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Phytochemical and Antioxidant Analyses of Selected Edible Mushrooms, Ginger and Garlic from Ebonyi State, Nigeria.

Udu-Ibiam, O. E.[1], Ogbu, O.[1], Ibiam, U. A.[2], Nnachi, A. U.[3], Agah, M. V.[1], Ukaegbu, C. O.[4], Chukwu, O.S.[5], Agumah, N. B.[1], Ogbu, K. I.[6]

[1]*Department of Applied Microbiology, Faculty of Biological Sciences, Ebonyi State University, Abakaliki*
[2]*Department of Biochemistry, Faculty of Biological Sciences, Ebonyi State University, Abakaliki, Nigeria*
[3]*Department of Medical Microbiology and Parasitology, Faculty of Medicine, Nnamdi Azikiwe University,Awka*
[4]*Department of Microbiology, Faculty of Natural Sciences, University of Jos, Jos, Nigeria*
[5]*Deparment of Biology, Microbiology and Biotechnology, Federal University, Ndufu-Alike, Ikwo, Nigeria*
[6]*Department of Biotechnology, Ebonyi State University, Abakaliki, Nigeria*

Abstract: *Antioxidant defense and repair systems naturally found in humans as well as in other living organisms are insufficient to completely prevent biological injuries caused by free radicals because of the relative ease with which we are constantly exposed to processes that generate them. The advantage of dietary antioxidants over synthetic ones (which are associated with gross side effects) as free radical scavengers has been documented worldwide. In this study, the phytochemical and antioxidant analyses of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria, were carried out using dried extracts of the samples. The results revealed the presence of valuable phytochemicals including: phenolics, flavonoid, proanthocyanidin, saponin, alkaloid, tannin, phytic acid, and cyanogenic glycosides. Ginger (Zingiber officianale) had the highest phenolics content of 64.42±0.91 mg/g, closely followed by the mushroom, Tricholoma nudum, (64.122±1.20) and garlic (Allium sativum) (12.42±0.61 mg/g). Least phenolics content of 2.65±0.006 mg/g was observed in the study with the mushroom, Boletus spp. Ginger also had the highest flavonoid content of 0.045±0.001 µg/g. The presence of these bioactive or phytochemical compounds supports the possible antimicrobial and antioxidant potentials of mushrooms, ginger and garlic and high phenolics and flavonoid contents of ginger and mushroom suggests high antioxidant potentials.*

Keywords: *Antioxidants, Garlic (Allium sativum), Ginger (Zingiber officianale), Mushrooms, Nigeria Phytochemicals*

I. Introduction

Edible mushrooms have gained worldwide recognition and increasing popularity owing to their nutritional and medicinal values since Greek and Roman antiquity. [1],[2] They are macroscopic fungi with distinctive fruiting bodies which can be hypogeous or epigeous, large enough to be seen with unaided eyes and to be picked by hands.[3] Mushrooms, divided into edible and poisonous types, grow in various places such as wet environments, decayed plants and animal sites, termites' nests, palm wastes, leaf litters, under shades, to mention but a few.[4]

Herbs and spices (including ginger and garlic) are very important and useful as therapeutic agents against many pathological conditions.[5] Spices have been defined as plant substances from indigenous or exotic origin, aromatic or with strong taste used to enhance tastes of foods.[6] Ginger (*Zingiber officianale*) is a rhizoid white to yellow in colour and very aromatic with lots of beneficial uses.[7] Garlic (*Allium sativum*) is a species of onion; it possesses characteristic hot, pungent flavor and has been know down history to be used for both culinary and medical purposes.[8]

Besides nutritional and medicinal values, mushrooms, ginger and garlic have been shown to have antioxidant potentials.[6],[9]-[12] Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate unpaired condition of electrons thereby protecting the body from damages caused by free radicals.[13] These free radicals are independent chemicals with one or more unpaired electrons and are responsible for biological injury[14] and contribute to many non-communicable diseases.[2] They are constantly formed in human body during energy production, in the mitochondrial electron transport chain, phagocytosis, arachidonic acid metabolism, ovulation, fertilization and in xenobiotic metabolism[15] and from external sources such as food, drug, smoke and other pollutants in the environment,[12] making them constant threats to human well being.

Naturally, humans as well as other living organisms are endowed with antioxidant defense and repair systems are insufficient to completely prevent oxidative damage, considering the relative ease with which we are daily exposed to processes that generate them.[16] Hence, there is constant need for external aids to these systems if we must be free of the damages caused by free radicals. Most available synthetic antioxidants which

act as free radical scavengers are associated with gross side effects,[17] making the need for natural and safe antioxidants sources even greater. Polyphenols and other phytochemicals have been shown to have antioxidant activities; it has then been suggested that consumption of polyphenol-rich foods is associated with a reduced risk of cardiovascular diseases, stroke and certain types of cancer.[18],[19] The consumption of dietary antioxidants would help to prevent free radical oxidative damage[2] by inhibiting the initiation step or interrupting the propagation step of oxidation damage.[20]

Although the antimicrobial activities of edible mushrooms, ginger and garlic have been increasingly researched upon, analysis of their antioxidant importance has received insignificant attention. The present study, therefore, was carried out to determine the phytochemical and antioxidant potentials of extracts of mushrooms, ginger and garlic from Ebonyi State, Nigeria.

II. Materials And Methods

2.1 Collection and Identification of Materials

Five species of mushroom including *Tricholoma nudum, Psalliota campestris, Flammulina* sp, *Trichaptum* sp, and *Boletus* sp, were collected from different parts of Egugwu, Umuchima and Umunaga all in Uburu, Ohaozara Local Government Area of Ebonyi State, Nigeria.The garlic and ginger used were bought from Abakpa Market in Abakaliki, Ebonyi State, Nigeria. All chemicals used for various analyses were purchased from Aldrich chemicals, Poole, UK.

2.2 Extraction of Mushroom, Ginger and Garlic

The extraction was carried out in accordance with the procedure of Fasidi and Jonathan.[21] The respective mushroom, ginger and garlic samples were cut into bits and sun dried for 6 days and then pulverised into powder using manual grinder. 40 g of each of the pulverised mushroom were respectively weighed and soaked in 200 ml of cold water, hot water, and ethanol. The ginger and garlic were soaked in 200 ml of cold water only. Cold water preparation was allowed to stand for 2 days only with intermittent shakings at 30 minutes intervals (this is to prevent mold from growing in the extraction water). The hot water was allowed to stand for 24hrs (same reason as above). The ethanol and diethyl ether were allowed to stand for 7 days. Then the four preparations were filtered using Whatman filter paper no 1. The filtrate was poured in crucible and air dried at room temperature to recover extracts.

2.3 Quantitative Determination of Phytochemicals

The following phytochemicals were quantitatively determined in the mushrooms, ginger and garlic extracts: Phytic acid, tannin, alkaloids, saponin, flavonoid, phenolics, proanthocyanidins, and cyanogenic glycoside.

2.3.1 Phytic Acids (Phytate) Determination

The method adopted was as described by Harbone.[22] Briefly, the plant material (mushroom, ginger and garlic) was extracted. Solution was made up to give up to 3-30 $mgml^{-1}$ phytate solution. 0.5ml of extract was pipetted into a test tube filled with a ground glass stopper. On it 1ml of Ferric solution was added, the tube was covered with the stopper and fixed with a clip. The tube was heated in a boiling water bath for 30 minutes. Within the first 5 minutes, care was taken that the tube remained well stoppered. The set up was covered in ice water for 15 minutes. It was then allowed to adjust to room temperature. Once the tube has reached room temperature, about 2ml of 2, 2-Bipyridine solution was added to the test tube and mixed. The bipyridine reacted with the iron phytate and the color changed with time. The absorbance was measured after about 0.5-1 min at 519 nm against distilled water.

2.3.2 Determination of Tannins

The method used was according to that described by Maga.[23] A measured weight of each sample (1.0 g) was dispersed in 10 ml of distilled water and agitated. This was left to stand for 30 minutes at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the supernatant decanted. 2.5 ml of the supernatant extract was dispensed into 50 ml volumetric flask. Similarly 2.5 ml of standard tannic acid solution was dispensed into a separate 50 ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask followed by 2.5 ml of saturated Na_2CO_3 solution. The mixture was diluted to 50 ml mark in the flask, and incubated for 90 minutes at room temperature. The absorbance was measured at 250 nm in a Genway model 6000 electronic spectrophotometer. Readings were taken with the reagent blank at zero.

The tannin content was calculated as follows:

$$\% \text{ Tannin} = [(An/As)x\ (C\ x100)]/[W\ x\ (Vf/Va)]$$

Where An = absorbance of test sample

As = absorbance of standard solution

C = concentration of standard solution
W = weight of sample used
Vf = total volume of extract
Va= volume of extract analyzed

2.3.3 Determination of Alkaloids

The gravimetric methods of Harbone [22] were adopted. A given weight (5.0) of each sample was weighed out and dispersed into 50ml of 10% acetic acid solution in ethanol. The mixture was well shaken and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one quarter (¼) of its original volume. Then a concentrated NH_4OH was added drop-wise to precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper. The precipitate in filter paper was dried in an oven at 60^{o}C for 30 minutes and reweighed. The weight of alkaloids is normally determined by weight difference.
The percentage alkaloids were calculated thus:

Percentage Alkaloids = $\frac{W_2 - W_2}{W}$ x 100

Where W = weight of sample
W_1 = weight of empty filter
W_2 = weight of paper plus precipitate

2.3.4 Determination of Saponin

The spectrophometric method of Brunner [24] was used for saponin analysis. Exactly I g of finely ground sample was weighed into a 250 ml beaker and 100 ml of ethyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter, the mixture was filtered through a Whatman No. I filter paper into a 100 ml beaker and 20 ml 0f 40 % saturated solution of $MgCO_3$ was added. The mixture obtained with saturated $MgCO_3$ was again filtered through a Whatman No 1 filter paper to obtain a clear colorless solution. About I ml of the colorless solution was pipetted into 50 ml volumetric flask containing 5 % $FeCl_3$ solution and made up to mark with distilled water. It was allowed to stand for 30 minutes. 0-10 ppm standard saponin was prepared from saponin stock solution. The standard solutions were treated similarly with 2 ml of 5% $FeCl_3$ solution as done for 1ml sample above. The absorbances of the sample as well as standard saponin solution were read after colour development on an Agilient spectrophometer at a wavelength of 380 nm.

Calculation:

Saponin (ppm) =

$$\frac{\text{Absorbance of sample x Gradient factor x dilution factor}}{\text{Weight of sample}}$$

2.3.5 Determination of Flavonoids

Aluminum chloride colorimetric method was used for flavonoid determination based on Chang *et al.* [25] Each plant extracts (0.5 ml of 1: 10 g/ml) in method were separately mixed with 1.5 ml of methanol, 0.1 ml of 10 % Aluminum chloride, 0.1 ml of 1 ml of potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 413 nm with a 6405 UV/visible spectrophotometer.

2.3.6 Determination of Phenolics

The total phenolics were determined using spectrophotometric method described by Brunner.[24] To each test tube containing 10 ml of 50 % aqueous ethanol, 30 g of dry finely ground mushroom and spice was added and extracted for two hour by shaking every 15 minutes using vortex. 200ml of initial extract was added to a 50ml volumetric flask containing about 25 ml of water. At time zero, 2.5 ml of Folin-Denise reagent was added and mixed well. After 3 minutes, 5 ml of saturated sodium carbonate was added and brought to volume with water and then mixed well. After exactly 20 minutes, the absorbance was measured at 760nm.

2.3.7 Determination of Proanthocyanidin

The total proanthocyanidin was determined according to Maga.[23] To a test tube, 1.0 ml of initial extract 30 mg of finely ground sample material in 10 ml of 50 % aqueous ethanol) was added. 1.0 ml of n-butanol–HCl reagent was added and mixed well using a vortex. It was then placed on a dry bath at 100^{o}C for 1 hour. It was allowed to cool to room temperature and the absorbance was measured at 550 nm.

2.3.7 Determination of Cyanogenic Glycosides

The alkaline picrate method was used according to Harbone. [22] Briefly, about 5.0 g of the sample was ground to paste. The paste was dissolved in 50 ml distilled water in a corked conical flask and allowed to stay overnight. The extract was filtered and the filtrate used for the cyanide determination. Alkaline picrate solution

was prepared by dissolving 1g picrate and 5 g sodium carbonate in a volume of minimally warm water and then made up to 200 ml with distilled water. Exactly 1 ml of sample filtrate was put in a test and corked firmly. This was followed by addition of 4 ml of alkaline picrate and incubated in a water bath for 5 minutes. After colour development (reddish brown colour), the absorbance read at 490 nm using a Spectrophotometer against a blank solution which contained only 1ml distilled water and 4ml alkaline picrate solution.

III. Results

The study showed that the extracts of mushrooms, ginger and garlic contained that following phytochemicals, though in varying proportions: phenolics, flavonoid, proanthocyanidin, saponin, alkaloid, tannin, phytic acid and cyanogenic glycosides. Ginger (*Zingiber officianale*) had the highest phenolics content of 64.42±0.91 mg/g, closely followed by the mushroom, *Tricholoma nudum*, (64.122±1.20) and garlic (*Allium sativum*) (12.42±0.61 mg/g). Least phenolics content of 2.65±0.006 mg/g was observed in the study with the mushroom, *Boletus* spp. Ginger also had the highest flavonoid content of 0.045±0.001 µg/g, followed by *Psalliota compestris* (0.031±0.02 µg/g) and then *Flammulina* spp. (0.0199±0.003 µg/g). Garlic had the least flavonoid content of (0.0021±0.0002 µg/g). Highest contents of proanthocyanidin (10.82±0.14 mg/g), saponin (5.13±0.05 µg/g), alkaloid (3.01±0.01 %), tannins (0.014±0.003 %), phytic acid (0.821±0.021 %) and Cyanogenic glycosides (259.57±1.11) were observed with ginger, *Boletus* spp., *Trichaptum* spp., *Tricholoma nudum*, *P. compestris* and *P. compestris* respectively. Details of the results are shown in the following TABLE 1.

Table 1: Phytochemical contents of selected edible mushrooms, ginger and garlic from Ebonyi State, Nigeria.

Extract	Proportion of Phytochemicals Present							
	Phenolics (mg/g)	Flavonoid (□g/g)	Proanthocyanidin (mg/g)	Saponin (□g/g)	Alkaloid (%)	Tannins (%)	Phytic acid (%)	Cyanogenic glycosides
Tricholoma nudum	64.122±1.2	0.0164±0.001	4.41±0.24	0.28±0.04	1.0±0.04	0.014±0.003	0.21±0.03	130.69±0.04
Psalliota compestris	6.012±0.91	0.031±0.02	6.015±0.31	0.27±0.008	2.0±0.01	0.014±0.0	0.821±0.021	259.57±1.11
Flammulina spp.	4.81±0.09	0.0199±0.003	10.02±0.05	0.27±0.006	0.2±0.01	0.011±0.004	0.093±0.04	177.67±1.21
Trichaptum spp.	4.41±0.51	0.0174±0.001	8.42±0.21	4.82±0.05	3.01±0.01	0.0114±0.002	0.162±0.02	115.28±0.59
Boletus spp.	2.65±0.06	0.0164±0.004	3.61±0.11	5.13±0.05	0.1±0.003	0.013±0.003	0.162±0.02	24.13±0.92
Ginger (*Zingiber officianale*)	64.42±0.91	0.045±0.001	10.82±0.14	4.96±0.06	2.0±0.02	0.0059±0.0	0.394±0.021	103.73±0.92
Garlic (*Allium sativum*)	12.424±0.61	0.0021±0.0002	2.004±0.05	0.587±0.02	3.0±0.022	0.0008±0.0004	0.232±0.09	33.38±0.58

IV. Discussion

The results of the phytochemical analyses of some selected edible mushrooms (*Tricholoma nudum*, *Psalliota compestris*, *Flammulina* spp., *Trichaptum* spp.), ginger and garlic from Ebonyi State, Nigeria, revealed the presence of phenolics, flavonoid, proanthocyanidin, saponin, alkaloid, tannins, phytic acid and cyanogenic glycosides, though in varying concentrations. These phytochemicals play a vital role in the medicinal properties of many plants. Spices are rich sources of polyphenolic compounds having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food systems and offer additional health benefits. Consumption of spices has been implicated in the prevention of many chronic diseases such as cardiovascular diseases, cancer and inflammation.[26] The results are in agreement with several previous works of Hamzah *et al.*[12] and Egwim *et al.*[11] both in Minna, Nigeria, Ehssan and Saadabi[27] in Sudan, Panpatil *et al.*[26] in India as

well as Wandati *et al.*[28] in Kenya who independently detected these phytochemicals in their studies. However, Iwalokun *et al.*[29] in Lagos, Nigeria and Mattila *et al.*[30] did not detect flavonoid in their study. The variation in these results could be attributed to the species of mushrooms under study as well as the variations in their growth conditions since they did not grow in the same place. Studies have shown that the absence of flavonoid in mushrooms may be of biological advantage in their various ecological niches.[31]

Ginger and the mushroom, *T. nudum*, showed very high contents of total phenolics, followed by garlic, possibly making them better antioxidants than garlic and the other species of mushrooms. The low and varied concentrations of these phytochemicals among the tested mushrooms may be attributed to mycelia wet and wet weights. Phenolics are good antioxidants and exhibit a wide range of pharmacological properties such as anti-cancer, anti-inflammatory and anti-diabetic effects.[32],[33] Flavonoids are group of more than 4000 polyphenolic compounds that occur naturally in food of plant origin, possessing a number of beneficial effects to human health.[34] They are potent water-soluble super antioxidants and free radical scavengers that prevent oxidative damage, and have anti-cancer, anti-inflammatory, anti-allergic, antiviral, vasodilating properties and inhibition of platelet aggregation.[12],[34],[35] The large quantities of flavonoid observed in ginger against mushrooms and garlic is in conformity with the work of Atai *et al.*[36]

Also, it is known that Saponins inhibit Na+ efflux by blockage of the influx of concentration in the cells, activating a $Na^+ – Ca^{2+}$ antiporter in cardiac muscles. The increase in Ca^{2+} influx through this antiporter strengthens the contraction of cardiac muscles.[10],[37] They are as well reported to have antibacterial, antihaemolytic, anti-diabetic, anti-inflammatory and cholesterol-binding properties.[34],[38] Tannins may elicit antibacterial activities via cell membrane lysis, inhibition of protein synthesis, proteolytic enzymes and microbial adhesions.[39] They are also reported to have physiological effects like anti-irritant, anti-secretolytic and anti-parasitic effects. Plants containing tannins are used to treat non specific diarrhea and inflammation of the mouth.[40],[41] The rich tannin content of *Trichaptum* species observed in this study is in collaboration with the work of Westerndarp[40].

Proanthocyanidin inhibits lipid peroxidation, platelet aggregation, capillary permeability and bacterial fragility.[42] The value of proanthocyanidin is higher in ginger than in mushrooms. The valuable pharmacological properties of mushrooms, ginger and garlic have been attributed to the presence of alkaloids on the autonomic nervous system, blood vessels, respiratory system, gastrointestinal tract, uterus and it has been shown to be effective against malignant diseases and malaria.[12] In ophthalmology, it acts as anaesthesia, pain reliever, antipuretic action among others.

Steroids and anthraquinones were not detected in this study. This correlates with the previous works of Egwim *et al.*[10] The absence of steroids does not agree with the findings of Hamzah *et al.*[12] while the absence of anthraquinones disagrees with the results of Akindahunsi[43] and Schneider and Wolfling[37].

V. Conclusion

This study has further elaborated our knowledge of medicinal and health benefits of mushrooms, ginger and garlic. The presence of the phytochemicals in the tested materials could possibly account for these benefits. Also observed is the fact that mushrooms and ginger could be better antioxidants than garlic because of their high phenolics and flavonoid contents. It is therefore, recommended that ginger, mushrooms and garlic be properly harnessed in the management and treatment of oxidative stress induced conditions, bacterial and viral diseases, cancer, diabetes, allergy, platelet aggregation, arthritis, asthma, atherosclerosis and used as blood cleansers and warm expellants.[44],[45]

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