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Effect of solvents with different polarity on the extraction of bioactive compounds from reishi mushroom (*Ganoderma lucidum*) and their antioxidant and free radicals scavenging activities: application in buffalo meatballs

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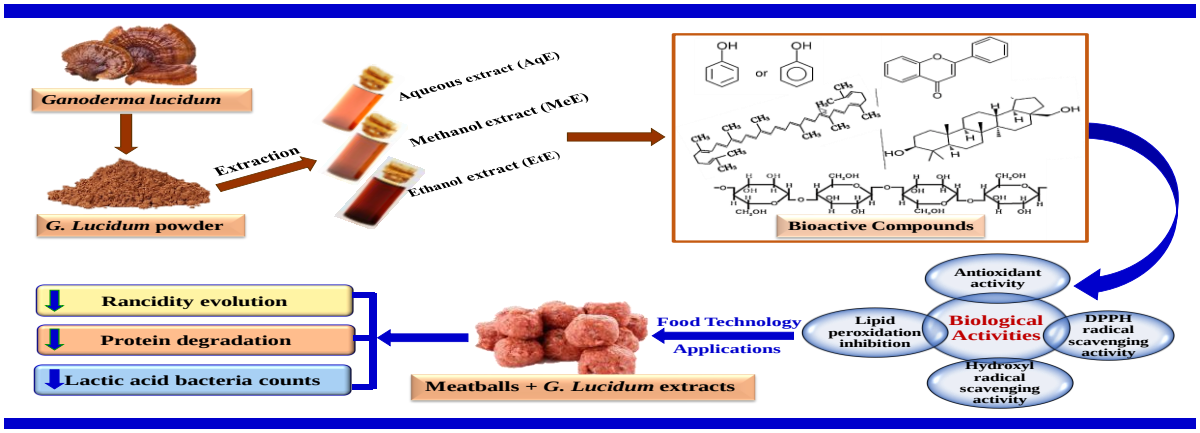
Abstract

This study aimed to evaluate the effect of solvents with different polarities on the extraction on extraction of bioactive compounds from *Ganoderma lucidum* (*G. lucidum*) and their antioxidants and free radical scavenging activities (FRSAs). Different solvents (water, methanol and ethanol) were used for the extraction of phenolic, flavonoids, lycopene and triterpenoids and the antioxidants and FRSAs of the extracts were determined in vitro by several standard methods. Among the extracts, ethanol extract showed the highest value for total phenolic, flavonoids, lycopene and triterpenoids were 98.78± 2.98 mg GAE, 51.69 mg CE, 39.07 µg and 87.63 mg UAE. g-1 dry extract, respectively. Ethanol extract displayed high scavenging action based on DPPH and **HRSA** (93.20± 0.98 and 62.89 ±1.57%) with IC50 of 10.11±0.75 and 44.98±1.17 µg/mL, respectively when compared with standard BHT (IC50 of 7.53±0.34 µg/mL) and CA (70.11±2.11 µg/mL). Also, in the lipid peroxidation inhibition method, ethanol extract showed the highest inhibitory activity (83.77±2.04%) with an IC50 of 11.84±1.10µg/mL, similar to standard CA (IC50 of 10.83 ± 0.78 µg/mL). These results demonstrate that EtE exhibited the highest radical scavenging and lipid inhibitory properties among the extracts, which closely correlated with the standards. The amount of phenolics and flavonoids was found to have a substantial positive connection (p value 0.001) with free radicals (DPPH and OH) scavenging and lipid peroxidation inhibitory capabilities. On the other hand, extracts of this mushroom were applied to one of the important meat products, meatballs, as natural antioxidants and they showed high efficiency in control the degradation of protein molecules and the development of rancidity and off flavors, thus improving product acceptability and prolonging shelf life. Finally, *G. lucidum* extracts could be useful as a plant-based functional food or medicine for a variety of disorders induced by free radicals.

Keywords: Phenolics, free radicals, DPPH, lipid peroxidation inhibition, TBARs, TVB-N, lac.

Graphic abstract



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1. Introduction

Bioactive compounds are naturally existing substances primarily found in the plant kingdom, which participate in protecting against attacks by pathogens [1]. Such compounds include phenolic, carotenoids, alkaloids, polysaccharides, organosulfurs, essential oils, terpenoids, and phytosterols [2-5]. These bioactive substances have received significant attention due to their ability to protect the human body from oxidative stress (OS) that can cause a variety of illnesses. OS was first defined as a significant imbalance between antioxidants and oxidation, as "a disturbance in the prooxidant/antioxidant balance in flavour of the former, potentially causing damage". OS was initially characterized as a significant imbalance where prooxidants outweigh antioxidants, posing a risk of potential damage [6]. As a result, it implies a mismatch among a biological system's produced reactive oxygen species (ROS) and its capacity to detoxify and adequately fix the associated damage. Disruptions in a cell's normal redox state may lead to free radicals (FRs) or the peroxides, which can destroy all of the cell's components, including lipids, proteins, and DNA. Changes in a cell's usual redox balance can lead to the formation of FRs or peroxides, which have the capacity to degrade all cellular components, including lipids, proteins, and DNA. Oxidative metabolism's OS can cause base damage and strand breakage in DNA [7 and 8]. ROS such as H2O2 (hydrogen peroxide), O2 (superoxide radical), and OH (hydroxyl radical) cause base damage in the majority of cases [8]. Furthermore, in redox signaling, some ROS operate as cellular messengers. As a result, OS has the potential to disrupt normal cellular signaling pathways [9].

OS is believed to play a role in in the development of variety of diseases in humans, including atherosclcrosis, malaria, rheumatoid arthritis, cancer, neurogical diseases like, Alzheimer's, Parkinsons's and Huntington's disease and chronic fatigue syndrome [10-13]. Also, it contributes to tissue harm after hyperoxia and irradiation, additionally, it is associated with the increased risk of cancer in diabetic patients [14]. Infection with *Helicobacter pylori,* that enhances generation of nitrogen species and reactive oxygen in the stomach's human , is thought to contribute to gastric cancer's development [7 and 16]. Moreover, connections have been identified in humans between the susceptibility of lipid to oxidative modification, obesity and OS indicators [17].

Antioxidants are substances taha mitigate OS prevents and sustains the adverse effects of FRs by delivering electrons from antioxidants to these damaged cells. Also, acting's antioxidants as radical scavengers protect the human body against FRs which may cause pathological conditions such as asthma, Parkinson's diseases, neurodegeneration, and inflammation. Furthermore, antioxidants turn FRs into waste by-products, which are eliminated from the body. [18 and 19].

Our previous studied with others found that consuming antioxidant-rich plant components reduces the incidence of numerous free radical-related disorders ([20,21, 13, 16, 22 and 23]).

All of the health advantages are mostly attributed to the existence of phytochemicals including phenolics, alkaloids, organosulfurs, carotenoids, and phytosterols [2]. Bioactive chemicals are present in herbs, fruits, spices, vegetables, grains, and oils [24 and 25].

The reishi mushroom, scientifically known as *G. lucidum*, is a type of fungus that decomposes wood and is classified under the Polyporales family of Ganodermaceae. It typically produces sturdy fruiting bodies. G. *lucidum* is mainly made up of protein, fat, carbohydrate, and fiber. The mycelia, spores and fruiting body of G. *lucidum* comprise approximately 400 diverse bioactive or phytochemical compounds, with polysaccharides, sterols, triterpenoids, nucleotides, proteins, peptides, fatty acids, steroids, and trace elements being the most prominent among them [26 and 27]. *G. lucidum* has many pharmacological effects, such as anti-inflammatory, immunomodulating, chemopreventive, radioprotective, hepatoprotective, hypolipidemic, analgesic, antioxidase, diabetic, antibacterial, sleep-promoting, antifibrotic, antiviral (including anti-HIV), antiatherosclerotic, and antitumor, (reviewed in [27- 31].

Meat is an important and excellent source of protein with high biological value. Its nutritional value depends on a large percentage of high-quality proteins with all the essential amino acids, in addition to the other nutrients present, which are important minerals and vitamins. Although meat is an almost complete food, it is considered a rapidly spoilage food, as the actions of microorganisms and internal enzymes lead to changes in the chemical composition during handling or storage. The most important of these chemical changes is the decomposition of proteins and other compounds containing nitrogen due to the aforementioned damage mechanisms. Causing the accumulation of total volatile basic nitrogen (TVB-N) [32 and 33]). Almost of these compounds are toxic and cause considerable changes in flavour and colour [34] that can influence on meat products' acceptability. Alternatively, lipids of meat become rancid due to oxidation, a process known as oxidative rancidity, which can also be caused by microorganisms. [35 and 36]. Several studies have reported on the effects of malonaldehyde (MDA), a significant product of polyunsaturated fatty acid (PUFA) oxidation, indicating its mutagenic and carcinogenic properties in human health [37 -39]. The levels of TVB-N and MDA in meat increase with storage time, and their accumulation pattern often mirrors other indicators of spoilage, such as microbial growth, overall quality deterioration, and changes in sensory attributes. Synthetic additives are extensively employed in the meat industry to inhibit fat oxidation, protein degradation, and microbial proliferation. However, there is a current trend toward reducing their usage due to increasing consumer concerns about the potential health risks and diseases associated with these chemical additives [40 and 41]. As a result, there has been a notable increase in the search for natural additives, particularly those derived from plants, in recent years. [42].

Despite all the previous studies, there is still a lack of information on the natural extracts of *G. lucidum*, specifically those related to the best extraction methods and conditions that ensure the highest concentration/content of the bioactive compounds,. So, this study aimed to determine the effect of solvents with different polarity on extraction of some bioactive compounds contents of reishi mushroom (*G. lucidum*) on the one hand and on the other hand to study the antioxidant and FRSAs of these extracts, by the use of *in vitro* assays. Also, *G. lucidum* extracts, natural products, will be applied in meat

products to prevent chemical and bacteriological spoilage agents and extend their storage life within the scope of this investigation**.**

2. Materials and Methods

G. lucidum collection

Dried fruits of reishi mushroom (*G. lucidum*) were purchased from Haraz Company of Herbs and Medical Plants, Cairo, Egypt. Identification of the mushroom was confirmed by taxonomist experts at the Department of Botany, Faculty of Agriculture, Menoufia University. Mushroom samples were ground into coarse powder by a grinding machine (AlAraby Toshiba Co., Benha, Egypt) and the materials that passed through an 80 mesh sieve were retained for use.

Chemicals

Potassium acetate, H2SO4, potassium chloride, thiobarbituric acid (TBA). phosphate buffer, 2-deoxy-d-ribose, H2O2, and HCl were acquired from Sigma-Aldrich, sodium carbonate and folin-ciocalteus's phenol reagent had been purchased from Merck (Dam-stadt, Germany); potassium ferricyanide, ferrous ammonium sulphate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), ascorbic acid (AA), butylated hydroxytoluene (BHT), gallic acid (GA), sodium phosphate, catechin (CA), sodium nitrate, ammonium molybdate, AlCl3, ethylenediamine tetraacetic acid (EDTA), trichloro acetic acid (TCA), FeCl3 and sodium hydroxide were acquired from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of extracts

Powders derived from the chosen plant parts were utilized for their various extracts using the method of [43], with slight adjustments. For the aqueous extraction, 20 g of dried plant material was mixed with 180 ml of deionized water. The mixture was homogenized, transported to a beaker, and stirred at 200 rpm using an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for one hour at ambient temperature. The resulting extract was The extracts were then separated from the residue by filtration through Whatman No. 1 filter paper. The remainder underwent two additional rounds of extraction, and the resulting extracts were mixed. The filtrates were concentrated using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany) under reduced pressure at 55°C. For organic solvent extraction, the same process was followed, but different organic solvents, including ethanol and methanol, were used individually as the extraction medium.

Determination of Bioactive Compounds

Determination of total phenolic as (mg GAE.g-1dry extract), total flavonoids as (mg CAE. g-1 dry extract) by using the colorimetric test, total triterpenoids (mg UAE. g-1 dry extract), lycopene (µg. g-1 dry extract) , and polysaccharides as (mg starch. g-1 dry extract-1) as described by described by Wolfe et al. [44]; Zhishen et al., [45]; Chang et al., [46]; Gordon and Diane, [47] and Vazirian et al., [48] respectively.

Determination of Antioxidant activties

Antioxidant activity (AA) of *G. lucidum* was assessed using the α-carotene bleaching (BCB) assay, DPPH radical scavenging assay, Hydroxyl radical scavenging activity (HRSA), and Lipid peroxidation inhibition (LPI) assay as described by described by Marco, [49], Desmarchelier et al., [50], Halliwell and Gutteridge technique [11], and Haenen and Bast, [51] respectively.

Technological studies

Meat samples

Samples of buffalo meat were purchased fresh, immediately after slaughter, from the slaughterhouse in the city of Shebin El-Kom, Menoufia Governorate, Egypt. The samples were shipped in refrigerated boxes directly to the lab, where manufacturing processes, technological treatments, and storage experiments were immediately conducted.

Meatball formulation and processing

Buffalo meatballs were processed such as mentioned by Fernandez-Lopez *et al*., [52] by using the following recipes: 78% minced buffalo meat (about 22% fat content), 5.5% water, 2.5% salt, and 14.5% flake potatoes. Minced meat (control samples), minced meat + 0.50 % (w/w) of aqueous extract (AqE), minced meat + 0.50 % (w/w) of ethanol extract (EtE), and minced meat + 0.50 % (w/w) of methanol extract were created as a set of three treatment samples that varied only in the G. *lucidum* extracts added. The concentration of mushroom extracts in the current study was chosen in light of many previous studies [53, 54, 29, and 31].

The meatball products were prepared in the lab similar to the commercial processing conditions applied. Samples of rose buffalo meat were minced using an electrical mixer and small-cut with a sharp knife (Moulinex Egypt, Al-Araby Co., Egypt). For six minutes, the ingredients were combined in a bowl mixer fitted with a spiral dough hook (Moulinex Egypt). The appropriate *G. lucidum* extract was added for each treatment at the chosen concentrations, and the mixture was again stirred for six minutes. The meatballs (20 g, 22–27 mm in diameter) were made by hand and cooked in two stages. To give the meatballs their distinctive browned appearance, they were first flash-fried in maize oil at 185 ^{0}C for 30 sec. Subsequently, second, the meatballs were cooked to an internal temperature of 70 ^{0}C in the middle, which took 6 minutes at 230 ^{0}C in a forced draft oven (Zanussi, Italy). In order to get the samples' final temperature down below 12 ^{0}C, they were immediately put in a chiller (4 ^{0}C).

Meatballs product quality examination

Thiobarbituric acid (TBA) determination

Lipid oxidation was assessed as TBA content according to Tarladgis et al [55]. After two minutes of mixing, 10 g of the sample was moved to a distillation tube along with 50 ml of distilled water. After adding 2.5 ml of 4N HCl to the same distillation tube, 47.5 ml of distilled water were used to cleanse the mixing cup. 50 cc of the distillate were collected after the mixture was distilled. Following a two-minute mixing period, 50 ml of distilled water and 10 g of the sample were transferred to a distillation tube. 47.5 ml of distilled water were used to clean the mixing cup after adding to the same distillation tube 2.5 ml of 4N HCl. After the mixture was distilled, 50 cc of the distillate were collected. Using a PV 8625 spectrophotometer (Philips, UK), then measured the absorbance at 538 nm in relation to a blank that was made up of 5 ml TBA-reagent and 5 ml distilled water. Thiobarbituric acid-reactive substances (TBARS) were computed using a freshly constructed malondialdehyde (MDA) standard curve (5–50 nmol) that was created by acidifying 1,1,3,3-tetraethoxy propane. The TBA values were computed using the formula mg MA/kg sample.

Total volatile basic nitrogen (TVB-N)

TVB-N was extracted using the method of Winton and Winton [56] such as follow: 50 g of ground meatball sample were blended with 100 ml of distilled water and left to stand for 24 h at 4^0C. The sample was then vigorously shaked and filtered through cheese cloth. The resulted filtrate was used for determination of the TVB-N. as follow: 400 ml of the filtrate were put into 1000 ml of distillation Erlenmeyer flask, plus 30 ml of ethanol and 2 g of Mg0. The distillate was collected in 25 ml at 0.1N H_2 SO_4. After the distillation was completed, the distillate was boiled for 10 - 15 min to remove the CO_2. The distillate was cooled to ambient temperature and 0.2 ml of resolic acid indicator (0.2%) was added then the excess of H_2SO_4 was immediately titrated with NaOH, 0.1 N. Finding were expresed as mg TVB-N /100 g sample according to the following equation:

TVN (mg / 100 g sample) = [(V1xN1- V_2xN_2) x 0.014 x 1000/400 x W] x100.

Where: V_1 = volume of H_2SO_4 by ml, V_2 = volume of NaOH by ml, N_1 = normal concentration of H_2SO_4, N_2 = normal concentration of NaOH and W = weight of sample.

Microbiological analysis (Lactic acid bacteria, LAB)

Portions of at least three meatballs were combined to create a composite sample (10 g), which was then homogenized for one minute in sterile 1.5% peptone water using a Stomacher 400 (Colworth, London, UK). In accordance with conventional procedures, aliquots were serially diluted in peptone water and plated out [57]. The plates were incubated under anaerobic conditions at 30 ^{0}C for two days in order to assess LAB counts on MRS Agar (pH 5.6). Oxoid (Oxoid Unipath Ltd., Basingtoke, and Hampshire, UK) provided the culture media. The expressed results were $\log_{10}$ cfu/ml.

Statistical Analysis

The experiments were conducted three times independently, and the results were documented as mean±SD. To calculate significant relationships between tested parameters via regression and correlation analysis, the student F- and *t* -tests were used.

3. Results and Discussion

Total phenolics, flavonoids, lycopene, triterpenoids and Polysaccharides content

The content of the total phenolics, flavonoids, lycopene, triterpenoids and Polysaccharides compounds were determined in *G. lucidum* extracts as shown in Table (1). Results in this table show phenolics was reported the most numerous ones in all extracts, followed by triterpenoids, flavonoids and lycopene. These compounds also recorded different significant values according to the type of extract/solvent as the highest values were recorded for the ethanolic extract (EtE) and came close to it to a large extent with the methanolic extract (MtE) then the lowest values were of the share of the aqueous extract (AqE). Such variations recorded in the bioactive compounds amount measured for the primary cause of the *G. lucidum* extracts is the variation in polarity of the extraction solvents. According to Sulaiman *et al.,* [58]. The solubility and extraction yield of chemical constituents in a sample can be affected by the polarity differences of the extracting solvents. The polarity parameters of different solvents used in the present study, water, methanol and ethanol were 1.82, 1.70 and 1.69 (dipole moment, μ), 80.1, 32.70 and 24.55 (Dielectric constant, ε) and 9, 6.6 and 5.2 (polarity index, PI), respectively. Our data confirmed that *G. lucidum* almost bioactive components were found in lipophilic phase i.e. accordance with the known rule "like dissolves like" This implies that compounds with comparable polarity and chemical properties will dissolve in one another. For instance, non-polar and polar substances are immiscible, meaning they do not mix, while non-polar solvents tend to dissolve non-polar solutes and polar solvents tend to dissolve polar solutes. Therefore, one of the most important steps in enhancing the recovery of bioactive chemicals from extracts, such as total phenols, flavonoids, lycopene, and triterpenoids, is identifying a suitable solvent system.

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Table 1. Total amount of phenolics, flavonoids, lycopene and triterpenoids compounds in *G. lucidum* extracts.

Bioactive compounds	Water /Aqueous extract (AqE)	Methanol extract (MeE)	Ethanol extract (EtE)
Phenolics (mg GAE.g^{-1}dry extract)	60.15 ± 5.30 ^{b}	98.78± 2.98 ^{a}	101.33 ± 3.11^{a}
Flavonoids (mg CAE. g^{-1} dry extract)	33.23 ± 3.67 ^{b}	51.69±1.95 ^{a}	53.39 ± 2.76 ^{a}
Lycopene (µg. g^{-1} dry extract)	32.04± 2.15 ^{c}	39.07± 1.91 ^{b}	44.98± 1.87 ^{a}
Triterpenoids (mg UAE. g^{-1} dry extract)	54.90 ± 4.81 ^{b}	87.63± 3.02 ^{a}	87.45 ± 3.11 ^{a}
Polysaccharides (mg starch. g^{-1} dry extract^{-1})	162.15 ± 5.30 ^{b}	119.11± 2.98 ^{a}	116.33 ± 3.11^{a}

Each value represents the mean of four replicates ±SD. Varied letters within the row indicate significant variations ($p \leq 0.05$). GAE, gallic acid equivalent, CAE, catechin equivalent and UAE, ursolic acid equivalent.

Antioxidant activities

Table 2 displays AA of *G. lucidum* extracts. The reduction in β-carotene absorbance when exposed to various *G. lucidum* extracts (and well-known antioxidants used as standards) with the oxidation of β-carotene and linoleic acid is shown in Figures (1). The *G. lucidum* extracts showed significant differences in AA 78.32- 91.96% when assessed using the four different methods. EtE showed strong activity, even equal to α -tocopherol at 50 mg/L and higher than that of BHA mg/L. MtE had high antioxidant activity, proximate α -tocopherol at 50 mg/Land BHA, while AqE was lower AA.

Table 2. Antioxidant activity of different extracts of G. lucidum.

Extract*	Antioxidant value AOX (A/h)	Antioxidant activity AA (%)	Oxidation rate ratio (ORR)	Antioxidant activity coefficient (AAC)
AqE	0.117± 0.011	78.32± 2.77^{d}	0.206± 0.010	551.26± 7.59
MeE	0.077± 0.013	86.43± 3.11 ^{c}	0.135± 0.022	674.86± 6.10
EtE	0.045± 0.009	91.96± 1.98 ^{b}	0.080± 0.012	771.00± 8.54
Control	0.565± 0.036	0.00± 0.00	0.998± 0.067	0.00± 0.00
α-Toc, 50 mg/L	0.006± 0.002	99.01± 0.97^{a}	0.010± 0.004	893.56± 6.54
α-BHT, 50 mg/L	0.041± 0.006	92.73± 0.84^{b}	0.072± 0.006	784.38± 5.12

Each value represents the mean of three repetitions ± standard deviation. Varied letters within the row indicate significant variations ($p \leq 0.05$).

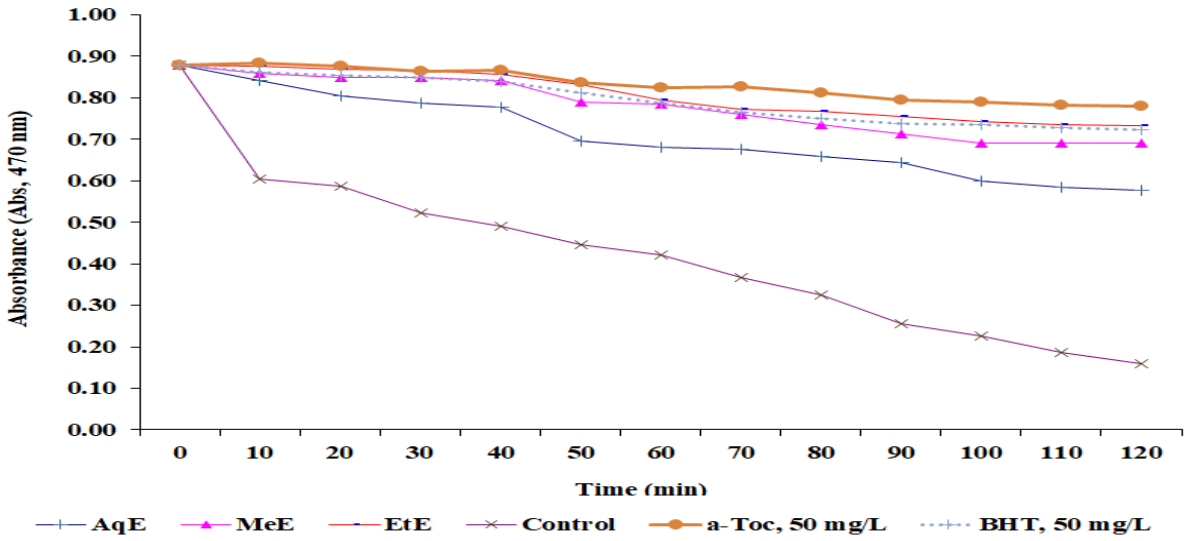

Figure 1. Antioxidant activity of different extracts of *G. lucidum* evaluated using the β-carotene bleaching technique (BHT and α–tocopherol at 50 mg/L Concentration used as a benchmark).

BCB assay, developed by Taga *et al.*, [59], The process involves oxidizing both linoleic acid and β-carotene. The method evaluates antioxidants' ability to scavenge radical of linoleic acid peroxide (LOO•) which causes oxidation of β-carotene (loss

of double bonds) in the emulsion phase, approach has also been used to determine the antioxidant potential in different plant parts through in vitro methods [24), 60-67]. All of these searches found a strong correlation between polyphenols, flavonoids, carotenoids, and terpenoids concentration and radical-scavenging antioxidant activity. So, the variance in degrees of *G. Lucidum* extracts' antioxidant activity is mostly attributable to the varying content of each of the bioactive chemicals, including phenolics, flavonoids, carotenoids, triterpenoids, and others.

Several studies have shown that such chemicals are the primary plant components with antioxidant activity. Wachtel-Galor et al. [67], Yuen et al. [68], Wu and Wang [69], and Oludemi et al. [70] discovered that several components of *G. lucidum*, specifically phenols, flavonoids, carotenoids, polysaccharides, and triterpenoids, exhibit antioxidant activity in vitro. Also, Polysaccharides present in *G. lucidum* have also been shown to protect immune cells against oxidative damage [71]. In vitro, aqueous extracts of *G. lucidum* shown excellent antioxidant activity, considerably protecting cells from H2O2)-induced DNA damage ([72]). While, Sheena et al. [73] discovered that the antioxidant properties of methanol extracts of *G. lucidum* reduce kidney damage caused by cisplatin by restoring the renal antioxidant defense system. In addition, Lu et al., [74] mentioned the antioxidant properties of water and ethanol extracts of *G. lucidum* inhibited the development of focal adhesion complexes and stress fiber in bladder cancer cells, resulting in the suppression of carcinogen-induced cell migration.

Furthermore, antioxidants from *G. lucidum* were rapidly absorbed after intake; this led to an elevation in the overall antioxidant levels in the plasma of the participants [75]. Also, data showed that, when methanol and ethanol extracts of *G. lucidum* were compared to standards such as tocopherols and BHT, they showed essentially identical FRSAs., our findings indicate that all *G. lucidum* extracts exhibited radical scavenging activity due to their ability to donate hydrogen.

DPPH radical scavenging activity

Figure 2 and Table 3 shows the FRSAs of the different extracts of *G. lucidum* and standard BHT. Among the extracts, MtE possessed the highest activity. At a concentration of 100 µg/mL, the scavenging activity of AqE, EtE and MeE was 83.15± 1.34, 93.20 ± 0.98 and 89.68 ± 1.11 %, respectively, while at the same concentration, the standard BHT was 98.32 ± 0.39 % (Figure 2). The IC50 of different extracts of AqE, EtE and MeE was 19.24±1.45, 10.11±0.75 and 12.21±1.07 µg/mL, respectively. FRSAs of different extracts and BHT was in the following order: BHT > EtE > MeE > AqE**.** The IC50 of BHT (standard) was 7.53 ± 0.34 µg/mL.

The theory of the previous test is based the radical scavenging activity of antioxidants toward the DPPH• which thought to be because of their hydrogen donating ability [76]. Several studies have regarded this assay as a straightforward and precise method for assessing antioxidant capacity. in plant parts samples (Reviewed in [77] Also, the DPPH assay were used successively to assess AA of the all bioactive compound found in *G. lucidum* such phenolics, flavonoids, carotenoids, terpenoids and polysaccharides [77 - 80]. Therefore, DPPH free radical scavenging is an accepted assay for screening AA of *G. lucidum* extracts. The ability to scavenge radicals is crucial in preventing the detrimental effects of FRs in various diseases. Such as cardiovascular, neurological, pulmonary, nephropathy, pulmonary and cancer diseases [81]. Our findings demonstrated that the various *G. lucidum* extracts exhibited higher FRSAs when compared to conventional BHT.

Polyphenols, flavonoids, carotenoids, terpenoids, polysaccharides, and tocopherols scavenge DPPH radicals through their hydrogen-donating capacity [82 and 77]. Also, Huang *et al.,* [82] mentioned that overall polyphenol concentration and radical scavenging antioxidant activity are significantly associated. The results of this investigation indicate that all *G. lucidum* extracts demonstrated radical scavenging activity through hydrogen donating ability or electron transfer.

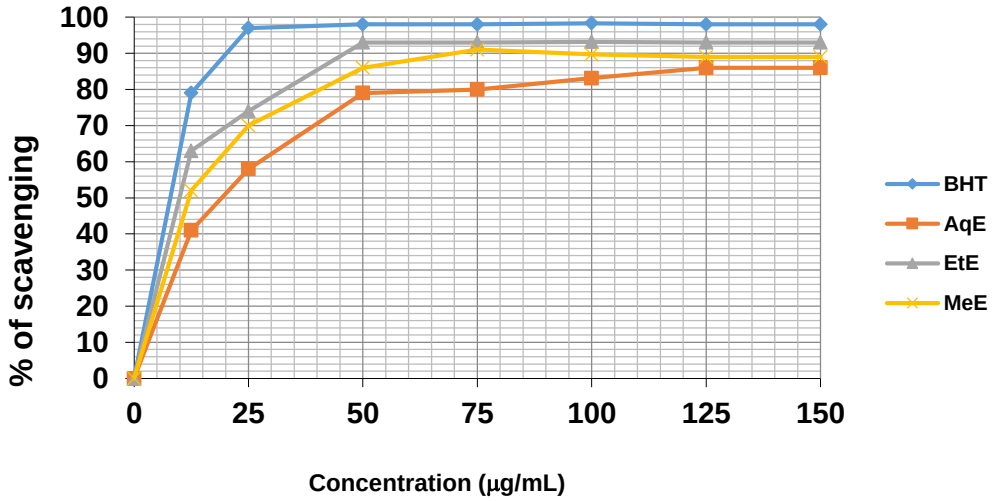

Figure 2. DPPH radical scavenging activity of *G. lucidum* extracts.

Table 3. IC50 (DPPH) of G. lucidum extracts.

Name of sample	BHT	AqE	EtE	MeE
IC_{50} (µg/mL)	7.53±0.34 [c]	19.24±1.45[a]	10.11±0.75 [b]	12.2±1.07 [b]

Each value represents the mean of three repetitions ± standard deviation. Varied letters within the row indicate significant variations ($p \leq 0.05$).

Hydroxyl radical scavenging activity (HRSA)

HRSA of the different extracts of *G. lucidum* was dose dependent. EtE had the highest activity, the scavenging activity of AqE, EtE and MeE was 54.23±2.12, 62.89±1.57 and 57.98±2.56%, respectively at a concentration of 100 µg/mL, while CA was 63.96± 0.61 % (Figure 3). The IC50 of AqE, EtE and MeE was 88.97±1.85, 44.98±1.17 and 62.52±2.11 µg/mL, respectively. The data in Table 4 shows that the inhibitory potency of EtE and MeE exceeded that of the standard CA. **HRSA** assay the mutagenic capacity of FRs which due to the direct interactions of hydroxyl radicals (OH•) with DNA, resulting in DNA breakdown and cancer formation [83]. According to Laura *et al.*, [77]) this assay based on the formation of the OH• by combining ferric chloride (FeCl3), ascorbic acid, hydrogen peroxide, and EDTA in aqueous solution at pH 7.4, causing 2-deoxyd-ribose degradation and generating MDA-like product (pink chromogen when heated and absorbed at 532 nm). Additions of the different extractions of *G. lucidum* to the reaction mixture scavenge the hydroxyl radicals and prevent the formation of this chromogen by competing for the radical and prevent further damage. In similar research, certain plant extracts with varying polarity, extracts derived from pomegranate along with its three primary anthocyanidins (pelargonidin, delphinidin, and cyanidin), and blood orange juice have likewise been assessed for their antioxidant capacity using this approach [84,85], Also, data show the *G. lucidum* extracts, EtE and MeE, showed more **HRSA** than the conventional antioxidant, CA, and might be used as anticarcinogenic and antimutagenic agents by blocking the interaction of hydroxyl radicals with DNA. The capacity of extracts to quench/scavenge hydroxyl radicals may be directly related to lipid peroxidation prevention.

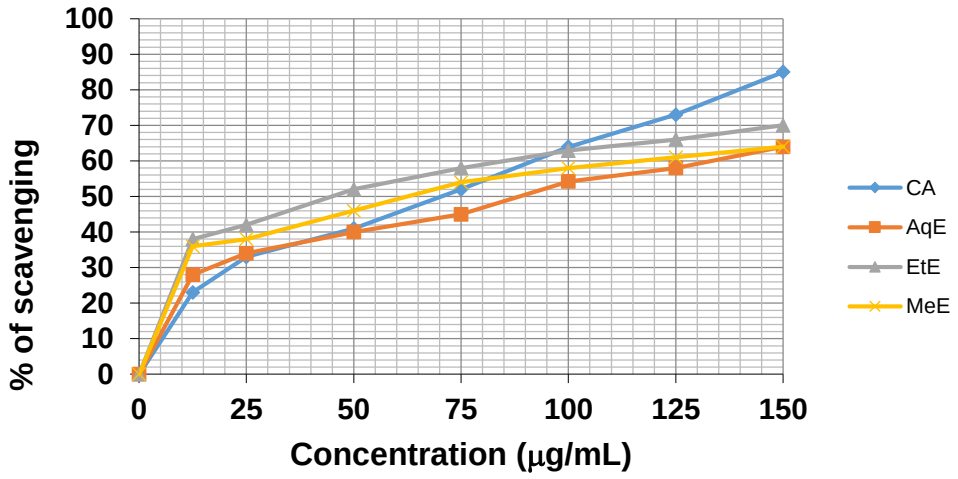

Figure 3. Hydroxyl radical scavenging activity of *G. lucidum* extracts.

Table 4. IC_{50} (Hydroxyl radical scavenging activity) of *G. lucidum* extracts.

Name of sample	CA	AqE	EtE	MeE
IC_{50} (µg/mL)	70.11±0.49 [b]	88.97±1.85 [a]	44.98±1.17 [d]	62.52±2.11[c]

Each value represents the mean of three repetitions ± standard deviation. Varied letters within the row indicate significant variations ($p \leq 0.05$).

Lipid peroxidation inhibition (LPI) assay

LPI activity of different extracts of *G. lucidum* was contrasted with CA., the inhibitory activity of AqE, EtE and MeE was 70.22±2.85, 83.77±2.04 and 79.96±3.01%, respectively at a concentration of 100 µg/mL; whereas that of the CA was 86.92±21.36% (Figure 4). The IC50 of AqE, EtE, MeE and CA were 21.15± 0.88, 11.84±1.10, 12.50±1.44 and 10.83±0.61 µg/mL, respectively (Table 5). EtE had higher inhibitory activity than other extracts.

ROS cause harm to cellular membranes (such as lysosomes, mitochondria, and the cell wall) by initiating a chain reaction called lipid peroxidation, predominantly affecting PUFA within these membranes [81]. Oxidative damage to membrane

PUFA produces a variety of lipid peroxidation products, that quantified as an indicator of OS, such as ketones, alcohols, short carboxylic acids, aldehydes, and hydrocarbons, causing cellular abnormalities. Therefore, preventing lipid peroxidation is considered the most crucial indicator of antioxidant activity. This in vitro **LPI** experiment is based on the stimulation of lipid peroxidation in rat liver by ferric ion (Fe+3) plus potassium chloride, which generates hydroxy radicals via Fenton's reaction. The current investigation found that EtE and MeE of *G. lucidum* had stronger **LPI** activity than AqE extract and were nearly identical to the standard, CA. These findings showed that *G. lucidum* extracts can prevent cellular abnormalities/damage induced by FRs by collapsing the chain processes responsible for lipid peroxidation.

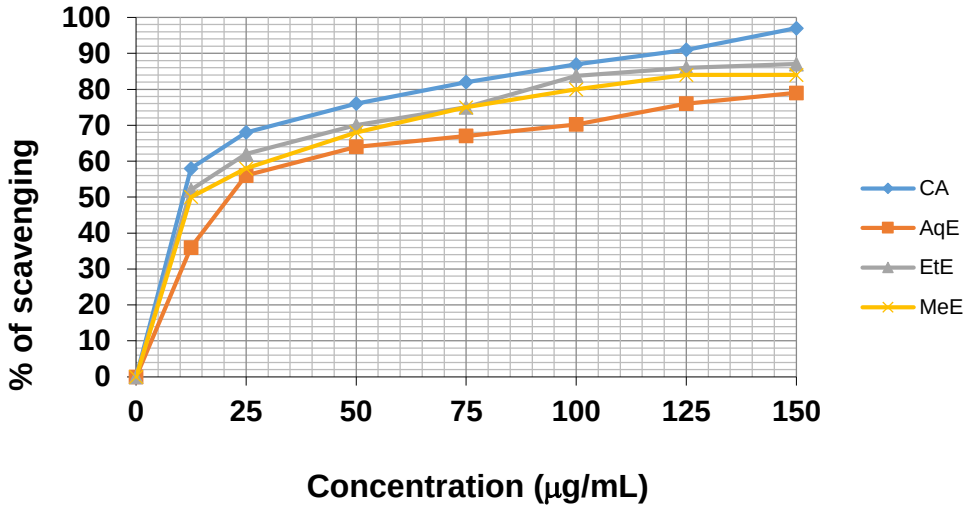

Figure 4. Lipid peroxidation inhibition activity of *G. lucidum* extracts.

Table 5. IC_{50} (Lipid peroxidation inhibition activity) of *G. lucidum* extracts.

Name of sample	CA	AqE	EtE	MeE
IC_{50} (µg/mL)	10.83±0.61 ^{b}	21.15± 0.88^{a}	11.84±1.10 ^{a}	12.50±1.44 ^{b}

Each value represents the mean of three repetitions ± standard deviation. Varied letters within the row indicate significant variations ($p \leq 0.05$).

Relationship between bioactive compounds content and antioxidant and free radical scavenging Activities

Table 6 displays the correlation coefficients (R2) linking the content of bioactive compounds to both antioxidant activity and the effectiveness of FRs scavenging, as well as to the lipid peroxidation inhibition (LPI) of *G. lucidum*. Total phenolic s and flavonoids content of the extracts showed significant (*p value <0*.001) and strong positive correlation with antioxidant activity (β-carotene bleaching rate), LPI**,** and FRSAs. Also, lycopene and triterpenoids showed significant (*p value <0*.01) and strong positive correlation for the identical association. Our findings align with the findings presented by [86 and 70] regarding DPPH radical scavenging and reducing power, despite the authors employing a distinct extraction method (methanol: water, 80:20, v/v). They also conducted investigations on polysaccharide and phenolic extracts from *G. lucidum*, with AA being marginally lower in the latter. This implies that phenolic compounds have a more significant influence on the scavenging activities of FRs. Thus, The phenolic and flavonoid components within the extracts are likely significant contributors to both LPI and FRSAs. Other compounds including lycopene and triterpenoids were also participated the same bioactive activities but to a lesser degree.

Table 6: The correlation coefficients (R^2) between bioactive compounds content and antioxidant and free radical scavenging Activities of *G. lucidum* extracts.

Compound	Antioxidant activity (β-carotene bleaching rate)	DPPH radical scavenging activity	**HRSA**	**LPI**
Total phenolics	0.9702±0.085^{aA}	0.9664±0.065 ^{aA}	0.9745±0.088 ^{aA}	0.9145±0.090 ^{b}
Flavonoids	0.9131±0.076 ^{aB}	0.9223±0.082 ^{aB}	0.9343±0.090 ^{aB}	0.8742±0.058 ^{bB}
Lycopene	0.8637±0.021 ^{aC}	0.8721±0.071 ^{aC}	0.8830±0.052 ^{aC}	0.8515±0.046 ^{aB}
Triterpenoids	0.9253±0.087 ^{aB}	0.9027±0.140 ^{aB}	0.8952±0.097 ^{abC}	0.8704±0.052 ^{abB}
Polysaccharides	0.9513±0.085 ^{aA}	0.9501±0.065 ^{aA}	0.9602±0.068 ^{aA}	0.9037±0.093 ^{bA}

Food Technology applications

The effect of adding *G. lucidum* extracts, as a natural antioxidant, on the chemical and microbial quality parameters of the meatballs, during storage time

Rancidity evolution

Table (7) and Figure (5) showed that rancidity (thiobarbituric acid content, TBA) evolution in meatballs with different *G. lucidum* extracts added, during storage time. The results of the analysis of variance for the TBA data show that both the extracts treatment and the storage period had a significant (P≤0.05) impact on the TBA values in the meatball samples. All meatball samples treated with extracts had initial TBA values that were substantially lower (P≤0.05) than the control group. At the conclusion of the 12-day storage period at 4 0C, the MDA for the control meatball samples increased from 0.27 mg/kg to 4.12 mg/kg (1415.24%).

All the *G. lucidum* extracts treatment leads to significant (p≤0.05) decrease in the formation of TBA in meatball samples after storage periods. The highest decreasing rates were recorded for samples treated with EtE followed by MeE and AqE, respectively. Several decades ago, Concern over the existence and harmful effects of some hazardous chemicals, such as MDA, present in TBARs grew on a global scale. It develops in fresh and prepared foods, such as meats, as a result of the oxidation of PUFA content during storage, processing, and heating (Reviewed in [87 and 36]. Also, Chen et al., [41] mentioned that iron was released from blood heme during cooking and elevation in non-heme iron levels which responsible for lipid oxidation. Furthermore, non-heme iron was discovered by [88] to be the active catalyst in cooked meats. Numerous authors have demonstrated that MDA has mutagenic and carcinogenic effects on human health [38,39,89]. The data from this study indicate that extracts from *G. lucidum*, a naturally selected product, slowed down lipid oxidation both during and right after cooking.

This finding is in accordance with that observed by several authors for other natural antioxidants applied to cooked beef [90-93]. Data from the current study with the others indicated that all the *G. lucidum* extracts applied in cooked meatball shows a strong antioxidant activity property due to their highly contain of several bioactive compounds, including phenolics, lycopene, polysaccharides, flavonoids and Triterpenoids [95-98]. Additionally, these bioactive substances shown the capacity to scavenge free radicals and limit the oxidation of lipids [64,99 and 4].

Table 7. The effect of adding G. lucidum extracts on thiobarbituric acid content (TBA, mg/kg sample) of meatball samples storage at 4 0C for 12 days *

Product	Storage period (days)									
	0		3		6		9		12	
MB	0.27±	0.08	1.69±	0.10 ^{a}	2.91±	0.15 ^{a}	4.27±	0.16 ^{a}	4.12±	0.19^{a}
MB + AqE	0.27±	0.08	0.56±	0.04 ^{b}	0.79±	0.36 ^{ab}	0.98±	0.26 ^{b}	1.39±	0.24 ^{b}
MB + EtE	0.27±	0.08	0.41±	0.10 ^{c}	0.65±	0.08 ^{c}	0.75±	0.14 ^{c}	1.07±	0.19 ^{b}
MB +MeE	0.27±	0.08	0.45±	0.14 ^{c}	0.72±	0.12 ^{b}	0.77±	0.13 ^{c}	1.13±	0.11 ^{b}

* Each value represents the average of three replicates ± standard deviation. Varied letters within the row indicate significant variations (p ≤ 0.05).

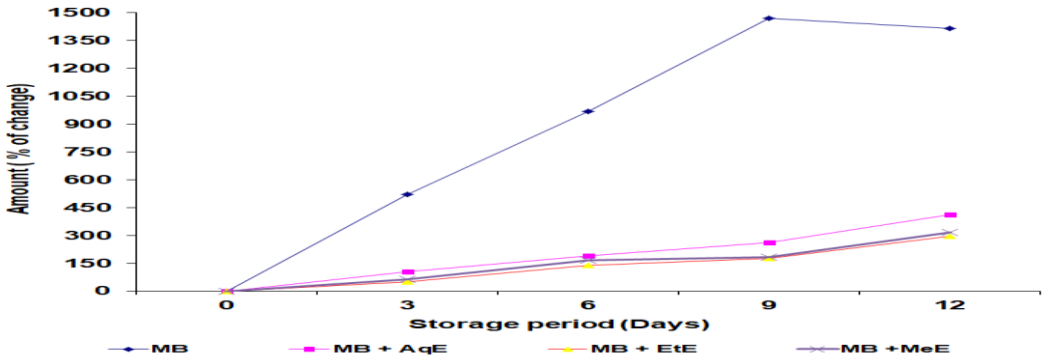

Figure 5. The effect of adding *G. lucidum* extracts on thiobarbituric acid content (TBA, as a % of control) of meatball samples storage at 4 ^{0}C for 12 days.

Total volatile basic nitrogen (TVB-N) formation

Table (8) and Figure (6) indicated TVB-N content in meatballs with added *G lucidum* extracts during storage time. Such data FRSAs that TVB-N values in meatball samples were significantly (P≤0.05) affected by the extracts treatment and the storage period. For all extracts treated meatball samples, TVB-N values were significantly (P≤0.05) lower than the control ones. The high effect was recorded for EtE followed by MeE and AqE, respectively. The findings are in accordance with that observed by several authors for other natural antioxidants applied to cooked meats products [100,101 and 94]. TVB-N often applied as an noticeable biomarker for the loss of muscle foods freshness and safety as well as the suitability for consumption

[102]. Almost of these nitrogenic producds are toxic and cause considerable colour and flavour changes that affect the acceptability and sanitation of the meat products [33]. Several studies indicated that the formation rate of TVB-N is affected by several factors involving the microbial contamination, types of meat, and conditions and length of storage period. Our data in this study with other studies confirmed that several technological treatments could delay the degradation of proteins and decrease the rate of TVB-N formation such as ermentation process and using the tenderization agents in addition to applying the natural antioxidant extracts ([32, 103, 101, 93 and 94] Also, our data showed that all the *G lucidum* extracts applied in cooked meatball shows a strong antioxidant activity due to their highly content of several bioactive constituents such lycopene, phenolics, flavonoids, Triterpenoids, and polysaccharides. Also, such bioactive compounds exhibited FRSAs which could be effective against the total volatile basic nitrogen fractions formation (TVB-N) ([64, 99 and 4]).

Table 8. The effect of adding *G. lucidum* extracts on total volatile base nitrogen (TVB-N, mg/kg sample) content of meatball samples storage at 4 ^{0}C for 12 days *

Product	Storage period (days)									
	0		3		6		9		12	
MB	5.66±	0.58	8.15±	0.70 ^{a}	9.13±	0.95 ^{a}	12.03±	1.08 ^{a}	16.08±	1.09 ^{a}
MB + AqE	5.66±	0.58	7.09±	0.37 ^{ab}	8.50±	0.60 ^{a}	10.24±	0.87 ^{a}	12.51±	1.42 ^{b}
MB + EtE	5.66±	0.58	6.15±	0.71 ^{b}	7.38±	0.55 ^{ab}	7.91±	0.53 ^{b}	10.18±	0.86 ^{b}
MB +MeE	5.66±	0.58	6.36±	0.86 ^{b}	7.74±	0.87 ^{b}	8.67±	0.96 ^{ab}	11.24±	0.56 ^{b}

*Each value represents the average of three replicates ± standard deviation. Varied letters within the row indicate significant variations ($p \leq 0.05$).

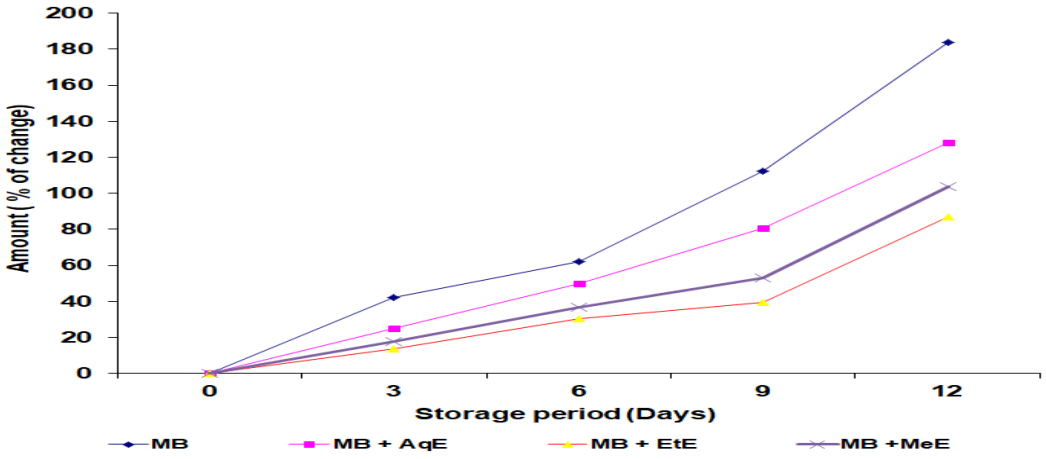

Figure 6. The effect of adding *G. lucidum* extracts on total volatile base- nitrogen content (TVB-N, as a % of control) of meatball samples storage at 4 ^{0}C for 12 days.

Lactic acid bacteria (LAB) counts

LAB counts in meatballs with added ***G. lucidum*** extracts during storage time are shown in Tables (9) and Figure (7). Such data indicated that LAB count in meatball samples were significantly ($P \leq 0.05$) affected by the type of extracts treatment and the storage period. Initial LAB values for all *G. lucidum* extracts treated samples were significantly ($P \leq 0.05$) lower than those for the control ones. The LAB was recorded 1.04 $\log_{10}$ cfu/g for the control meatball samples, sample which increased to 4.95 $\log_{10}$ cfu/g (increasing rate, 376.95%) after 12 days storage. All the *G. lucidum* extracts treatments leads to decrease the LAB in meatball samples during storage. By the conclusion of the storage period, significant declines in rates were observed for EtE- treated samples (103.86%) followed by MeE (121.72%) and AqE (217.0%), as the percent of the starting point. These findings align with those reported by multiple authors for other natural antioxidants applied to cooked meats products [92-94]. Findings from the current investigation with others confirmed that several factors contribute to LAB counts such as fermentation process, storage conditions, fat in meat, physical interactions with the food matrix, water activity within the product, in addition to applying the natural antioxidant extracts [104-108, 93 and 94]. For the *all G. lucidum* extracts, have several bioactive compounds. Also, such bioactive compounds exhibited antibacterial and antifungal activities which could be effective against growth of LAB subsequently reduce the formation of the total volatile basic nitrogen fractions formation (TVB-N) and TBARs [99 and 4]).

Table 9. Lactic acid bacteria (LAB) counts (log_{10} cfu/g) in meatballs with added *G. lucidum* extracts, during storage time (12 days at 4 ^{0}C).

Product	Storage period (days)					
	0	3	6	9	12	
MB	1.04± 0.11	2.56± 0.19 ^{a}	3.04± 0.29 ^{a}	3.34± 0.21 ^{a}	4.95± 0.36 ^{a}	
MB + AqE	1.04± 0.11	1.97± 0.06 ^{ab}	2.09± 0.10 ^{b}	2.40± 0.14 ^{b}	3.29± 0.31 ^{b}	
MB + EtE	1.04± 0.11	1.11± 0.20 ^{b}	1.31± 0.23 ^{c}	1.77± 0.28 ^{c}	2.12± 0.29 ^{c}	
MB +MeE	1.04± 0.11	1.14± 0.19 ^{b}	1.81± 0.17 ^{bc}	1.96± 0.18 ^{bc}	2.30± 0.28 ^{c}	

* Each value indicates the mean of three replicates ±SD.

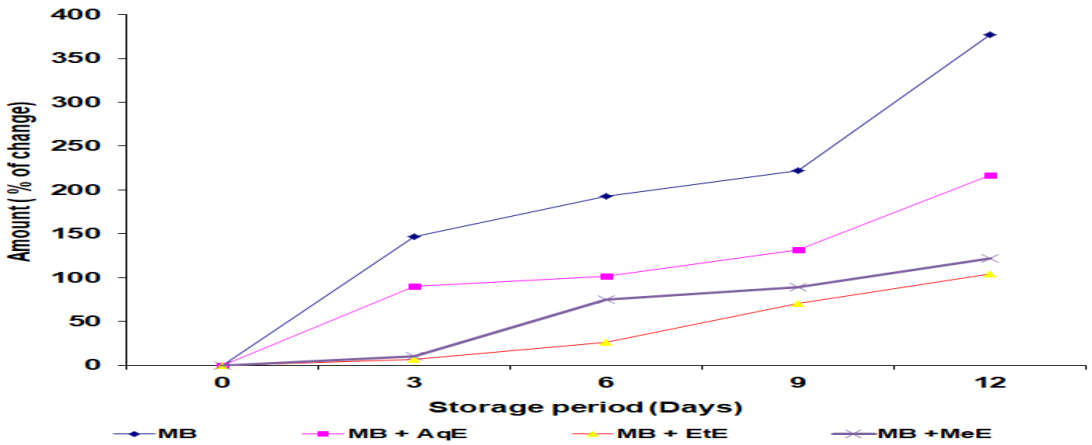

Figure 7. Lactic acid bacteria (LAB) counts (as a of control) in meatballs with added *G. lucidum* extracts, during storage time (12 days at 4 ^{0}C).

4. Conclusion

To obtain an overview of the phytochemical composition and biological activities of any plant part, it is important to choose the appropriate extraction method that ensures obtaining the largest amount of those bioactive compounds from this plant part. Here, we examined the effect of extracts of different polarities on *G. lucidum* and found that ethanolic and methanolic extracts contain large amounts of phenolics, flavonoids, lycopene and triterpenoids. These extracts also showed the greatest levels of antioxidant and FRSAs, as well as the ability to prevent lipid peroxidation in an in vitro model system. There was a positive association between measured bioactive quantities, FRSAs (DPPH and OH), and lipid peroxidation. These *in vitro* tests demonstrate that extracts of *G. lucidum* are an important source of natural antioxidants, which could help prevent the development of several illnesses induced by FRs, such as cancer. On the other side, the application of these extracts to one of the important peat products, meatballs, was beneficial to control the degradation of protein molecules and the development of rancidity and off flavors, thus improving product acceptability and prolonging shelf life. Hence, further investigation is needed to expand the base of using *G. lucidum* extracts as good plant-based functional foods and pharmaceutical products for several diseases caused by FRs.

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6. Ethical Approval

Procedure applied in this investigation for the use of rats as animal model for LPI assay was accepted by the Scientific Research Ethics Committee (Approval # 11- SREC- 07-2022), Faculty of Home Economics, Menoufia University, Egypt.

7. Competing interests

The authors declare that they have no competing interests.

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