

# PHYTOCHEMICAL ANALYSIS AND *IN-VITRO* ANTIOXIDANT ACTIVITIES OF SOME SELECTED HIGHER FUNGI FROM OYO STATE, SOUTH WEST OF NIGERIA

## ABSTRACT.

Higher-fungi (Hf) of the polypore mushrooms are considered to have unique secondary metabolites, making them reservoirs of therapeutically significant bioactive compounds. Phytochemical and antioxidant properties of the Hf were accessed in this study.

Four Hf, which were found in several wild locations in Oyo state, Nigeria, were collected. At the University of Ibadan Botany Department Laboratory, the species of the four Hf were determined. In-vitro antioxidant activity were assessed using the 1,1-Diphenyl-2-picrylhydrazyl (DPPH), Ferric Reducing Antioxidant Power (FRAP), and Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) assays using methanol extracts of air-dried and powdered Hf. Results were displayed as Mean SEM, graphs were drawn using Microsoft Excel, one-way ANOVA was used for analysis, and  $p < 0.05$  was considered significant.

These Hf were identified as *Lycoperdon rimlatum* (*Lr*) FFUI1, *Trametes versicolor* (*Tv*) FFUI2, *Ganoderma lucidum* (*Gl*) FFUI3, and *Daedelia quarcina* (*Dq*) FFUI4 and were recorded on the NCBI with accession numbers EU833664.1, JQ621899, JQ520179.1 and KP171209.1, respectively. All the Hf showed positive tests for the presence of saponin, tannin, alkaloid, terpenoid, carbohydrate, tannin and flavonoids. The Hf showed antioxidant activities, the highest DPPH inhibition was by *Tv* (94.48%), FRAP was by *Gl* (0.16 mg/g) and H<sub>2</sub>O<sub>2</sub> inhibition was by *Lr* (70.90%).

The antioxidant activities observed were due to the presence of useful phytochemicals making them therapeutically significant.

**Key words:** Higher- fungi, Phytochemicals, Bioactive compounds and *In-vitro* Antioxidant activities.

## 1.0 INTRODUCTION.

Fungi constitute an undescribed, undocumented lineage of eukaryotes with huge ecological and economic effects (Carris, *et al.*, 2012). Higher fungi have been considered one of the highly diversified biological resources in the world (Zhong, & Xiao, 2009). They are diverse, heterotrophic organisms with unique nutritional and ecological requirements (Okaroh, 2017). These Mushrooms have been reported as foods with therapeutic value used in the management of hypercholesterolemia, hypertension, and cancer (Kumar, 2015). Natural products produced by microorganisms, plants, and animals that play important roles in defense are secondary metabolites (Tiwari & Rana, 2015).

This metabolite from fungi has been receiving great consideration globally since the 1940s when antibiotics were discovered (Manganyi & Ateba, 2020). Oxidation is essential for energy production in several living organisms for the fueling of physiological processes (Augusto & Miyamoto, 2011)). Although oxygen is essential for life, it can also worsen the harm done by oxidative events within the cell. Oxygen's oxidative property is vital in a range of biological activities. The production of ATP (adenosine triphosphate) by the mitochondria, which is used by the cell to produce energy, results in the synthesis of free radicals. Reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are byproducts of the biological redox process, are both deadly and beneficial molecules in humans. Free radicals can be produced in humans all around the world by environmental factors like noise, radioactivity, smoke, and weedicides. Most endogenous and physiological reactive oxygen species (ROS) are by-products of the oxidative reaction process that occurs in the mitochondrial respiratory chain (Nathan & Cunningham-Bussel, 2013). Reactive oxygen species (ROS) have a variety of consequences on cell physiology, including aiding in the death of invasive bacteria, wound healing, and regenerative processes (Bhattacharyya *et al.*, 2014). Consuming antioxidants on a regular basis can help to reduce free radicals' potential for harm. Unquestionably, the delicate balancing act between these two opposing effects is crucial in life. At low to moderate

46 concentrations, reactive species have positive impacts on immune response and cellular redox signaling;  
47 yet, at high concentrations, they can impair cell structures and function by inducing oxidative stress. The  
48 hydrogen-based scavenging, regulation of free radicals, and radical peroxide oxidation processes are all  
49 part of the mushroom's antioxidant action (Ferreira *et al.*, 2009). By strengthening the immune system, these  
50 antioxidants lower the risk of infection, cancer, and cardiac issues. External antioxidants from food  
51 supplements are required when endogenous antioxidants are insufficient to sufficiently protect the  
52 organisms from free radicals (Sen & Chakraborty, 2011).

53 God designed plants and other herbal products with specific therapeutic or curative properties, which are  
54 known as "phytochemicals," to benefit people (Venkataramaiah, 2020). Phytochemicals, as their name  
55 implies, are chemicals produced by plants from their primary or secondary metabolites (Anulika *et al.*, 2019).  
56 These phytochemicals come in various kinds depending on their medicinal capabilities. Many plants and  
57 herbal items include a number of chemical components known as secondary metabolites that combine to  
58 produce therapeutic effects. Given that they have fewer or no side effects as compared to conventional  
59 synthetic medications and that many natural therapies have their own curative powers, the therapeutic  
60 properties of many plants and herbal items are becoming more and more well-known and preferred  
61 (Ogunmefun, 2018). The phytochemicals that medicinal plants and herbs create for defense are quite  
62 abundant, and it is these components that give them their therapeutic properties (Alamgir, 2017). Medicinal  
63 plants and herbs may contain a range of phytochemicals, including saponin, which can be used to lower  
64 blood cholesterol, nitrogen-rich alkaloids, which can be used as stimulants, tannins, which act as natural  
65 antibiotics, anthraquinones, which act as laxatives and dyes, cardiac glycosides for cardiac drugs,  
66 flavonoids, and antioxidant phenols, as well as other compounds (Oyugi, 2016). The objective of this  
67 research is to assess the phytochemical and antioxidant properties of the selected higher fungi.

68

## 69 **2.0. MATERIALS AND METHODS.**

### 70 **2.1 Collection and identification of mushroom samples**

71 Fresh fruiting parts of the selected macro-fungi were collected from the wild in Saki, Ibadan, Ogbomosho,  
72 and Iseyin in Oyo State, southwest of Nigeria between August and September 2016 and 2017. They were  
73 validated at the University of Ibadan's Botany Department Laboratory. They were identified using  
74 descriptions from Alexopoulos *et al.* (1998). The macro-fungi DNA was extracted with Hexadecyl trimethyl  
75 ammonium bromide (cTAB) (Moller *et al.*, 1992). The internal transcribed spacer (ITS) region was amplified  
76 using the primer pairs, pITS4-F (5'-TCCGTAGGTGAACCTGCCG-3') and pITS1-R (5'-  
77 TCCTCCGCTTATTGATATGC-3'). The PCR data was analyzed by electrophoresis on a 1 % agarose gel at  
78 the International Institute of Tropical Agriculture in Ibadan, Oyo state, Nigeria, prior to sequencing. Using the  
79 NCBI basic alignment search tools (BLAST), the generated sequences were analyzed to determine the  
80 closest sequence matches for taxonomy classification (Thomson *et al.*, 1997). Their basidiocarps were  
81 gathered and kept in the macro-fungi collection owned by Jonathan Gbolagade at the University of Ibadan's  
82 Botany Department.

83

### 84 **2.2. Extraction of phytochemicals from selected Higher Fungi**

85 To obtain the fraction of the methanol extract, 1.5 kg of the air-dried powdered sample was placed  
86 separately into the soxhlet chamber extractor and extracted with 7.5 liters of 95 % methanol for 24 hours at  
87 40° C. As reported by Evans, (2009), the filtrate was evaporated using a rotating electrical evaporator in a  
88 vacuum. Crude methanol extract yield was measured and correctly labeled and maintained in hygienic glass  
89 containers at room temperature until it was ready for use. The crude methanol yield was calculated after the  
90 above extraction, and determined by deducting the final weight of the extract obtained from the initial weight  
91 multiply by 100.

92 % yield =  $W1 - W2 \times 100$ , Where W1 initial weight, W2 =final weight.

93

### 94 **2.3. Qualitative Phytochemical analysis**

95 Methanol extracts from medicinal mushrooms were **subjected** to numerous chemical tests to classify  
96 particular bioactive constituents using standard procedures (Evans, 2009).

### 97 **2.4. Quantitative determination of phytochemical constituents (Evans, 2009).**

98 Under lower pressure, filtered raw mushroom extracts (200 ml) are concentrated and segmented with 70  
99 percent (V/V) sequential extractions of n-hexane, chloroform, ethyl acetate, and ethanol. **These four**  
100 **fractions were tested on secondary metabolites using qualitative phytochemical**  
101 **reactions. Triterpene/steroids, alkaloids, flavonoids, saponins, carbohydrates, tannins, and terpenoids are**  
102 **measured.** As an empirical response to those measures, colour intensity was used.

### 103 **2.5 In-vitro antioxidant assays**

104 2.5.1 DPPH radical scavenging activity method.

105 Following the protocol established by Shimada, *et al.*, (1992), the scavenging ability of mushrooms was  
106 assessed with some modifications. First, 0.5 ml of the aliquot sample extract was put in the test tubes with  
107 radical 2.9 ml of 200 **µmol** DPPH at various ethanol concentrations. The mixture was shaken vigorously and  
108 allowed to stand for 30 minutes at room temperature in the dark. Using a UV spectrophotometer, the  
109 reaction blend was measured at 515 nm. The solvent extraction was used as a blank, without an extract.  
110 The base norm used as ascorbic acid. The scavenging effect was determined based on the following  
111 formulation:

112 Scavenging effect (%) =  $1 - [(Absorbance\ sample / Absorbance\ control) \times 100]$

113 2.5.2 Ferric reducing antioxidant power (FRAP) assay.

114 The process described in (Benzie & Strain, 1996; Huang, *et al.*, 2005) was applied. The freshly formed  
115 FRAP reagent was regulated and incubated in a water bath for 10 minutes at 30 °C. At 0 min., absorbance  
116 was then recorded (**t<sub>0</sub>**). The test tube was directly exposed to 100–500 l of mushroom sample extract and  
117 100 l of distilled water for 30 minutes at about 30 °C. The absorbance was then measured at a wavelength  
118 of roughly 700 nm (**t<sub>30</sub>**). The reference substance was ferrous sulphate. The sample extract's antioxidant  
119 capacity was assessed using a conventional ferrous sulfate curve, and the FRAP value was determined as  
120 being equal to M Fe2 + per gram of extract using the formula:

121 FRAP value = Absorbance (sample + FRAP reagent) – Absorbance (FRAP reagent)

122 2.5.3 H<sub>2</sub>O<sub>2</sub> radical scavenging assay.

123 The Ruch *et al.*, (1989) method was used to examine the extract's capacity to scavenge hydrogen peroxide.  
124 In the phosphate buffer, a solution of hydrogen peroxide (2mol/l) was made (pH 7.4). Extracts were added to  
125 the hydrogen peroxide solution at a rate of 1 to 10 g per ml (0.6 ml). The hydrogen peroxide absorbance at  
126 230 nm was estimated using a blank solution devoid of hydrogen peroxide and comparing the results with  
127 ascorbic acid and the reference substance after 10 minutes. H<sub>2</sub>O<sub>2</sub> activity (%) =  $(Abs\ control - Abs\ sample) /$   
128  $Abs\ (control) \times 100$

129

## 130 2.6 Statistical Analysis

131 Results were presented as Mean  $\pm$  SEM, graphs plotted using Microsoft Excel, analysed using one-way  
132 ANOVA and  $P < 0.05$  was significant.

## 133 3.0 RESULTS AND DISCUSSIONS

134 After the sequenced data was edited using bio edit and blasted in the NCBI blast data based. The identity of  
135 our fungi were revealed. as *Lycoperdon rimulatum* FFUI1, *Trametes versicolor* FFUI2, *Ganoderma lucidum*  
136 FFUI3, and *Daedelia quarcina* FFUI4 and were recorded on the NCBI with accession numbers  
137 EU833664.1, JQ621899, JQ520179.1 and KP171209.1, respectively.

138 Table 1

139

140 The percentage yield of metabolites from the Higher Fungi is represented in Table 2. The highest  
141 percentage yield was observed with the organism *Lycoperdon rimulatum* at 46.98%, while the lowest was  
142 observed with *Trametes versicolor* at 26.26%.

143 Table 2

144

145 The qualitative phytochemical analysis of the Higher fungi samples is represented in Table 3. In the  
146 analysis, it was observed that all the organisms showed positive tests for the presence of saponin, tannin,  
147 alkaloid, terpenoid, carbohydrate and tannin. The steroid tested positive with only *Trametes versicolor*  
148 while anthocyanin and phlobatannin tested positive with only *Ganoderma lucidum*. Flavonoids tested  
149 positive with all the Higher fungi except *Daedelea quercina*.

150 The quantitative phytochemical analysis of the Higher fungi samples is represented in Table 4. In the  
151 quantitative analysis of carbohydrate content, the highest amount of carbohydrate was observed with  
152 *Lycoperdon rimulatum* at  $2.38 \pm 0.03$ , while the lowest was observed with *Trametes versicolor* at  
153  $0.19 \pm 0.01$ . For the flavonoid content, *Ganoderma lucidum* had the highest amount with  $0.2 \pm 0.01$  while the  
154 lowest amount was observed with *Trametes versicolor* with  $0.11 \pm 0.01$ . In the quantitative analysis of  
155 Terpenoid content, *Lycoperdon rimulatum* has the highest amount of terpenoid with  $0.60 \pm 0.03$ , while  
156 *Trametes versicolor* has the lowest with  $0.20 \pm 0.0$ . For saponin content, *Ganoderma lucidum* has the  
157 highest amount with  $0.18 \pm 0.03$  while the lowest amount was observed with *Lycoperdon rimulatum* with  
158  $0.02 \pm 0.01$ . For alkaloid, the highest amount was observed with *Trametes versicolor* while the lowest  
159 amount was observed with *Daedelea quercina* with  $0.02 \pm 0.01$ . For the quantitative presence of tannin, the  
160 highest amount was observed with *Ganoderma lucidum* with  $7.73 \pm 0.0$  while the lowest was observed with  
161 *Trametes versicolor* with  $2.6 \pm 0.02$ .

162 The link between phytochemistry and pharmacology is critical to consider when designing studies on the  
163 medicinal potential of plants and herbal medicines. In general, the results showed that phytochemicals  
164 were present in all samples, but at varying quantities. Alkaloids, tannins, saponins, and phenols are

165 considered anti-nutrients since they are poisonous when consumed in large doses. However, it has been  
166 shown that several of the phytochemicals found in mushrooms have therapeutic properties. The presence  
167 of bioactive phytochemical constituents found in all of the Higher fungi studied has been suggested as the  
168 reason for their traditional uses in the treatment of inflammation, pains, hemostatic, diuretic, nutrition,  
169 antibiotics, and antitumor agents, which is supported by the findings of Edeoga and Erita (2001), who  
170 discovered alkaloids' significant pharmacological modulation. The presence of alkaloids in the samples  
171 shows that they have analgesics and bactericides medicinal value, confirming Stary's (1998) findings.  
172 Furthermore, presence of phenols in the Higher fungi samples makes them antiseptics and antifungal  
173 (Gill, 1992). The presence of flavonoids in the Higher fungi suggests that they have antioxidant healing  
174 properties, which backs up Okwu, (2004) findings that flavonoids can prevent cancer and oxidative cell  
175 damage. External antioxidants from food supplements are required when endogenous antioxidants are  
176 insufficient to protect organisms from free radicals. This exogenous antioxidants can be obtained naturally  
177 from flavonoids, according to Litescu *et al.* (2011). The presence of tannin in the sample, on the other  
178 hand, is linked to wound healing, supporting Okwu, (2004) results that the concentration of tannin present  
179 in mushrooms can draw tissues together to aid wound healing.

180

181 Table 3

182 Table 4

183

184 The percentage inhibition of radical of methanolic extract of the selected higher fungi and Vitamin C,  
185 using DPPH assay are represented in Figure 1. At 100 µg/ml, the highest percentage inhibition was  
186 observed with Vitamin C, a standard antioxidant by 94.63% followed by *Trametes versicolor* by 94.48  
187 % while the least percentage inhibition was observed with *Lycoperdon rimulatum* by 89.44  
188 %,meanwhile at 500 µg/ml, the standard sample, vitamin c had the highest percentage inhibition by  
189 87.1% followed by *Trametes versicolor* by 85 % while the least percentage inhibition was observed  
190 with *Lycoperdon rimulatum* by 48.3 %.Overall, it was observed that percentage inhibition reduced with  
191 an increase in the concentration of the extracts. The DPPH test assesses the reactivity of substances  
192 using a stable free radical called DPPH, which produces a potent visible-range absorption band at  
193 517 nm. The absorbance decreases and the color of the DPPH solution changes from deep violet to  
194 light yellow when the odd electron pairs off in the presence of a free radical scavenger. The extent of  
195 the absorbance reduction reflects the extract's antioxidant strength. (Barreira *et al.*, 2008).

196

197 The ferric reducing power activity of methanolic extract of the selected higher fungi and Vitamin C  
198 assay are represented in Figure 2. At 100 µg/ml, the highest ferric reducing power activity was  
199 observed with *Ganoderma lucidum* by 0.14 while the least activity was observed with *Lycoperdon*  
200 *rimulatum* by 0.01. .At 500 µg/ml, the standard sample, vitamin c had the highest ferric reducing  
201 power activity by 0.37 followed by *Ganoderma lucidum*, by 0.36, while the least was observed with  
202 *Lycoperdon rimulatum* by 0.16.Overall, it was observed that ferric reducing power activity increased

203 with an increase in the concentration of the extracts and the highest reducing power was observed  
204 with Vitamin C at 500 ( $\mu\text{g/ml}$ ) with 0.37 while the lowest reducing power was observed with  
205 *Lycoperdon rimulatum* with 0.01 at 100 ( $\mu\text{g/ml}$ ). Since a compound's reducing power is correlated with  
206 its capacity for electron transfer, it may be a useful predictor of its potential antioxidant action (Abbasi  
207 et al., 2013). The extract's ability to reduce happened in a dose-dependent way. This can be ascribed  
208 to the extract's polyphenols' ability to donate electrons.

209 The percentage inhibition of methanolic extract of the selected higher fungi and Vitamin C using,  
210 Hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) radical assay are represented in Figure 3. At 20  $\mu\text{g/ml}$ , the highest percentage  
211 inhibition was observed with Vitamin C, a standard antioxidant by 83.5% followed by *Lycoperdon*  
212 *rimulatum*, *Trametes versicolor* and *Daedelia quarcina* by 70.9 %, 65.3 %, and 65.3 % respectively while  
213 the least percentage inhibition was observed with *Ganoderma lucidum* by 55.28 %. At 100  $\mu\text{g/ml}$ , the  
214 standard sample, vitamin C had the highest percentage inhibition by 54.1 % followed by *Daedelia*  
215 *quarcina* by 35.2 %, while the least percentage inhibition was observed with *Trametes Versicolor* by 24.1  
216 %. In the overall, it was observed that percentage inhibition reduced with an increase in the concentration  
217 of the extracts, and *Lycoperdon rimulatum* gave the highest inhibition at 20( $\mu\text{g/ml}$ ) with 70.9 % while the  
218 least was observed at 100( $\mu\text{g/ml}$ ) with *Trametes Versicolor* at 24.1%. A precursor to the formation of  
219 hydroxyl radicals in cellular components is hydrogen peroxide. One of the quick initiators of the lipid  
220 peroxidation process, hydroxyl radicals take hydrogen atoms from polyunsaturated fatty acids to cause  
221 peroxidic reactions of membrane lipids. (Lipinski, 2011).

222 The capacity of a chemical to transport electrons influences its reducing power. Reducing power is  
223 frequently used to assess the anti-inflammatory properties of polyphenols, such correlated with  
224 reductones' existence, which exerts antioxidant activity by severing the cycle of free radicals by giving  
225 atom of hydrogen (Duan and Jiang, 2007). The FRAP value acts as a Fe (II) TPTZ extract's reduction  
226 power as measured.

227 **A protective framework that counteracts unpaired radicals fortifies living things. Oxidative enzymes make**  
228 **up the safety structure. The body's anti-oxidative defense system has so far successfully adjusted free**  
229 **radicals in controlled environments.** A defense mechanism that balances unpaired radicals strengthens  
230 living things, and this defense mechanism is made up of oxidative enzymes. Accordingly, antioxidant food  
231 supplements can help the body's defense mechanism in neutralizing or mitigating oxidative harm,  
232 supporting the findings of the American Dietetic Association (2003) that a healthy diet can provide all of  
233 the antioxidants needed and Cadenas' (1997) early findings that dietary antioxidant intake can improve  
234 protection against free radicals. All the higher-fungi tested positive for antioxidant properties *in vitro* using  
235 DPPH, FRAP, and Hydrogen peroxide assays, which is consistent with Mau *et al.*,(2002) findings, that  
236 natural bioactive products generated by microorganisms, macro-fungi, plants, and animals have  
237 antioxidant properties. The hydrogen peroxide scavenging activity of the Higher-fungi can be attributed to  
238 the proton donating potential of their phytochemical components. It also corroborated the findings of  
239 Chang *et al.* (2007) that hydrogen scavenging is a key component of the antioxidant action of mushrooms,  
240 keeping free radicals and radical peroxide oxidation in check.

241

242 Figure 1

243 Figure 2

244 Figure 3

245

#### 246 **4.0 CONCLUSION**

247 According to what has already been stated, the primary objective of researchers today is to discover  
248 natural antioxidants that will displace synthetic ones in the food, medicinal, and industrial applications.  
249 (Shebis, *et al.*,2013). Finding novel natural products from wild sources could benefit the food business  
250 by introducing better and safer antioxidants that offer strong defense against oxidative damage, which  
251 happens in both the body and our everyday foods. Therefore, new wild non- poisonous mushrooms  
252 could be introduced as natural supplies for this purpose. The researched Higher-fungi seems to be  
253 viable sources of bioactive substances that might have intriguing antioxidant effects in animal  
254 systems. Due to the Fungi's high phenolic content, it exhibits substantial antioxidant action. As a  
255 result, *Ganoderma lucidum*, *Trametes versicolor*, *Daedelia quercina*, and *Lycoperdon rumilatum* are  
256 recognized as superb source of bioactive substances that can be turned into medicines to treat  
257 oxidative stress.

258 The current research was able to identify indigenous mushrooms using molecular analysis, enriching  
259 and adding to our knowledge of mushroom biodiversity in Nigeria. The methanolic extracts of the  
260 studied Higher-fungi (*Trametes versicolor*, *Daedelia quercina*, *Ganoderma lucidum*, and *Lycoperdon*  
261 *rimulatum*) indicated the existence of flavonoids as well as antioxidant activity. Consuming these  
262 mushrooms can therefore serve as a source of exogenous antioxidants to supplement endogenous  
263 antioxidants in nutritionally supplemented diets, which can be extremely beneficial as protection  
264 against cancer, heart disease, boosting immunity, and anti-aging, supporting the findings of Omeonu  
265 *et al.*, (2022). Preclinical and clinical studies are also needed to assess the effectiveness of the  
266 natural extracts of these mushrooms in the treatment or prevention of a variety of human diseases.

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#### 271 **CONFLICT OF INTEREST**

272 There are no competing interests among the writers of this work.

273  
274

## **AUTHORS' CONTRIBUTIONS**

275 All authors contributed to the completion of this work. The study's design, statistical analysis,  
276 protocol, and initial writing of the manuscript were all handled by author FCO. The study's  
277 analysis was supervised and handled by authors SGJ and ATS. The discussions, editing, and  
278 literature searches were overseen by authors VOA and SAL. The final manuscript was read and  
279 approved by all authors.

280

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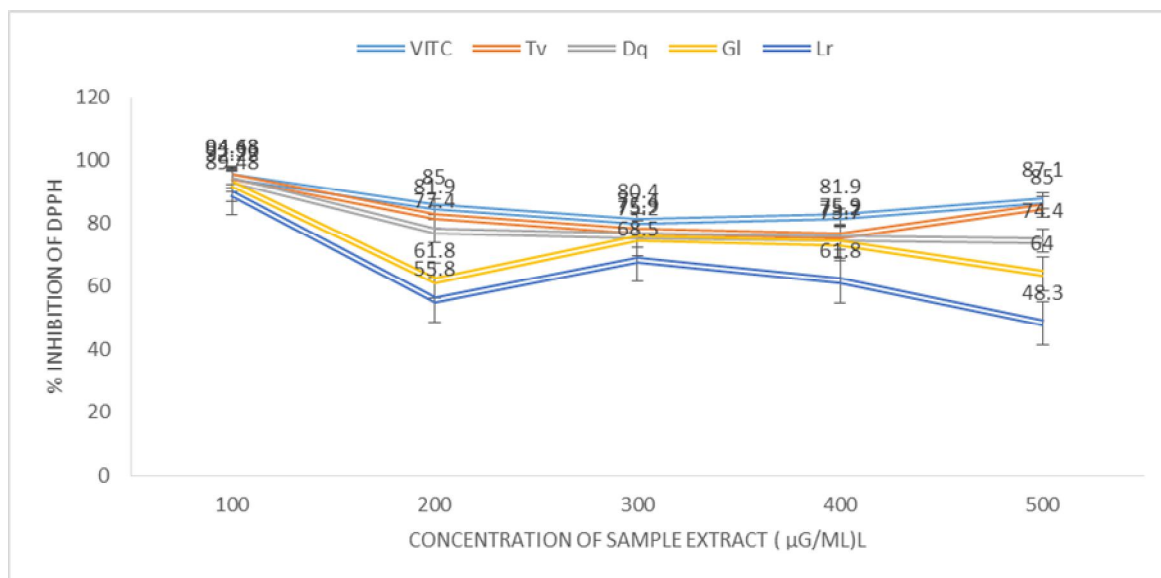
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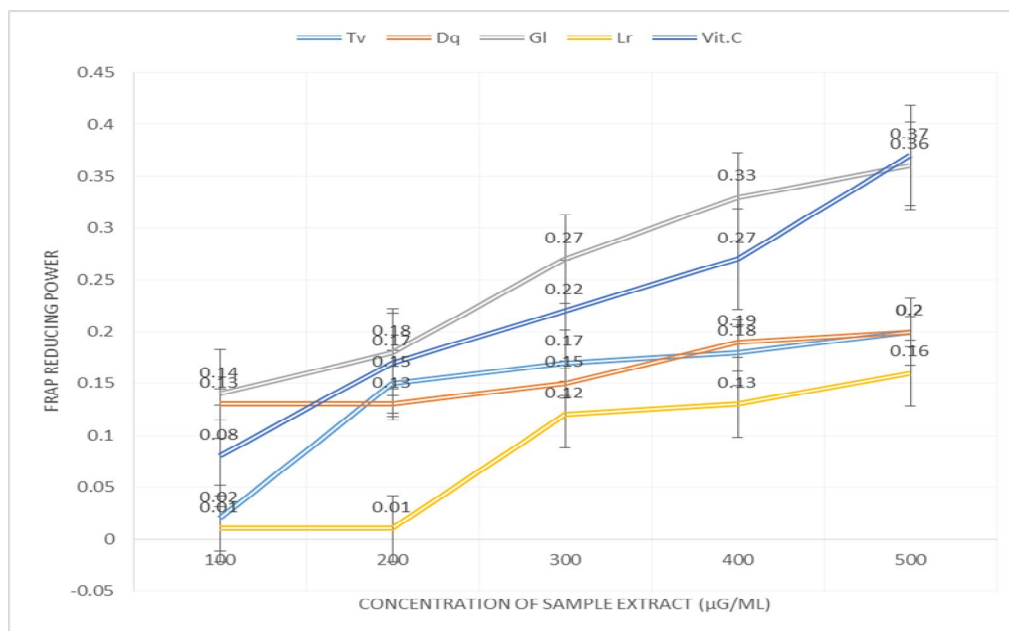


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**Fig. 1. Inhibition of radical by methanolic extract of the selected higher fungi and Vitamin C, using DPPH assay.**

409 *T.v* - *Trametes versicolor*, *D.q* - *Daedelea quercina*, *G.l* - *Ganoderma lucidum*, *L.r* - *Lycoperdon*  
410 *rimulatum*

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**Fig. 2. Ferric Reducing Power activity of the selected higher fungi and Vitamin C.**

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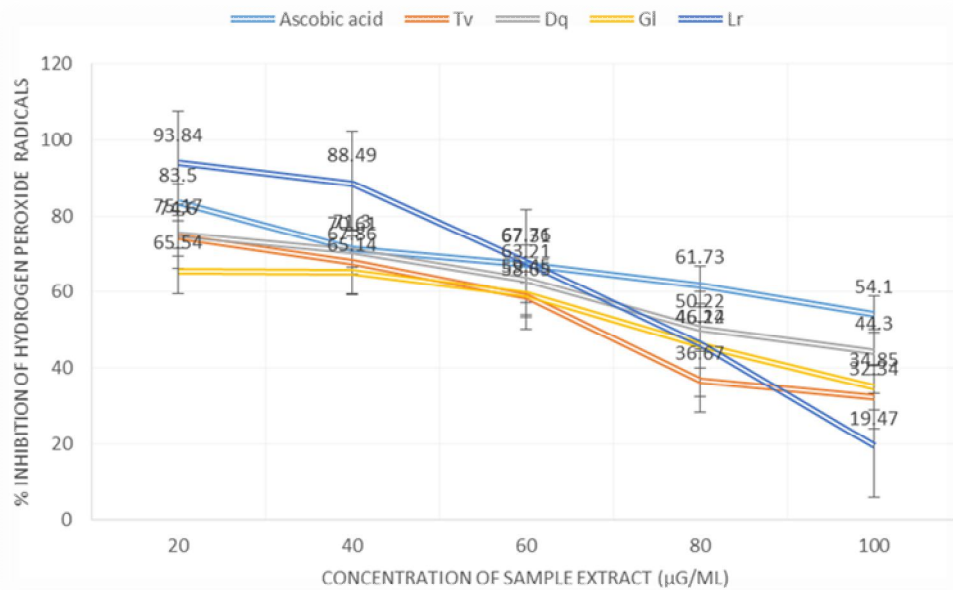
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*T.v* - *Trametes versicolor*, *D.q* - *Daedelia quercina*, *G.l* - *Ganoderma lucidum*, *L.r* - *Lycoperdon rimulatum*.



418

419 **Fig.3. Percentage inhibition of methanolic extract of the selected Higher fungi and Vitamin C**  
 420 **using Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) radical assay.**

421 *T.v* - *Trametes versicolor*, *D.q* - *Daedelia quercina*, *G.l* - *Ganoderma lucidum* , *L.r* -  
 422 *Lycoperdon rimulatum*

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424 **LIST OF TABLES**

Strain	ID(NCBI) SUBMISSION	Accession number	Identified name	Blast search similarity
STB112	F1	EU833664.1	<i>Lycoperdon rimulatum</i>	99%
EMB5	F2	JQ621899	<i>Trametes versicolor</i>	99%
IUM4100	F3	JQ520179.1	<i>Ganoderma lucidum</i>	99%
Dai12697	F4	KP171209.1	<i>Daedelia quercina</i>	99%

425 **Table 1. Identity of the fungi strains**

426

427 **Table 2. Percentage yield of metabolites**

ORGANISMS	% YIELD

<i>Trametes versicolor</i>	26.26	428
<i>Daedelia quercina</i>	38.68	
<i>Ganoderma lucidum</i>	36.19	429
<i>Lycoperdon rimulatum</i>	46.98	430

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TEST	<i>T.v</i>	<i>D.q</i>	<i>G.l</i>	<i>L.r</i>
<b>SAPONIN</b>	+	+	+	+
<b>TANNIN</b>	++	+++	+++	++
<b>STEROID</b>	+	-ve	--ve	-ve
<b>ALKALOID</b>	+	+	+	+
<b>FLAVONOID</b>	+	-ve	+	+
<b>COUMARIN</b>	-ve	-ve	-ve	-ve
<b>EMODIN</b>	-ve	-ve	-ve	-ve
<b>TERPENOID</b>	+	+	+	+
<b>CARBOHYDRATES</b>	+	+	+	+
<b>ANTHRAQUINONE</b>	-ve	-ve	-ve	-ve

