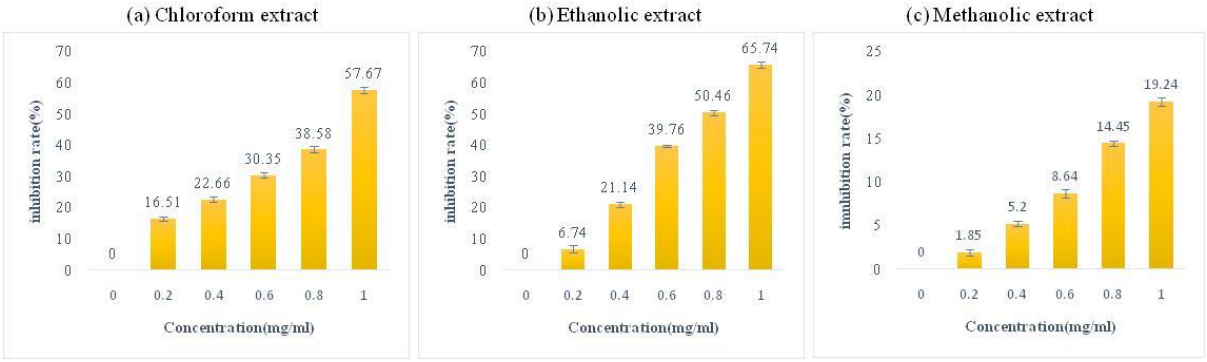

Figure 1. DPPH scavenging assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

3.2.2. ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid)) assay.

In vitro, antioxidant activity is measured using the ABTS radical scavenging assay, based on the reaction between ABTS and a hydrogen-donating oxidizing agent, such as potassium persulfate, which produces a blue-green chromophore. Among the extracts, the

highest ABTS free radical scavenging activity in ethanol (50.38±0.65%), followed by chloroform (42.31±0.42%) and lastly, methanol (35.09±0.41%) (Figure 2).

A comparison was made between the comparative antioxidant potential for scavenging the radical ABTS+ and the standard ascorbic acid, and the results are quite significant.

It is essential to note that the results obtained by the ABTS assay for chloroformic and ethanolic extracts of plant species were noticeably less than that of values obtained by the DPPH assay for the same plant species. On the other hand, methanolic extracts demonstrated a higher ABTS inhibition rate than the DPPH inhibition rate. The data shows this to be the case.

The DPPH and ABTS assays were used to examine the antioxidant power of the selected solvents, and the findings revealed that the solvents helped treat diabetes. In addition, the findings of the ABTS test provide further evidence that the extract contains phenolic chemicals, which have a role in the radical scavenging capabilities of the plant extracts.

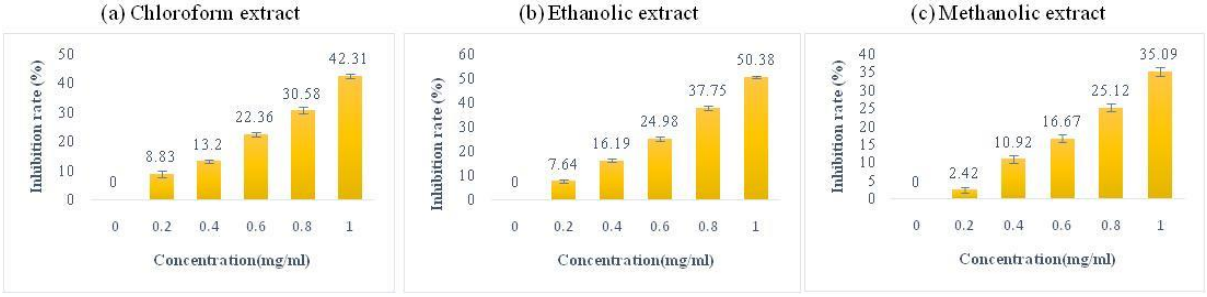

Figure 2. ABTS assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

3.2.3. Nitric oxide inhibitory assay (NO inhibitory assay).

The various mushroom extracts utilized in this experiment suppressed NO generation in a concentration-dependent way, as demonstrated in the experiment results. The ethanolic extract demonstrated the greatest NO inhibitory action among the extracts, with an inhibition efficiency of 35.5% at a 100 mg/ml concentration (Figure 3). This was followed by the chloroform extract (27.050.85%) and the methanol extract (14.841.4%).

Sodium nitroprusside generates nitric oxide, a free radical when it combines with oxygen to make nitrite; nitric oxide triggers the inflammatory response; and peroxynitrite, a by-product of the reaction between nitric oxide and $O_2^{\bullet}$ radicals, increases the toxicity of nitric oxide [33]. These findings point to the selected extracts as potential innovative pharmaceutical substances for NO scavenging and managing pathological diseases induced by excessive NO and peroxynitrite production.

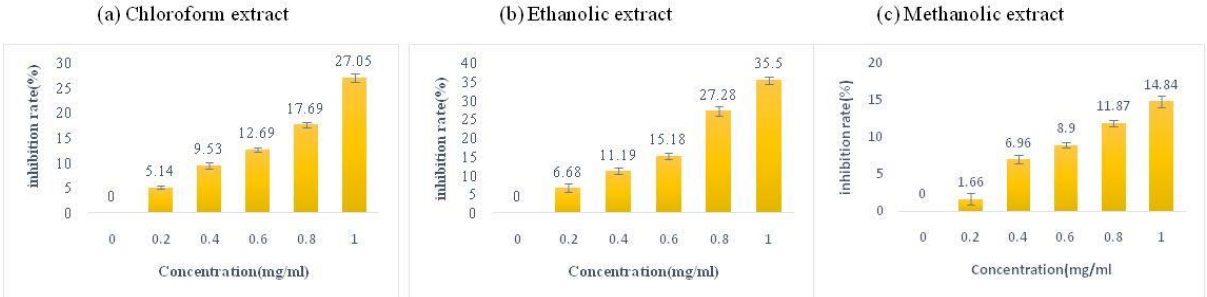

Figure 3. Nitric oxide inhibitory assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

3.2.4. H_2O_2 Scavenging assay.

The scrounging impact of different extracts of *Morchella esculenta* on hydrogen peroxide was concentration-dependent (0.2-1 mg/mL), as shown in (Figure 4). Ethanolic extract (46.15%) had slightly higher scavenging efficacy than chloroform (38.89%) and methanol (15.81%). Extracts showed their activity in a concentration-dependent manner. The ability to scavenge H_2O_2 followed the same pattern observed for DPPH, with ethanol being the most effective, followed by chloroform and methanol [34].

In addition, the ethanolic, chloroform, and methanolic extracts of *Morchella esculenta* have respective IC50 values for scavenging H_2O_2 of 1.10, 1.28, and 3.27 mg/ml. The highly reactive hydroxyl radical (•OH), which is produced as a by-product of a biological Fenton reaction (hydrogen peroxide with Fe^{2+} and Cu^{2+}), is what causes the cytotoxicity effects caused by the disruption of [Ca^{2+}] homeostasis [35]. Based on the findings, it was determined that *Morchella esculenta*, which exhibited hydrogen peroxide scavenging activity, may be a valuable source of antioxidants for removing H_2O_2.

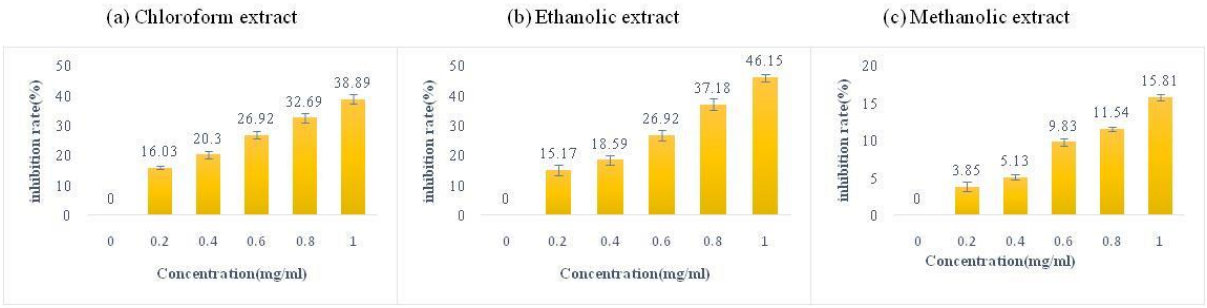

Figure 4. H_2O_2 scavenging assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

3.2.5. Lipid peroxidation(LPO) assay.

As per experimental results, we ended up finding that ethanol had the most lipid peroxidation activity with the lowest IC50 value, i.e., 43.18±0.71% (IC50 1.18), followed by chloroform with 35.56% and then ethanol with 28.79 % Ethanolic extract has highest IC50 value 1.74 mg/ml. It was shown that the extracts' activity levels increased with increasing concentration.

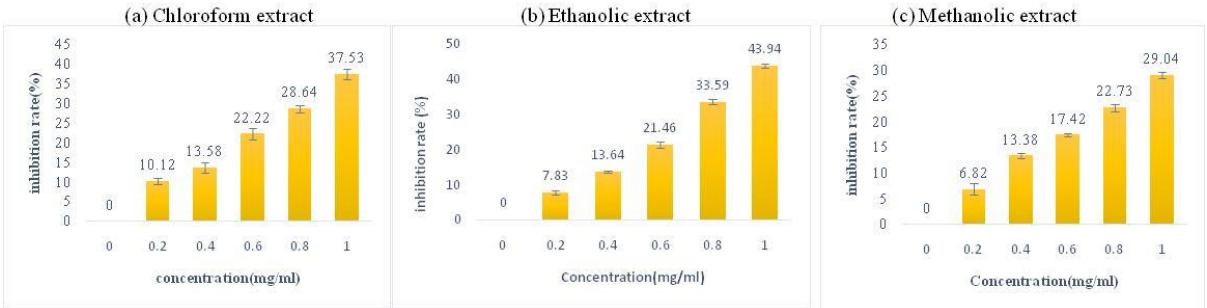

Figure 5. Lipid peroxidation (LPO) assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

Lipid peroxidation ability exhibited the order (Ethanol > Chloroform >Methanol) (Figure 5). The hydroxyl radical serves as the most reactive form of reactive oxygen species (ROS), and it may trigger lipid peroxidation via targeting polyunsaturated fatty acids (PUFA); hence, lipid peroxidation is regarded to be a viable target for assessment of oxidative stress.

Unchecked lipid peroxidation results in membrane rupture and cell death by generating lipid peroxyl radicals, hydroperoxides, and other oxidation products [36]. This LPO inhibitory activity is thus highly important and useful in the field of medicine [37,38].

3.2.6. α-glucosidase inhibitory assay

We carried out this experiment with ethanolic extract, methanolic extract, and chloroform extract at different concentrations to take a gander for inhibition. Ethanol had the highest level of inhibition, i.e., 49.5%, followed by methanol with a 37.8% inhibition rate and chloroform with a 30.7% inhibition rate (Figure 6).

Remarkable α-glucosidase inhibitory actions have been attributed to iridoid glycoside and other components in the crude extract [39,40]. Inhibitory-glucosidase activity was quite high in *Morchella esculenta*. As a result, *Morchella esculenta* may hold the key to a previously unexplored medicine for improved diabetes management.

Based on these findings, *Morchella esculenta* extracts high in triterpenoids, phenolics, and flavonoids might be useful for lowering postprandial glucose levels by inhibiting -glucosidase. Therefore, additional research may shed insight into the anti-hyperglycemic capabilities of *Morchella esculenta*, notably in the therapy of type 2 diabetes [41].

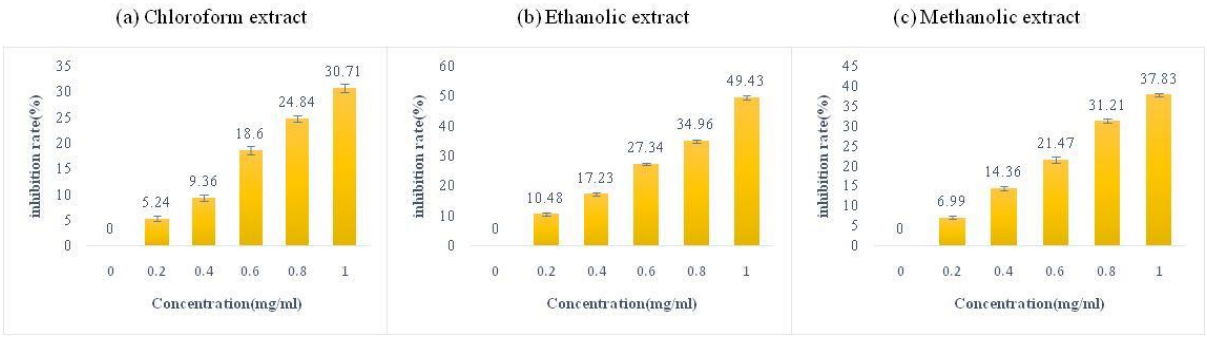

Figure 6. α-glucosidase inhibitory assay for **(a)** chloroform, **(b)** ethanolic, and **(c)** methanolic extract of *Morchella esculenta* at different concentrations.

4. Conclusions

Studies on *Morchella esculenta* revealed that three distinct solvents each contained their own unique set of chemical components. In response to radiation, the body's metabolic waste products, known as free radicals, become extremely reactive because they lack an electron pair. The free radicals create a chain reaction that destroys cell membranes and their contents. The free radicals also promote oxidation of lipids, which in turn diminishes the flavor of foods. Antioxidants' primary functions include scavenging free radicals, protecting cells from damage, delaying aging, and halting the progression of diseases like cancer linked to oxidative stress [42]. Accordingly, introducing a unique antioxidant may have therapeutic effects and help foods keep their original flavor. As a result of their lack of side effects, natural antioxidants have been deemed preferable to synthetic ones. Based on the findings, it appears that the combinatorial capability of the chemical ingredients present was responsible for the observed inhibitory activities. Furthermore, it is remarkable that the extracts have excellent antioxidant effects, which could be beneficial for further application.

Low quantities of H_2O_2 can be found in the environment, the human body, vegetation, bacteria, and food. Oxygen (O_2) and water (H_2O) are the immediate by-products of its breakdown, and the latter may generate hydroxyl radicals ($OH^{\cdot}$), which can set in motion lipid

peroxidation and damage DNA. Hydrogen peroxide was effectively scavenged by a selective extract of *Morchella esculenta*, which may be due to the existence of phenolic groups that might transfer electrons to hydrogen peroxide, neutralizing it into H_2O.

The study also showed that many plant extracts and their fractions possessed strong α-glucosidase inhibitory capacity, with evidence pointing to the combinatorial potential of chemical ingredients. The research confirmed some pharmacological bases for using *Morchella esculenta* in clinical applications for diabetes treatment. Inhibitors of beta-glucosidase, which are present in extracts of *Morchella esculenta*, have been implicated in the management of hyperglycemia. However, before *Morchella esculenta* extract is widely used in reducing hyperglycemia in diabetic patients, more study is needed. This includes in vivo tests and separation of the active ingredients.

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Conflicts of Interest

The authors declare no conflict of interest.

List of Abbreviations

WHO, World Health Organisation; ABTS, 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonicacid); DPPH, 1,1-diphenyl-2-picrilhydrazil; IDF, International Diabetes Federation; DM, Diabetes mellitus; O_2, Oxygen; H_2O, Water; TBA, Thiobarbituric acid; TBARS, Thiobarbituric acid reactive substances; OD, Optical density; LPO, Lipid peroxidase; NO, Nitric oxide; PUFA, Polyunsaturated fatty acids.

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