Original Research Article

Evaluation of pharmacological potentials of the ethanolic extract of a mushroom (Ganoderma lucidum) grown in FCT.

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ABSTRACT

The aim of this research work is to evaluate the pharmacological potentials of the mushroom Ganoderma lucidum grown in Federal Capital Territory Abuja, Nigeria. Phytochemical, antioxidant and antibacterial properties of ethanolic extract were carried out. The phytochemical analysis of the ethanolic extract revealed the presence of pharmacological constituents such as: steroids, triterpenoid, carbohydrate, cardiac glycosides and glycosides. The antibacterial activity of the extract was conducted using well agar diffusion method on four clinical bacterial isolate which are identified using various standard biochemical tests. The extract shows activity on: Escherichia coli (12mm), Klebsiella pneumonia (12mm), Proteus mirabilis (13mm) and Streptococcus spp (14mm) at 1000mg/ml respectively. The antioxidant potentiality of the extract was also evaluated using the stable radical 1,1-Diphenyl-1-pieryl hydroxyl (DPPH) and the IC<sub>50</sub> of the standards and extract was obtained at 0.06, 0.13, and 0.23 respectively.

1. Introduction

Mushrooms are found growing on decaying or dead organic matter, on rotten logs of woody tree trunks and dump soil that is rich in organic substances. Mushroom is defined as “a macro fungus with a distinctive fruiting body which can be hypogenous or epigeous, large enough to be seen with the naked eye and to be picked by hand [1]. Mushrooms have long been used as a valuable food source and as traditional medicines around the world, especially in Japan and China [2]. Records of health promoting properties such as antioxidant, antimicrobial, anticancer, cholesterol lowering and immuno-stimulatory effects have been reported for some species of mushrooms [3, 4, 5]. Higher fungi have been identified as a major source of biologically active natural products which provide varieties of active secondary metabolites [6, 7, 8, 9]. They are highly nutritious, low-calorie food with good quality proteins, vitamins and minerals [10, 11].

Mushrooms have continued to generate a lot of interest particularly in its consumption as food, in cure of diseases, in biodegradation and as important items of commerce in Nigeria and all over the world [12, 13]. Mushrooms represent a major and as yet largely untapped source of potent pharmaceutical products [14, 15, 16]. Ganoderma species are found all over the world, and different characteristics, such as shape and color (red, black, blue/green, white, yellow, and purple) of the fruit body, host specificity, and geographical origin, are used to identify individual members of the species [17, 18, 19]. Ganoderma lucidum, an oriental fungus, has a long history of use for promoting health and longevity in China, Japan, and other Asian countries. It is a large, dark mushroom with a glossy exterior and a woody texture. The Latin word lucidus means “shiny” or “brilliant” and refers to the varnished appearance of the surface of the mushroom [20]. In China, G. lucidum is called
lingzhi, whereas in Japan the name Lingzhi has been recognized as a medicinal mushroom for over 2000 years, and its powerful effects have been documented in ancient scripts [21]. From the *Ganodermataceae* family is reishi or manenntake [22]. *Ganoderma* are characterized by basidiocarps that are large, perennial and woody bracket. They are leathery and the fruit bodies typical grow in a fan-like form on the trunks of living or dead trees, and on soil. Over the years some scientists have been able to carry out some well structured studies on the medicinal properties of mushrooms found in Nigeria. The effects of aqueous extract of *Ganoderma lucidum* collected from Zaria, Nigeria on blood glucose levels of normoglycemic and alloxan induced diabetic wistar rats had been reported by Mohamed *et al.*, [23]. The anti-microbial properties of several other mushroom species in Nigeria have also been reported [6, 24, 25, 26]. Mushroom species have been shown to possess antagonistic effects against bacteria, fungi, viruses and cancer [27, 6]. It is interesting that during the last three decades; more than 150 triterpenes and more than 50 carcinostatic polysaccharides have been isolated and are known to be unique compounds in these mushrooms [28, 29]. Reishi, polyopus and cordyceps sinensis are mushrooms of medicinal importance in china [30, 31].

This study was conducted to evaluate the pharmacological potentials of *Ganoderma lucidum* Mushroom growing at Sheda Science and Technology Complex medicinal plant reserved garden

### 2. Materials and methods

#### 2.1 Study Area

The study area is Sheda Science and Technology complex (SHESTCO), in Kwali area council, which is located at the south western part of Federal Capital Territory of Nigeria and lies between latitude 8.9 degrees and longitude 78 degree east.

#### 2.2 Collection of Mushroom

Fresh fruiting part of *Ganoderma lucidum* was harvested from SHESTCO COMPLEX (Research garden) in Kwali area council Federal Capital Territory, Abuja Nigeria. Between (July and September 2011) and it was transported using a clean polythene bag to the Department of Biotechnology and Genetic Engineering Advanced Laboratory, Sheda Science and Technology Complex for identification, storage and further studies. The mushrooms were identified using standard conventional methods and books. The mushroom was further authenticated at the Mushroom Science Unit, Plant Pathology and Biotechnology Department, University of Benin, Nigeria. The dried mushroom was then grinded to fine powder using clean pestle and mortar, and the powder was stored in an air tight glass jar at 40°C until required for use.

#### 2.3 Phytochemical Screening

Chemical test were carried out on the Ethanolic extract and on the powdered samples using standard procedures to identify the constituents as described by [32, 33].

**Test for Triterpenoid**

To 0.5g each of the extract was added 2 ml of chloroform. Concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of triterpenoid.

**Test for phenols**

Test extract was first extracted with ethyl acetate and then filtered with Whatman filter paper No. 1. The development of blue-black or brown colouration on the addition of ferric chloride reagent to the filtrate indicates the presence of phenol.
Test for phlobatannins

10 ml of the extract of each plant sample is boiled with 1% Hydrochloric acid (HCl) acid in a test tube or conical flask. If the sample of plant carries phlobatannins, a deposition of a red precipitate will occur and indicates the presence of phlobatannins.

Test for steroid

0.2 ml of Concentrated H$_2$SO$_4$ was added to about the same volume of each of the test extracts in a test tube separately. A red colour indicates the presence of steroidal ring.

Test for carbohydrate

1 ml of Fehling’s solution A (aqueous solution of CuSO$_4$) and 1 ml of Fehling solution B (potassium tartrate), were added into 2 ml of sugar solution mix well and boil. The red precipitates indicate the presence of carbohydrate.

Test for Glycosides

5 ml H$_2$SO$_4$ was added to each of the test extracts in a separate test tubes. The mixture was heated in boiling water for 15 minutes. Fehling’s solution was then added and the resulting mixture was heated to boiling. A brick-red precipitate indicates the presence of glycosides.

Test for flavonoids

Few drops of 1% ammonium solution were added to 1g of the extract. A yellow colouration indicates the presence of flavonoids.

Test for alkaloids

0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions of 5mls each. Mayer’s reagent was added to one portion and Dragendroff’s reagent to the other. The formation of a cream (with Mayer’s reagent) or reddish brown precipitate (with Dragendroff’s reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides

To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of a deoxy-sugar characteristic of cardenolides. A violet ring appear below the brown ring, while in the acetic acid layer greenish ring was form just above the brown ring and gradually spread throughout this layer.

2.4 Determination of antioxidant activity using DPPH

The in vitro radical scavenging activities of the extract against 2, 2-Diphenyl-1-1-picrylhydrazyl radical (DPPH Sigma-Aldrich) were determined by UV-Visible spectrophotometer at 517 nm. Radical scavenging activity was measured by slightly modifying the method previously described by [34] and [35]. The following concentrations of extracts were prepared in duplicate, 0.5, 0.25, 0.125, 0.0625, 0.03125, and 5.0 mg/ml in methanol (Analar grade). Buthylated hydroxyl anisole (BHA) was used as the antioxidant standard at the same concentrations with the extracts and 5.0mg/ml. 1ml of each of the extracts were placed in 5 test tubes, and 3 ml of methanol were added each followed by 0.5 ml of 0.1 mM DPPH in methanol. Therefore, the absorbance was determined on a UV-Visible spectrophotometer at 517 nm. A blank solution was prepared containing the same amount of methanol and DPPH. The radical scavenging activity was calculated using the following formula:

\[ \% \text{Inhibition} = \left( \frac{A_b - A_a}{A_a} \right) x \frac{100}{1} \]

\(A_b = \text{Absorbance of blank},\) 
\(A_a = \text{Absorbance in the presence of extract}\)

2.5 Agar well diffusion assay

Four clinical bacterial isolates viz: - Streptococcus spp, E. coli, Kleb. pneumonia and Proteus mirabilis were tested to determine the antibacterial activity of the ethanolic extract, using agar well diffusion method. The media Mueller hinton agar (Sigma Adrich) was prepared based on the manufacture’s instruction. The agar plates were sterilized at 37°C. The sterilized Mueller Hinton agar plates were inoculated with the test culture by surface spreading using sterile cotton bud and each bacterium evenly spread on the entire surface of the plate to obtain
uniformity of the inoculum. The wells were made in each
was used as a positive control while DMSO was used as a
negative control. Approximately, 0.1 ml of the crude extract
concentrations from (1000, 500, 250, 125 mg/ml) respectively
was dispensed in each well and incubated for 24 h at 37°C.

The plates were examined for the presence of bacterial
inhibition zones around each well. The zones of inhibition were
measured using a ruler and the results were reported in
millimeters (mm).

3. Result and Discussion

The ethanolic extract of *Ganoderma lucidum* photochemical
constituents shows the presence of (triterpenoid, steroids,
glycosides, alkaloids), while the flavonoids, phenol and
saponins were not detected which indicated their absence in the
effect of the extract (Table 1). Mushroom contains a huge amount of active
secondary metabolites including phenolic compounds,
polyketides, triterpenoids and steroids [36]. Steroids are organic
culture plates using a sterile 6mm cork borer. Chloramphenicol
compounds related to sterols found in animal tissues, eggs,
yeasts and plants. They play a vital role in enhancing the well-
being of animals and humans and act as sex hormones [36].
Cardiac glycoside on the other hand is cardio-active and
increases the function of myocardial circulation [36]. Alkaloids
generally exert pharmacological activity particularly in
mammals such as humans and many of our most commonly
used drugs are alkaloids from natural sources [37].

From the antioxidant result of the crude extract of *Ganoderma
lucidum* which was compared with other two standard
antioxidant viz; Vit C and BHA. The result showed a higher
antioxidant potential at 0.125mg/ml. The antioxidant potential
of Vitamin C was (0.0625), while BHA was (0.5), the result for
*Ganoderma lucidum* was (0.125). The antioxidant bar chart of
the standards Vit C, BHA and the extract MME IC 

Table 1: *Ganoderma* Ethanolic extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Triterpenoid</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Steroids</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Anthracene</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Cardiac glycosides</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Carbohydrate</td>
<td>+</td>
</tr>
</tbody>
</table>

Key:
+ Present
- Absent

From the antioxidant result of the crude extract of *Ganoderma
lucidum* which was compared with other two standard
antioxidant viz; Vit C and BHA. The result shows a higher
antioxidant potential at 0.125mg/ml. The antioxidant potential
of Vitamin C was (0.0625), while BHA was (0.5), the result for
*Ganoderma lucidum* was (0.125). The antioxidant bar chart of
the standards Vit C, BHA and the extract MME IC 

Table 2: Percentage of inhibition of the extract and Antioxidant standard
The antibacterial assay of the crude ethanolic extract on clinical isolates shows (Table 3) that the extract activity was concentration dependent with higher activity indicated on *Streptococcus spp* (14mm) at 1000mg/ml, *proteus mirabilis* (13mm), while *E. coli* and *kleb pneumonia* (12mm) at 1000mg/ml each respectively. This result correlates with work done on different species of mushrooms including *Russula vesca, Auricularia auricular, Pleurotus squarrosulus, Volvariaella vulvae* and *Cantharellus cibarius* on the antimicrobial properties of ethanol, cold water and hot water extract [38, 9]. Antimicrobial property of several mushrooms had also been reported [6, 25, 26, 27].

**Table 3: Antibacterial sensitivity test**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Test Organism</th>
<th>Concentrations (mg/ml)</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>500</td>
</tr>
<tr>
<td>1.</td>
<td><em>Streptococcus spp</em></td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>2.</td>
<td><em>E. coli</em></td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>3.</td>
<td><em>Kleb. pneumonia</em></td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>4.</td>
<td><em>Proteus mirabilis</em></td>
<td>13</td>
<td>9</td>
</tr>
</tbody>
</table>

KEY: CL- Chloramphenicol.

**4. Conclusion**

The demonstration of activity against different category of clinical bacterial isolates as well as the antioxidant potentiality by ethanolic extract of *Ganoderma lucidum* proved its scientific justification of the local application as a health remedy. Thus it can therefore, be concluded that there is need for further in depth pharmacological research on this species of mushroom in order to discover more from its natural product.

**Conflict of interest statement**

We declare that we have no conflict of interest.
Acknowledgement

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