**Comparative Study on the Physio-chemical and Toxicological Properties of *Agaricus bisporus* (edible) and *Amanita phalloides* (non-edible) Mushrooms**

**ABSTRACT**

Comparative studies on the physio-chemical and toxicological properties of *Agaricus bisporus* (edible mushroom) and *Amanita phalloides* (non-edible mushroom) were carried out in this study. Samples used in this study were air-dried at room temperature for three days. The air-dried samples were then ground into powder. Twenty grams (20 g) of each sample was extracted using distilled water and ethanol as extraction solvents. Phytochemical and toxicological analyses were carried out on the mushroom samples. Results from the study showed that phenols, anthraquinone glycosides, tannins, terpenes, and saponins were present in the ethanolic extract of *Amanita phalloides*, while alkaloids, flavonoids, and steroids were absent. However, phenols, anthraquinone glycosides, tannins, steroids, and terpenes were absent in *Agaricus bisporus*, whereas alkaloids, cardiac glycosides, flavonoids, and saponins were present in its ethanolic extract. Anthraquinone glycosides were noted to be present in *Amanita phalloides* but absent in *Agaricus bisporus*, while alkaloids were present in *Agaricus bisporus* but absent in *Amanita phalloides*. The toxicological properties of the two mushrooms showed that *Amanita phalloides* contains various toxins, such as amatoxins, phallotoxins, phalloidin, phalloin, phallacidin, and phallisin, with phallin B toxin being absent. In contrast, *Agaricus bisporus* was found to lack these toxins. Evidence from the study indicates that *Amanita phalloides* possesses several toxic substances capable of causing harm to humans when consumed, whereas *Agaricus bisporus* contains none of these toxic compounds or substances. This study recommends that further research be carried out on non-edible mushrooms to determine whether their poisonous content can be harnessed for beneficial purposes.

Keywords: Mushrooms, *Amanita phalloides*, *Agaricus bisporous*, Edible, Non-edible, Physio-chemical, Toxicological

**INTRODUCTION**

The word "mushroom" has been used in various ways at different times and in different countries. Generally, a mushroom refers to all larger fungi, those fungi that have stalks and caps, or all large fleshy fungi. The term "mushroom" is also used strictly to mean only the edible species of *Agaricus* (Hawksworth, 2001). Broadly, mushrooms are macro-fungi with a distinctive fruiting body, which may be found above or below the ground. They grow large enough to be easily recognized with the naked eye and can be picked by hand (Ukwuru *et al*., 2018).

Mushrooms are widely consumed as fresh vegetables, pickled for storage, canned, and in powder form. They are one of the most popular and highly valued foods due to their high nutritional value (Gbolagade *et al*., 2006; Ajay *et al.,* 2021) and numerous medicinal benefits. Mushrooms have great nutritional value, as they are rich in protein, with a significant content of essential amino acids, fiber, and low fat. Edible mushrooms also provide a nutritionally significant content of vitamins B1, B2, B12, C, D, and E (Heleno *et al.,* 2010). Edible mushrooms are a source of various nutraceuticals, including unsaturated fatty acids, phenolic compounds, tocopherols, ascorbic acid, and carotenoids. They have become an integral part of the human diet due to their safety, leading to their classification as functional foods or nutraceutical products (Heleno *et al*., 2010). Thus, edible mushrooms can be directly incorporated into the diet to promote health by taking advantage of the additive and synergistic effects of all their bioactive compounds (Pereira and Tibuhwa, 2012; Vaz and Singer, 2010). Picking mushrooms from the wild for food is an age-old practice in Africa. The recognition of edible versus non-edible mushrooms is simply an art that has been passed down from generation to generation. Occasionally, errors occur in this process due to the close resemblance of mushroom species, leading to the accidental consumption of non-edible mushrooms by families, resulting in serious consequences (Ukwuru *et al.,* 2018).

Mushroom poisoning (also known as mycetism or mycetismus) refers to the harmful effects caused by the ingestion of toxic substances present in certain mushrooms. Cases of mushroom poisoning have occurred in many rural populations in Africa, where health care facilities are either poorly equipped or completely absent. Poisonous mushrooms contain a variety of toxins, which can vary significantly in their toxicity levels. These toxins, whose potency is influenced by multiple intrinsic and extrinsic factors, are secondary metabolites produced through specific biochemical pathways in fungal cells. Mushroom poisoning usually results from the consumption of wild mushrooms due to the misidentification of a toxic species as an edible one. The most common reason for this misidentification is the close resemblance in color and general morphology between toxic and edible mushroom species (Ukwuru *et al*., 2018). Unfortunately, few records of mushroom poisoning and epidemiological studies have been conducted in rural areas, where the issue is most prevalent. The symptoms of mushroom poisoning can range from mild gastrointestinal discomfort and vomiting to fatal organ failure. The incubation period may vary from one day to several weeks, after which severe symptoms emerge, often after the toxins have already affected the kidneys or liver (Alagözlü *et al.,* 2002).

Edible mushrooms are often used as a garnish or delicacy and can be regularly incorporated into the human diet as healthy foods or functional foods. Mushrooms can also be cultivated to supplement the human diet—not as regular food but as health-enhancing products, classified under dietary supplements/mushroom nutraceuticals (Ukwuru *et al*., 2018). Many mushroom species produce secondary metabolites that can be toxic, mind-altering, antibiotic, antiviral, or bioluminescent, making such mushrooms non-edible (Filipa and Zang, 2012). Although only a small number of deadly species exist, several others can cause particularly severe and unpleasant symptoms. Toxicity likely plays a role in protecting the basidiocarp and serves as a defense mechanism against consumption and premature destruction. This is due to the evolution of chemicals that render the mushroom inedible, either causing the consumer to vomit the meal or learn to avoid consumption altogether (Aida and Piearce, 2009).

Mushrooms contain various polyphenolic compounds, which are recognized as excellent antioxidants due to their ability to scavenge free radicals through single-electron transfer (Hirano *et al*., 2010). Some common edible mushrooms, such as *Grifola frondosa*, *Termitomyces*, and *Pleurotus*, which are widely consumed in Asian culture, have been found to possess antioxidant activity, which correlates well with their total phenolic content (Mau, 2011). Phytochemicals, especially phenolics in mushrooms, are suggested to be the major bioactive compounds with antioxidant potential, providing health benefits and are associated with the inhibition of atherosclerosis and cancer (Onadou, 2010). Edible mushrooms have become an integral part of the human diet and have received increasing attention due to their safety, leading to their classification as functional foods or nutraceutical products. Additionally, they are an important food source contributing to income generation in both developing and developed countries.

Many mushrooms have also been reported to produce a wide range of secondary metabolites with high therapeutic value, including antioxidant, antitumor, antibacterial, antiviral, cholesterol-lowering, hematological, and immunomodulating properties (Jackson, 2017). The major challenge in mushroom consumption is the inability of mushroom gatherers or scientists to correctly identify poisonous mushrooms, which contain toxins that can be detrimental to human health. Research studies have reported fatalities caused by poisonous mushrooms in different parts of the world, including Nigeria, due to misidentification (Ukwuru *et al*., 2018). Therefore, the main objective of this study is to compare the physiological and toxicological properties of *Agaricus bisporus* (edible mushroom) and *Amanita phalloides* (non-edible mushroom), focusing on identification approaches.

**MATERIAL AND METHODS**

**Sample collection:** The sample (*Agaricus bisporous*- plate 1 and *Amanita phalloides*- plate 2) were procured from Maeve Farm in Okpuno Awka before being transported in sterile paper bags to the laboratory for analysis.

**Preparation of Samples for Phytochemical Extraction**

The samples were air dried at room temperature for three days. The air-dried samples were then ground into powder. Twenty grams (20g) of each sample was extracted using distilled water and ethanol as extraction solvents.

**Phytochemical Analysis**

Major physio-chemical metabolites classes such as alkaloids, cardiac glycosides, anthraquinone glycosides, flavonoids, tannins, saponins, steroids and terpenes were screened according to the methods described in A Guide to Modern Techniques of Plant Analysis, Medicinal Plants and Traditional Medicine in Africa, and Pharmacognosy (Harborne, 1973; Saviour *et al.*, 2024).

**Test for Phenol:** Five millilitres (5 mL) of the extract was pipetted into a test tube, and a few drops of dilute ferric chloride solution were added. The formation of a red, blue, green, or purple coloration indicates the presence of phenols.

**Test for Alkaloids:** Five millilitres (5 mL) of the extract was pipetted into a test tube. The filtrate was carefully tested with Mayer’s reagent (potassium mercuric iodide). A yellow-coloured precipitate indicates the presence of alkaloids.

**Cardiac glycoside:** Keller-Killani Test: To 5 mL of the extract, a few drops of glacial acetic acid were added, followed by a few drops of 10% ferric chloride and concentrated sulphuric acid. The appearance of a reddish-brown color at the junction of the two liquid layers indicates the presence of cardiac glycosides.

**Anthraquinone glycosides:** Borntrager’s Test: To 5 mL of the extract, dilute sulphuric acid was added, boiled, and then filtered. To the cold filtrate, an equal volume of benzene or chloroform was added. The organic layer was separated, and ammonia was added. The ammonia layer turns pink or red.

**Test for Flavonoids:** To 5 mL of the extract, a few drops of ammonia solution were added. The appearance of a yellow or orange color indicates the presence of flavonoids.

**Test for Tannins:** To 5 mL of the water extract from all plant parts, 2 mL of 10% ferric chloride solution was added to a test tube. A blue-black precipitate indicates the presence of tannins.

**Test for Saponin:** To 5 mL of the plant sample extract, 2 mL of distilled water was added in a test tube and vigorously shaken. The presence of persistent froth or bubbles indicates the presence of saponins.

**Test for Steroids and Terpenes** *(Liebermann-Burchard Reaction):*

To 5 mL of the sample extract, 2 mL of acetic anhydride and a few drops of concentrated sulphuric acid were added in a test tube. A blue-green ring between layers indicates the presence of steroids, while a pink-purple ring signifies the presence of terpenes.

**Toxicological Analysis**

**Phytate:** Phytate content was determined using the method of Young and Greaves (1940), as adopted by Lucas and Markakes (1975). 0.2 g of each differently processed sample was weighed into separate 250 mL conical flasks. Each sample was soaked in 100 mL of 2% concentrated HCl for 3 hours, and the samples were then filtered.

50 mL of each filtrate was placed in a 250 mL beaker, and 100 mL of distilled water was added to each sample. 10 mL of 0.3% ammonium thiocyanate solution was added as an indicator and titrated with standard iron (III) chloride solution, which contained 0.00195 g of iron per mL. The percentage of phytic acid was calculated using the formula:

Phytate acid (%) = Titre value x 0.00195 x 1.19x 100

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**Oxalate Determination by Titration Method**

This was determined according to Osagie (1998). This determination involves three major steps: digestion, oxalate precipitation, and permanganate titration.

This was determined according to Osagie (1998)

**Digestion**

I) 2 g of the sample was suspended in 190 mL of distilled water in a 250 mL volumetric flask.

II) 10 mL of HCl was added, and the suspension was digested at 100 °C for 1 hour. .

III) Cool and then made up to 250 mL mark before filtration.

**Oxalate precipitation**

Duplicate portions of 125 mL of the filtrate were measured into beakers, and four drops of methyl red indicator were added. This was followed by the dropwise addition of NH₄OH solution until the test solution changed from salmon pink to a faint yellow (pH 4–4.5). Each portion was then heated to 90 °C, cooled, and filtered to remove the precipitate containing ferrous ions.

The filtrate was again heated to 90 °C, and 10 mL of 5% CaCl₂ solution was added while being stirred constantly. After heating, it was cooled and left overnight at 25 °C. The solution was then centrifuged at 2,500 rpm for 5 minutes. The supernatant was decanted, and the precipitate was completely dissolved in 10 mL of 20% (v/v) H₂SO₄ solution.

**Permanganate Titration**

The total filtrate resulting from the digestion of 2 g of the sample was made up to 300 mL. Aliquots of 125 mL of the filtrate were heated until near boiling and then titrated against a 0.05 M standardized KMnO₄ solution to a faint pink colour, which persisted for 30 seconds. The calcium oxalate content was then calculated using the formula.

Tx (vme) (Df)x105(mg/100g)

(ME) x Mf

Where T is the titre of KMnO₄ (mL), Vme is the volume–mass equivalent (i.e., 1 mL of 0.05 M KMnO₄ solution is equivalent to 0.00225 g of anhydrous oxalic acid). Df is the dilution factor, ME is the molar equivalent of KMnO₄ in oxalate (KMnO₄ redox reaction), and Mf is the mass of the sample used.

**Quantitation of Trypsin Inhibitor**

The method of Erlanger *et al*., (1961) was used.

Trypsin inhibition by plant extract was indicated by a reduction in trypsin activity below its standard level. As a control, 0.9 mL of casein bovine substrate and 0.1 mL of trypsin were added to a test tube, mixed well, and incubated for 30 minutes at room temperature.

Next, 1.5 mL of 10% TCA was added to stop their action. After 15 minutes, the test tube was placed in boiling water for 10 minutes and then filtered using Whatman No. 1 filter paper. The filtrate (0.5 mL) was transferred to a test tube along with 1 mL of distilled water.

Finally, 5 mL of NaOH and 1.5 mL of diluted Folin C reagent (10%) were added.

**Preparation of Plant Sample**

Five grams (5 g) of the ground sample was extracted with 200 mL of distilled water for 24 hours at 37 °C. It was filtered using Whatman No. 1 filter paper. 0.1 mL of the filtrate was added to 0.9 mL of the substrate (bovine casein) and 0.1 mL of 0.25% trypsin.

Tyrosine (Sigma, USA) was used as the standard to measure trypsin activity. A blank was prepared by adding 5 mL of 0.5 N NaOH together with 1.5 mL of Folin C reagent. The absorbance of the control, the standard, and the test was measured at 650 nm against the blank.

The activity of trypsin was calculated as follows:

Activity of trypsin= O.D .test- O. D of +ve control X dil. factor

O.D. Standard - O.D. blank

**RESULTS**

**Physio-chemical** **Properties of Aqueous Extract of *Agaricus bisporus* and *Amanita phalloides***

Table 1 showed that in the aqueous extract of *Amanita phalloides*, phenols, alkaloids, flavonoids, and steroids were absent, while cardiac glycosides, anthraquinone glycosides, tannins, terpenes, and saponins were present.

On the other hand, the aqueous extract of *Agaricus bisporus* also lacked phenols, alkaloids, tannins, and steroids, but cardiac glycosides, flavonoids, terpenes, and saponins were present.

In the table below, tannins are present in *Amanita phalloides* but absent in *Agaricus bisporus*. Similarly, flavonoids are observed in *Agaricus bisporus* but are absent in *Amanita phalloides*. This result implies that there are differences in the physiological properties of the two mushrooms.

**Table 1: Physio-chemical Properties of Aqueous Extract of *Agaricus bisporus* and *Amanita phalloides***

|  |  |  |
| --- | --- | --- |
| **Parameter** | ***Amanita phalloides***  **(aqueous extract)** | ***Agaricus bisporus***  **(aqueous extract)** |
| Phenol | **-** | **-** |
| Alkaloid, | **-** | **-** |
| Cardiac glycoside | **+** | **+** |
| Anthraquinone glycoside | **+** | **+** |
| Flavonoid | **-** | **+** |
| Tannin | **+** | **-** |
| Steroid | **-** | **\_** |
| Terpene | **+** | **+** |
| Saponin | **+** | **+** |
|  |  |  |

**+ = Present - =Absent**

**Physio-chemical Properties of Ethanol Extract of *Agaricus bisporus* and *Amanita phalloides***

Table 2 showed that phenols, anthraquinone glycosides, tannins, terpenes, and saponins are present in the ethanolic extract of *Amanita phalloides*, while alkaloids, flavonoids, and steroids are absent.

However, phenols, anthraquinone glycosides, tannins, steroids, and terpenes are all absent in *Agaricus bisporus*, whereas alkaloids, cardiac glycosides, flavonoids, and saponins are present in its ethanolic extract. Anthraquinone glycosides are noted to be present in *Amanita phalloides* but absent in *Agaricus bisporus*, while alkaloids are found in *Agaricus bisporus* but absent in *Amanita phalloides*.

This clearly suggests that the two fungi have different physiological functions and properties.

**Table 2: Physio-chemical Properties of Ethanol Extract of *Agaricus bisporus* and *Amanita phalloides***

|  |  |  |
| --- | --- | --- |
| **Parameter** | ***Amanita phalloides***  **(Ethanolic Extract)** | ***Agaricus bisporus***  **(Ethanolic Extract)** |
| Phenol | **+** | **-** |
| Alkaloid | **-** | **+** |
| Cardiac glycoside | **+** | **+** |
| Anthraquinone glycoside | **+** | **-** |
| Flavonoid | **-** | **+** |
| Tannin | **+** | **-** |
| Steroid | **-** | **\_** |
| Terpene | **+** | **-** |
| Saponin | **+** | **+** |
|  |  |  |

**+ = Present - =Absent**

**Toxicological Properties of *Amanita phalloides and Agaricus bisporus***

Table 3 showed that *Amanita phalloides* contains various toxins, including amatoxins, phallotoxins, phalloidin, phalloin, phallacidin, and phallisin, while phallin B toxin is absent. In contrast, the results for *Agaricus bisporus* indicate the absence of these toxins.

**Table 3 Toxicological Properties of *Amanita phalloides and Agaricus bisporus***

|  |  |  |
| --- | --- | --- |
| **Toxins** | ***Amanita phalloides*** | ***Agaricus bisporus*** |
| Amatoxins | ✓ | **X** |
| Phallotoxins | ✓ | **X** |
| Phalloidin | ✓ | **X** |
| Phalloin | ✓ | **X** |
| Phallacidin | ✓ | **X** |
| Phallisin | ✓ | **X** |
| phallin B | X | **X** |
| Phytate | ✓ | X |
| Oxalate | ✓ | X |
| Trypsin Inhibitor | ✓ | **X** |

✓= present

**x** = absent



**Plate 1: *Agaricus bisporous* (edible) mushroom**



**Plate 2**: ***Amanita phalloides* (Non-edible/ Poisonous) Mushroom**

**DISCUSSION**

Findings from the study showed that the physio-chemical properties of *Amanita phalloides* and *Agaricus bisporus* were almost identical. Some of the physio-chemical characteristics of the *Amanita phalloides* aqueous extract include the presence of cardiac glycosides, anthraquinone glycosides, tannins, terpenes, and saponins, which help the mushroom survive in its habitat.

On the contrary, the physio-chemical properties of *Agaricus bisporus* slightly differ from those of *Amanita phalloides*. *Agaricus bisporus* contains cardiac glycosides, flavonoids, terpenes, and saponins but lacks phenols, which are present in *Amanita phalloides*. Similarly, *Amanita phalloides* lacks flavonoids, which are present in *Agaricus bisporus*.

The slight differences in the physio-chemical composition of these two mushrooms account for their variations in physio-chemical properties. The findings also indicate that the physio-chemical properties of both the ethanolic and aqueous extracts of these mushrooms remain unchanged, regardless of the solvent used for extraction.

This study also showed that *Amanita phalloides* contains poisonous toxins such as phallisin, phallacidin, phalloin, and phalloidin. These toxins are absent in *Agaricus bisporus*, which explains why *Agaricus bisporus* mushrooms are safe for human consumption compared to their toxic counterpart, *Amanita phalloides*.

This finding aligns with previous research indicating that edible mushrooms have become an essential part of the human diet, receiving more attention due to their safety, thus being considered functional foods or nutraceutical products. Additionally, mushrooms serve as an important food source, contributing to income generation in both developing and developed countries. They may be directly incorporated into the diet to promote health, benefiting from the additive and synergistic effects of all bioactive compounds present (Pereira and Tibuhwa, 2012; Vaz and Singer, 2010; Heleno *et al*., 2010).

The presence of toxins in *Amanita phalloides* makes it unsafe for human consumption and may serve as a defense mechanism. The poisonous compounds in this mushroom can cause severe illness and even death. This finding aligns with Jackson (2017), who reported that mushrooms produce a wide range of secondary metabolites, which may be harmful or possess high therapeutic value, including antioxidant, antitumor, antibacterial, antiviral, cholesterol-lowering, hematological, and immunomodulating properties.

**CONCLUSION**

Evidence from the study indicates that *Amanita phalloides* mushrooms possess several toxic substances that have the capacity to cause harm to humans when consumed, compared to *Agaricus bisporus*, which has none of these toxic compounds or substances. The presence of these toxins accounts for the non-edible nature of *Amanita phalloides*. In contrast, the absence of these toxins in *Agaricus bisporus* makes it safe for human consumption and serves as food for humans. It has also been noted that the physio-chemical properties of the two mushrooms studied slightly differ in their consistency. Hence, the toxicity of *Amanita phalloides* makes it a non-edible mushroom.

**RECOMMENDATION**

Further studies should be carried out on non-edible mushrooms to determine whether their poisonous content can be harnessed for beneficial purposes that would be of value to humans.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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