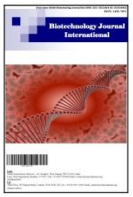

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Phytochemical Evaluation and Antioxidant Capacity of *Ganoderma lucidum* and *Pleurotus pulmonarius* in Ibadan, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors COA and STO designed the study. Authors GA and EOA performed the laboratory and statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors COA and STO managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To evaluate the phytochemical constituents and antioxidant properties of two selected mushrooms in Ibadan, Nigeria.
Study Design: An experimental and descriptive study was conducted using the fruit bodies of *Ganoderma lucidum collected from the Botanical garden University of Ibadan and Pleurotus pulmonarius* which was bought from a local store in Ibadan. Successive extraction was done on the mushrooms with four solvents of increasing polarity that is from the non-polar (N-hexane) to a more polar solvent(water).Phytochemical and antioxidant analysis were then carried out on the extract fractions.
Place and Duration of Study: Department of Botany, University of Ibadan, Ibadan, Nigeria and Department of Microbiology, University of Ibadan, Ibadan, Nigeria between May 2018 and February 2020.
Methodology: The N-Hexane, Ethyl acetate, Ethanol and Aqueous extract fraction of *Ganoderma lucidum* and *Pleurotus pulmonarius* were analyzed in different system, including DPPH, Ferric ion Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC). The various antioxidant

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activities were compared to the standard ascorbic acid. Phytochemical include: Phenol, Alkaloids, Flavonoids, Tannins, Steroids, Saponins, Terpenoids Anthraquinones, and Cardiac glycosides were also analysed.

Results: The Phytochemical screening result shows the presence of Phenol, Alkaloids, Flavonoids, Tannins, Steroids, Saponins, Terpenoids Anthraquinones, and Cardiac glycosides. The quantitative phytochemical screening revealed that the Ethyl acetate fraction of *Ganoderma lucidum* recorded the highest percentage of Alkaloids (41.70±0.14%) while the Ethanolic fraction of *Pleurotus pulmonarius* has the lowest percentage of Tannins (0.10±0.24%). DPPH scavenging activity of the Ethanolic extract fraction of *Ganoderma lucidum* (at 200 µg/ml) was 94.96% higher than that of *Pleurotus pulmonarius* (22.39%). The extract fraction of all the mushrooms possesses significant TAC content with N-Hexane and Ethyl acetate fraction having the highest. The results of the DPPH, FRAP, and TAC assays indicate that both mushrooms examined showed significant antioxidant activities. Among these, *Ganoderma lucidum* extract seems to be more effective antioxidant. The antioxidant activity of theses mushrooms has significant importance as it greatly contribute to their nutraceutical properties thus enhancing their nutritive value. Cultivation and production of dietary supplements from *Ganoderma łucidum* is highly recommended.

Conclusion: The Mushrooms species analyzed have demonstrated to be good sources of phytochemical and antioxidants hence it can be recommended to pharmaceutical industries for the manufacturing of drugs.

Keywords: Phytochemical; antioxidant; Ganoderma lucidum Pleurotus pulmonarius.

1. INTRODUCTION

Ganoderma lucidum is a type of mushroom that has been used as medicine by the Chinese for about 2000yrs. it has a woody texture, bright surface and reddish brown in colour. It is also called Mushroom of immortality as it believed to be mushrooms growing only in the houses of immortals and hence it is described as holy mushroom. In recent times it is majorly seen growing on dead wood and rarely available. Cultivation of this mushroom has been the major source due to increase in demand as a result of its medicinal value. It is claimed to be effective in the prevention and treatment of many diseases, and in addition, it exerts anticancer properties. Though most data on its benefits are based on laboratory and practical studies [1].

Pleurotus pulmonarius is an aggressive growing fungi with simple cultivation which gives higher yields with high nutritional values [2]. It is an edible as well as medicinal mushroom which is able to break down lignin and other aromatic compounds as it produces enzymes such as laccase, Manganese oxidizing peroxidase and versatile peroxidase. These enzymes allows *Pleurotus pulmonarius* grow easily on various lignocellulosic materials [3]. They are characterized by white spore print, attached to gills often with and eccentric stip and they are commonly known as Oyster mushrooms [4].

During the production of energy in the body-free radicals are formed constantly in the mitochondria electron chain and free radicals can also be formed from internal and external sources such as drugs, smoke, food and other pollutants. Living organisms possess both internal and external antioxidants capable of inhibiting the autoxidation of these free radicals [5].

The formation of free radicals beyond the capacity of the antioxidant present in the body system will lead to oxidative stress [5]. Oxidative stress can lead to disorders which in turn can lead to Cancer, Arthritis, and Hypertension. As a result of this, synthetic antioxidants to inhibit these free radicals have been produced, they are butylated hydrocytoluen (BHA), tert-butylated hydroxyquinone and butylated hydroxyltoluene of which are associated with adverse side effect. The adoption of naturally occurring antioxidants for the neutralization of these free radicals damage from different sources is being accepted as a modern therapy [6,7].

Phytochemicals screening and antioxidant activity of cultivated *Ganoderma lucidum* showed the presence of flavonoids, saponins, tannings, anthraquinones, terpenoids and steroids [8].

A lot of interest has been drawn to mushrooms due to their nutritive and therapeutic properties [9]. They can then be utilized as useful candidates in the search of phytochemicals with radical scavenging activities [10,4]. Numerous studies on the proximate analysis has been

carried out on mushrooms [4], however, there are scanty knowledge on the successive exhaustive extraction of the bioactive compounds and antioxidant activities of each of the extract fractions of *Ganoderma lucidum* and *Pleurotus pulmonarius.* This study was carried out to evaluate the phytochemical constituent and antioxidant activities of *Ganoderma lucidum* and *Pleurotus pulmonarius.*

2. MATERIALS AND METHODS

2.1 Collection of Samples and Preparation

Ganoderma lucidum (Curtis) P. Karst fruiting bodies were collected from the Botanical Garden University of Ibadan while, *Pleurotus pulmonarius* (Fr.) Quélet fruiting bodies were bought from a local producing farm in Ibadan Oyo State Nigeria. The samples were Oven dried at 50^0C for 24 hours (one day) and grind into powder.

2.2 Serial Exhaustive Extraction of the Mushrooms

This method of extraction was done according to Das et al. [11] which involve successive extraction with solvents of increasing polarity from a non-polar (N-Hexane) to a more polar solvent (water) to ensure that a wide polarity range of compound could be extracted. Equivalent weights of each blended sample were extracted with the following solvents successively: 1) N-Hexane, 2) Ethyl acetate, 3) Ethanol and 4) Water. Each time before employing the solvent of higher polarity the samples were re-dried. Each extract was then concentrated using rotary vacuum evaporator at 40-50^oC under vacuum and dried residue was collected and stored at 4^0C in an opaque glass bottle for further studies.

2.3 Phytochemical Analysis of the Mushrooms

The qualitative and quantitative phytochemical determination was done according to Hussain et al. [12]. Flavonoids, steriods, glycosides,terpenoids, alkaloids, anthraquinones, saponings, cardiac glycosides, tannin and terpenoids on the four fractions from each of the samples (eight fractions in total).

2.4 Determination of Antioxidant Activity of the Mushrooms

The ability of the extract fractions to scavenge for free radicals was done according to the method described by Manzocco et al. [13]. The Ferric Reducing Antioxidant Power (FRAP) of the extract fractions was determined by accessing their ability to reduce $FeCl_3$ solution as described by Oyiazu et al. [14,15] and the Total Antioxidant Capacity (TAC) of the extract fractions was determined according to the thiocynate method of Mitsuda et al. [16].

Photo A. Photograph of *Ganoderma lucidum* (Curtis) P. Karst

Photo B. Photograph of *Pleurotus pulmonarius* (Fr.) Quélet

2.5 Statistical Analysis

Experiments data were analyzed in triplicate. The data were reported as mean ± SD. The means of all parameters were examined for significance using ANOVA with Duncan's Multiple Range test. Statistical significance was tested at p =.05.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Analysis

The results of the qualitative phytochemical screening of the extract fractions of *Ganoderma lucidum*, and *Pleurotus pulmonarius* are shown in Table 1 and 2.

Saponins are present in Ethyl acetate, Ethanolic and aqueous fraction of *Ganoderma lucidum* but absent in N-Hexane fraction; Tannins, Anthraquinones, Terpenoids and Phenol are present in the entire four extract fraction. Cardiac glycoside is present only in the N-Hexane fraction but absent in the remaining extract fraction. Steroids are present in N-Hexane, Ethyl acetate and Ethanolic Fraction but absent in Aqueous while Alkaloids was present in Ethyl acetate and aqueous fraction but absent in N-Hexane and Ethanolic fraction (Table 1).

Table 2 shows the qualitative phytochemical screening of *Pleurotus pulmonarius* of which Saponins, Tannins, Flavonoids and Phenol are present in Ethyl acetate, Ethanolic and aqueous fractions but were absent in N-Hexane fraction of *Pleurotus pulmonarius*. Cardiac glycosides are strongly present in only the Ethanolic fraction. Anthraquinones are present in N-Hexane, Ethyl acetate and Ethanolic fraction. Terpenoids are present in all the extract fractions. Steroids are present in N-Hexane, Ethanolic and Aqueous fraction while Alkaloids is present in Ethyl acetate and Ethanolic fraction.

Serial exhaustive extraction of mushrooms using different solvents on the bases of polarity, from a non-polar solvent (N-Hexane) to a more polar solvent (Aqueous) was performed according to Das et al. [11]. The results from the qualitative phytochemical screening of the extract fractions of *Ganoderma lucidum* and *Pleurotus pulmonarius* reveals the presence of saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, Terpenoids, Steriods, alkaloids and phenol. The findings from this research revealed that most of the phytochemicals are present in Ethanol, Ethyl acetate and Aqueous extract fractions though at varying levels than N-Hexane fraction, this is in line with the work reported by Hussein et al. [17]. Cardiac glycoside which was absent in the powder of cultivated *Ganoderma lucidum* from the work reported by Ijimbili and Adenipekun [8] is present in N-Hexane fraction of *Ganoderma lucidum.* The method of extraction used in this study has advantage over crude extraction method as it allows more of the phytochemicals to be re-covered.

Table 1. Comparative qualitative phytochemical screening of *Ganoderma lucidum*

Phytochemicals	Extraction solvent			
	N-Hexane	Ethyl acetate	Ethanol	Aqueous
Saponins	-	+	+	++
Tannins	+	+	+	+
Flavonoids	-	+	+	+
Cardiac glycosides	+	-	-	-
Anthraquinones	+	+	+	+
Terpenoids	++	++	+	+
Steroids	++	++	+	-
Alkaloids	-	+	-	+
Phenol	+	+	+	+

+ Present, ++ Strongly Present, - Absent

Table 2. Comparative qualitative phytochemical screening of *Pleurotus pulmonarius*

Phytochemicals	Extraction solvent			
	N-Hexane	Ethyl acetate	Ethanol	Aqueous
Saponins	-	++	++	+
Tannins	-	+	+	+
Flavonoids	-	+	++	++
Cardiac glycosides	-	-	++	-
Anthraquinones	++	++	+	-
Terpenoids	++	++	+	+
Steroids	++	-	+	+
Alkaloids	-	+	+	-
Phenol	-	+	+	+

+ Present, ++ Strongly Present, - Absent

3.2 Phytochemical Quantification

The results of Table 3 show the comparative phytochemical quantification of *Ganoderma lucidum* and *Pleurotus pulmonarius* extracts. The two mushrooms contain alkaloids ranging from 41.70±0.14% in Ethyl acetate fraction of *Ganoderma lucidum* to 18.10±0.14% in Ethanolic Fraction of *Pleurotus pulmonarius;* Flavonoid content ranged between 19.90±0.14% in Ethyl acetate fraction of *Ganoderma lucidum* to 8.80±0.00% in Ethyl acetate fraction of *Pleurotus pulmonarius;* Terpenoids content in all the extract fractions of *Ganoderma lucidum and Pleurotus pulmonarius* ranged from 3.40±0.14 to 0.70±0.14, Saponins content range from 2.70±0.14 in Ethanolic fraction of *Pleurotus pulmonarius* to 0.70±0.14 in Aqueous fraction of *Ganoderma lucidum* while Tannins content ranges from 0.25±0.10 in N-Hexane fraction of *Ganoderma lucidum* to 0.10±0.24 in Ethyl acetate fraction of *Pleurotus pulmonarius.* Ethyl acetate fraction of *Ganoderma lucidum* has the most significant amount of alkaloids when compared to other extract fractions.

The quantitative evaluations of some of the phytochemicals show that the mushrooms has significant amount of Alkaloids, Flavonoids, Saponins Tannins and Terpenoids with *G. lucidum* having the highest percentage of alkaloids in Ethyl acetate fraction. According to Cheng and Miles [18], Alkaloids has been analyzed from many species of mushrooms and the quantitative phytochemical screening of *Pleurotus pulmonarius* and *Ganoderma lucidum* confirm these mushrooms to be among those that produced alkaloids. These phytochemical constituents play a major role in the medicinal properties of many mushrooms.

Mushrooms have been reported to have a wide range of pharmacological properties that are beneficial, such as anti-inflammatory and anti-diabetic effects. As a result theses mushrooms can be used in the controlling of diabetes and inflammation related diseases [19].

Terpenoids have been reported to display a wide range of pharmacological potentials that include anti-malarial, anti-inflammatory and anti-cancer effects among others [20,5].

The valuable medicinal properties of many mushrooms have also been attributed to the presence of alkaloids on the autonomic nervous

system, blood vessels, respiratory system, gastrointestinal tract, uterus, and have been shown to be effective against malignant diseases, infections and malaria [21]. Phenolic compounds are antioxidants, and show a wide range spectrum of medicinal properties such as anti-cancer, anti-inflammatory and diabetic effects [22]. Flavonoids are one of the most diverse groups of natural compounds that have been shown to possess a broad spectrum of chemical and biological activities including radical scavenging properties, antiallergenic, antiviral, anti-inflammatory, and vasodilating actions [23]. Thus the extracts of the studied mushrooms may be good alternatives for the treatment of diseases associated with excessive free radical generation and damage. The aqueous extract fraction of *Pleurotus pulmonarius* shows that flavonoid content agrees with the work of Hamzah et al. [5] and is higher than that of *Ganoderma lucidum*. These mushrooms can therefore be harnessed in the management of oxidative stress induced diseases since flavonoid have been shown to possess various antioxidant functions.

3.2.1 Antioxidant activities in the mushrooms extract fractions

The percentage scavenging activity of the different extract fractions of *Ganoderma lucidum* and *Pleurotus Pulmonarius* on 2,2-diphenyl-1-picrylhdrazyl (DPPH) are shown in Table 4. Both mushrooms possess the properties of free radical scavenging activity of which the Ethyl acetate, Ethanolic and Aqueous fraction of *Ganoderma lucidum* have percentage inhibition ranging from 96.41% to 90.00%, which is close to the standard (ascorbic acid) while N-Hexane fraction have the lowest percentage inhibition as the concentration increases. The percentage inhibition of DPPH radical scavenging activities of the different extract fractions of *Pleurotus pulmonarius* ranges from 66.87% to 20.33% as the concentration increases.
The result of the Ferric ion Reducing Antioxidant Power (FRAP) and Total Antioxidant Capacity (TAC) content is shown in the Figs 1 and 2.

The result of the reductive power of *Ganoderma lucidum* and *Pleurotus pulmonarius* shows that the activity of the extract fraction increases as the concentration increases. The N-Hexane extract fraction of *Pleurotus pulmonarius* had better reducing power at 20 µg/ml, 40 µg/ml, 80 µg/ml and 100 µg/ml while the Ethyl acetate and aqueous fractions had better reducing power at 60 µg/ml.

The N-Hexane extract fraction of *Ganoderma lucidum* perform better at 60 µg/ml, 80 µg/ml and 100 µg/ml while the Ethanolic and Ethyl acetate fraction perform better at 20 µg/ml and 40 µg/ml.

The results of the Total Antioxidant Capacity (TAC) of the both mushrooms show that the N-Hexane and Ethyl acetate fraction had better TAC content than Ethanolic and Aqueous fraction of both *Pleurotus pulmonarius* and *Ganoderma lucidum* as the concentration increases. The N-hexane fraction of the both mushrooms at 200µg/ml, 600µg/ml had better TAC content than the other fractions while the Ethylacetate fractions had better TAC content at 1000µg/ml.

The2, 2 diphenyl-1- picrylhydrazyl (DPPH) free radical Scavenging activity assay is a widely methods for the determination of antioxidant capacity. It relies on the reduction of methanolic DPPH solution in the presence of hydrogen donation compound (antioxidant). The resulting decolourisation upon the perception of hydrogen from the antioxidant is stoichiometric with respect to the degree of reduction and absorbance measurement after a certain time corresponds inversely to the radical scavenging activity of the antioxidant [5]. Although the Ethyl acetate extract fractions of *Ganoderma lucidum* specie had the highest percentage inhibition, which is closer to that of the standard (ascorbic acid) compared to the extract fractions of *Pleurotus pulmonarius*.

The N-Hexane Fraction of the mushrooms has the highest Ferric ion Reducing Antioxidant Power (FRAP) content, this is in contrary to DPPH radical investigation and could be as a result of Anthraquinones, Terpenoids and Steroids that are strongly present in the N-Hexane extract fraction. It could be concluded that the investigated mushrooms extract fractions are antioxidant products, but they possess reductive capabilities which are still lower than the standard antioxidant compounds (ascorbic acid) [24].

The observed Total Antioxidant Capacity (TAC) of the mushroom can be attributed to the presence of phytochemicals. Bioactive compounds found in edible and medicinal mushrooms are known to play a vital role in promoting health [19].

Table 3. Quantification of some of the Phytochemical in the extract fractions of *Ganoderma lucidum* and *Pleurotus pulmonarius*

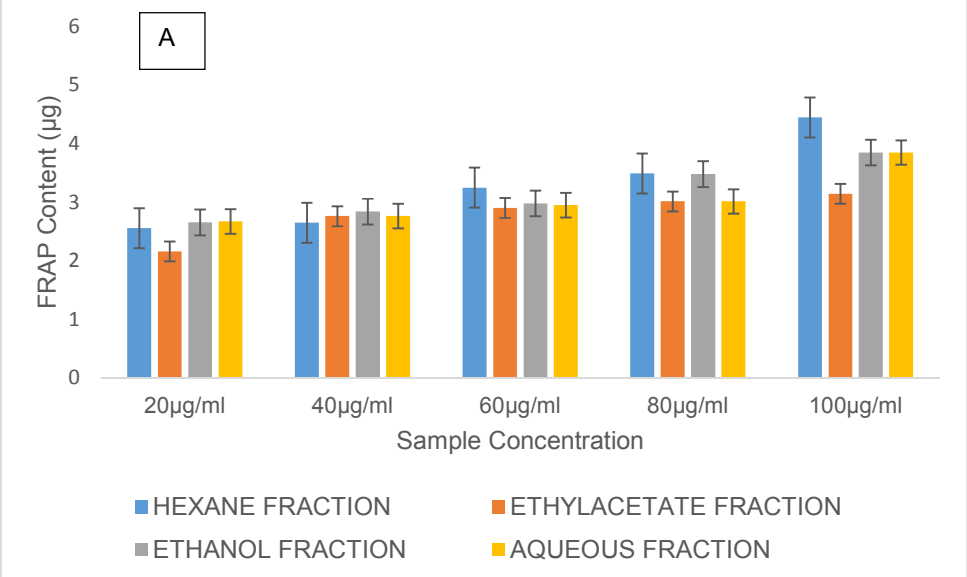
Mushrooms	Solvents	Phytochemicals				
		Alkaloids (%)	Flavonoids (%)	Terpenoids (%)	Saponins (%)	Tannins (%)
P.P	Hex.	-	-	3.13±0.42	-	-
	Ea	19.50±0.41	8.80±0.00	3.32±0.14	2.00±0.28	0.12±0.71
	Ethanol	18.10±0.14	15.10±0.71	1.50±0.42	2.70±0.14	0.10±0.24
	Aqueous	-	22.30±0.14	0.90±0.14	1.22±00	0.10±0.32
G.L	Hex.	-	-	3.40±0.14	-	0.25±0.10
	Ea.	41.70±0.14	19.90±0.14	2.13±0.13	0.60±0.00	0.09±0.52
	Et.	37.30±0.42	19.40±0.28	1.62±0.00	0.70±0.14	0.10±0.28
	Aqueous	-	13.20±0.00	0.70±0.14	1.52±0.10	0.13±0.00

Values are means ± Standard deviation of duplicate observations. Hex: N-Hexane, Ea: Ethyl acetate,P.P: Pleurotus pulmonarius G.L Ganoderma lucidum

Table 4. Percentage scavenging activity of the different extract fractions of *Ganoderma lucidum* and *Pleurotus pulmonarius*

Mushroom	Conc. (µg/ml)	Hex. (%)	Ea. (%)	Et. (%)	Aqueous. (%)	Standard (%)
P.P	200	20.33^{e}	33.33^{e}	22.39^{d}	24.20^{e}	96.97^{c}
	400	21.42^{d}	52.42^{d}	25.88^{c}	27.15^{d}	97.00^{c}
	600	25.83^{c}	57.97^{c}	26.69^{b}	44.73^{c}	97.02^{c}
	800	26.18^{b}	61.65^{b}	33.36^{a}	53.56^{b}	97.18^{b}
	1000	32.80^{a}	64.73^{a}	33.36^{a}	66.87^{a}	98.34^{a}
G.L	200	6.41^{e}	94.86^{e}	94.96^{d}	75.54^{d}	96.97^{c}
	400	27.02^{d}	95.04^{d}	95.11^{c}	75.62^{d}	97.00^{c}
	600	27.63^{c}	95.29^{c}	95.75^{b}	86.03^{c}	97.02^{c}
	800	34.43^{b}	95.45^{b}	95.88^{b}	90.00^{b}	97.18^{b}
	1000	36.23^{a}	96.41^{a}	96.03^{a}	94.91^{a}	98.34^{a}

Each value is a mean of 3 replicates. Means with different superscripts in each column are significantly different at P=.05 according to Duncan's multiple range tests. G. L.: Ganoderma lucidum, P.P: Pleurotus pulmonarius. Conc: Concentration, Hex. N-Hexane, Ea: Ethyl acetate and Et: Ethanol



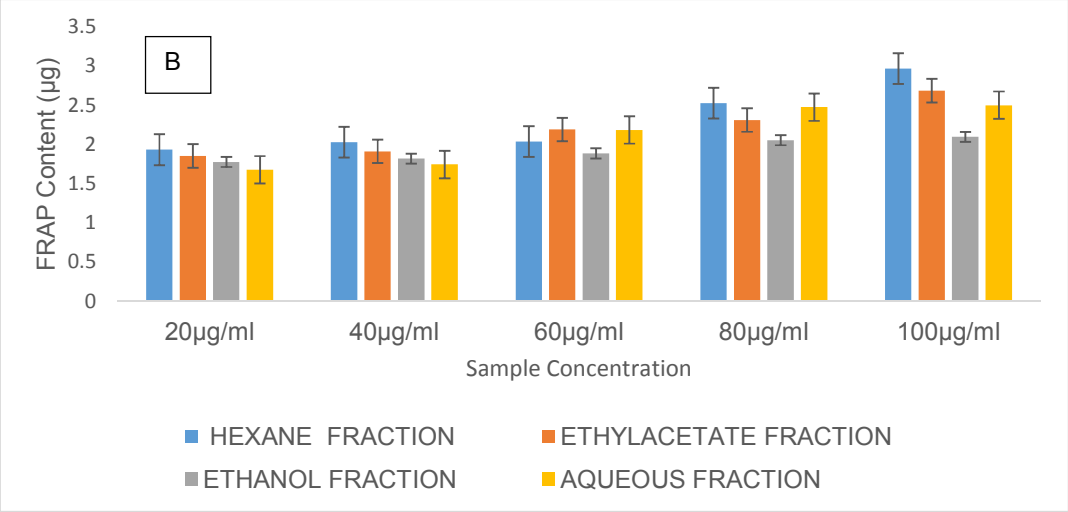


Fig. 1. Total Ferric ion Reducing Antioxidant Power (FRAP) for the different fractions of the mushrooms (A- *Ganoderma lucidum;* B- *Pleurotus pulmonarius*

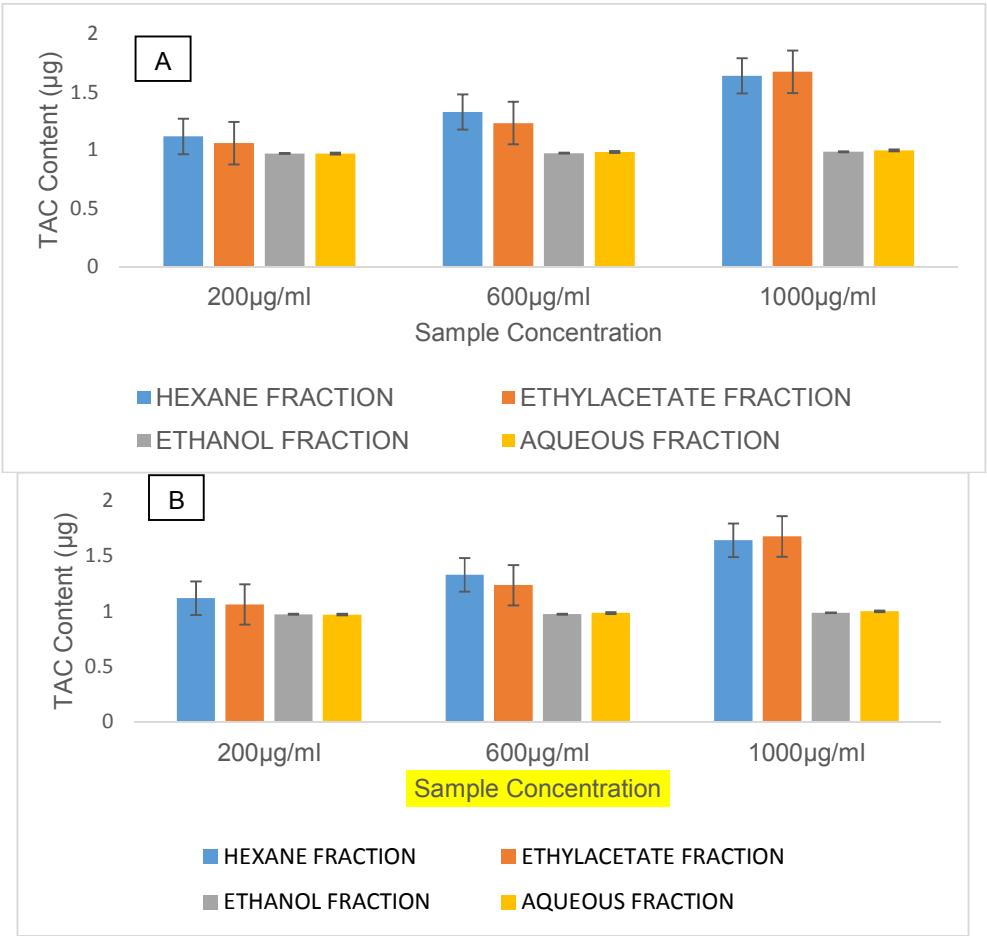


Fig. 2. Total antioxidant capacity of the different extract fractions of the mushrooms (A- *Pleurotus pulmonarius;* B- *Ganoderma lucidum*)

Fe (III) reduction is often used as an indicator of electron donating activity, which is an important mechanism of antioxidant action of Phenolics [25]. Therefore the in-vitro antioxidant properties exhibited by this mushroom extract may be due the presence of these antioxidant phytochemicals inherent in it.

4. CONCLUSION

Mushrooms possess numerous potential bioactive compounds and can serve as a rich source of natural antioxidants food for the enhancement of the immune system against oxidative damage and as possible supplements in pharmaceuticals industries for the production of drugs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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