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Odangowei Inetiminebi Ogidi
Department of Biochemistry, Federal Polytechnic, Ekowe, Bayelsa State

Lilian Maureen Oluchi Oguoma
Department of Biotechnology, Federal University of Technology, Owerri, Imo State

Patrick Chukwudi Adigwe
Department of Science Laboratory, Federal Polytechnic Ekowe, Bayelsa state

Blessing Bumein Anthony
Department of Science Laboratory, Federal Polytechnic Ekowe, Bayelsa state

Correspondence:
Odangowei Inetiminebi Ogidi
Department of Biochemistry, Federal Polytechnic, Ekowe, Bayelsa State
Email: ogidiodangowei@gmail.com

Phytochemical Properties and *In-vitro* Antimicrobial Potency of Wild Edible Mushrooms (*Pleurotus ostreatus*) obtained from Yenagoa, Nigeria

Odangowei Inetiminebi Ogidi*, Lilian Maureen Oluchi Oguoma, Patrick Chukwudi Adigwe, Blessing Bumein Anthony

ABSTRACT

Microorganism resistance to synthetic antibiotics is an increasing public health challenge, therefore, new antimicrobial agents from different plant and biological sources are sought-after. This research was aimed at evaluating the phytochemical properties and antimicrobial potency of wild edible mushrooms. Qualitative and quantitative phytochemical analysis was done using the standard method of Association of Official Analytical Chemist and agar well diffusion method was used for antimicrobial analysis. Phytochemical results of wild edible mushroom *Pleurotus ostreatus* obtained from Yenagoa, shows varying quantities of tannins, terpenoids, alkaloids, flavonoids, saponins, glycosides and steroids. The methanol and aqueous extracts of the samples reveals different degree of inhibition on both bacteria and fungi (*Shigella sp., Staphylococcus sp., Vibrio sp., Escherichia coli, Penicillium sp.,* Yeast and Moulds). Aqueous extracts were more effective on the test organisms when compared to methanol extracts. *P. ostreatus* showed good antimicrobial potency against all the microorganisms tested. The findings from this study show the efficacy of wild edible mushroom as a potent antimicrobial agent.

Keywords: Edible Mushroom, Phytochemicals, Antimicrobial, Yenagoa, *Pleurotus ostreatus*.

INTRODUCTION

Global microorganism resistance to synthetic antibiotics is an increasing public health challenge. Various specific plant and macro-fungi has been used since ancient times to alleviate human kind from various diseases [1]. Hence, this renewed interest in orthodox medicine and the continuously increasing demand for plant sourced drugs. This revival of interest in plant sourced drugs is mainly due to the belief that "green medicine" is safe and dependable more than the synthetic ones, many of which have adverse side effects [2]. Therefore, new antimicrobial agents from different plant and biological sources are sought-after which includes mushrooms [3].

Mushrooms are macro-fungi that have fruiting bodies (hypogeous or epigeous) and can be seen with the naked eye [4]. They are any rapid-growing, wood colonizing fleshy fungi, belonging to Basidiomycota phylum. Basidiospores formation is a major characteristic of this group of fungi. Some mushrooms live as saprophytes when they are alone or symbiotic association when they are found with plants. A lot of these macro-fungi are edible and possess beneficial medicinal characteristics while some are also poisonous (toadstools).

Today, wild edible mushrooms *Pleurotus ostreatus* is mostly accepted due to its nutritional and therapeutic potentials. Edible mushrooms are used for the treatment of several diseases such as bronchial inflammation, hepatitis, cancer, hypertension, allergies, flu and colds, fungal infection, diabetes and heart failure. Edible mushrooms are important source of biologically active compounds [5]. Recently, attention has been drawn to investigating natural drugs from various plant and biological sources such as wild edible mushrooms which are fleshy and edible fruit bodies of several fungi species [6].

Mushrooms do adapt their metabolism in their habitat for survival. That is why their metabolites have antimicrobial, antioxidation, and anti-inflammation activities [7]. Mushrooms contain a good number of bioactive compounds such as terpenoids, flavonoids, tannins, alkaloids, polysaccharides [8, 9]. The basidiocarps and mycelia of mushrooms promote health benefits such as antioxidative, antibacterial and immunostimulatory activities [10]. The combination of these properties usually increases therapeutic values of the mushroom of interest.

This research was aimed at evaluating the phytochemical properties and antimicrobial potency of wild edible mushrooms obtained from Yenagoa, Bayelsa state, Nigeria.

MATERIALS AND METHODS

Collection of Mushroom Samples

Edible mushrooms were obtained from the market in Yenagoa. It was sun-dried for three days and were pulverized and stored in airtight container for laboratory analysis.

Methods

Qualitative Phytochemical Screening

Phytochemical tests for the screening of bioactive chemical constituents in the mushroom samples under study were carried out using the standard procedures as previously described by Odangowei *et al.* [11].

Quantitative Phytochemical Analysis

Total Tannin Content Determination

Total tannins were determined by slightly modified Folin and Ciocalteu method. 0.5 ml of the sample was added with 3.8 ml of distilled water, 35% sodium carbonate solution and 0.25 ml of Folin Phenol reagent. The absorbance was measured at 725 nm. Tannic acid dilutions (0 to 0.5 mg/ml) were used as standard solutions. The result of tannins is expressed in terms of tannic acid in mg/ml of the sample.

Total Alkaloid Content Determination

Forty (40) ml of 10% acetic acid in ethanol was added to 1 g of the powdered sample, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to get 1/4th of its original volume. Concentrated ammonium hydroxide was added drop wise to the sample until the precipitation was complete. The whole solution was allowed to settle and collected precipitate was washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed.

Total Flavonoid Content Determination

Total flavonoid content of the samples was determined by following the Aluminum chloride method. Macro-fungi concentrate was mixed with $NaNO_2$ solution, distilled H_2O and $AlCl_3$ solution after 6 min. It was allowed to stand for 6 min, NaOH solution and distilled H_2O was added to the mixture to achieve the final volume. The mixture was vortexed extensively and stand for another 15 min. Optical density of the mixture was recorded at 510 nm. Rutin was used as a standard compound for the evaluation of total flavonoid. The total flavonoids were calculated using the standard curve, and expressed as rutin equivalent in mg/g of the sample.

Total Saponin Content Determination

Two (2) ml of 80 % methanol, 72 % sulphuric acid solution and vanillin in ethanol solvents were dissolved with the pulverized mushroom samples and mixed thoroughly and heated on a water bath at 60°C for 10 min, absorbance of 554 nm was measured against reagent blank. Diosgenin was used as standard material and assay compared with Diosgenin equivalents.

Total Terpenoid Content Determination

Mushroom sample (1 g) was marcarated with 50 ml of ethanol and filtered. The filtrate 2.5 ml of 5 % aqueous phosphomolybdic acid solution and concentrated H_2SO_4 was added and mixed which was left to stand for 30 minute, 12.5 ml of ethanol solvent was used to make up and 700 nm absorbance was taken.

Total Steroid Content Determination

The steroid content of the mushroom sample was determined using the method described by Trease and Evans [12]. A portion of 2 ml was taken from a solution of 2.5 g of powdered plant material prepared in 50 ml of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3 ml of 0.1M NaOH (pH 9) and later mixed with 2 ml of chloroform and 3 ml of ice cold acetic anhydride followed by the cautious addition of two drops of concentrated H_2SO_4. The absorbance of both sample and blank were measured using a spectrophotometer at 420 nm.

Mushroom Sample Extraction

The extraction reagents were methanol and aqueous. About 10 g of the mushroom sample was placed in a beaker and 25ml of methanol added and mixed by vortexing. It was centrifuged at 3000 rmp for 10 minutes. The supernatant was collected and transferred to a stoppered test tube by filtration. The resulting supernatant was evaporated to dryness with a gentle stream of nitrogen and reconstituted in 10 ml dimethyl sulphoxide and was mixed by vortexing. The same procedure was repeated for that of aqueous.

Mushroom Extract Disc Placement

Mushroom disc containing 3 ml (3 µl) concentration, as well as mushroom were made using filter paper and then placed on the plates using sterile forcep. One sterile antibiotic disc and an agar plate were placed on the surface by using a sterilized forcep. The disc was then gently pressed with the forcep to ensure complete contact with the agar surface and placed away from the edge of the plates so that it is easily measured. Once all discs were in place, the plates were inverted, and placed in a 37°C incubator for 24 hours.

Bacteria/ fungi suspension preparation

Media used: Nutrient agar, buffered peptone water, *shigella* agar, macconky agar and cetrimide agar. These media were prepared according to manufacturer's instruction. Using a sterile inoculating loop and needle for bacteria and fungi respectively, through aseptic techniques of the test organisms of each colony was taken from the subculture plate. The microorganisms were suspended (4 ml) in normal saline and vortexed for overall suspension. Mcfarland standard solution was used as a reference to adjust the turbidity of individual bacterium isolate in the suspension (1×10^8). And 10 fold serial dilutions was made and plated for the antimicrobial sensitivity test.

Inoculation of Isolates

Sterile swab stick was dipped into the test organism's suspension in 4 ml of buffered peptone water. The swab was rotated against the side of the tube using firm pressure to remove excess fluid, but the swab was not dipped wet. The dried surface of the plate was inoculated by streaking the swab over the entire surface of the agar by rotating the plate at 60° each time to ensure an even distribution of the inoculum.

RESULTS

Phytochemical constituents

Qualitative phytochemicals present are: tannin, terpenoid, saponin, flavonoid, alkaloid, steroids and glycoside. Quantitatively, tannin content was (0.04 ± 0.01%), terpenoid (0.06±0.01), saponin (0.15 ± 0.01%), alkaloids (0.44 ± 0.01%), flavonoid (0.05 ± 0.02%) and steroids (0.13± 0.02) (Tables 1 and 2).

Table 1: Qualitative Phytochemical Result of Mushroom

Sample code	Alkaloid	Tannin	Flavonoid	Saponin	Glycosides	Terpenoid	Steroids
Mushroom	+	+	+	++	+	++	+

Key: + (presence), ++ (abundance)

Table 2: Quantitative Phytochemical Results of Mushroom

Sample Code	Phytochemicals (%)					
	Alkaloid	Tannin	Flavonoid	Saponin	Terpenoid	Steroid
Mushroom	0.44±0.01	0.04±0.01	0.05±0.01	0.15±0.01	0.06±0.01	0.13±0.02

Antimicrobial sensitivity

Antibacterial sensitivity of Gram-positive bacteria shows the diameter of inhibition zone of *Staphylococcus sp (*10±1.0mm, and 9.3±0.3mm) and gram-positive bacteria, such as *E. coli, Shigella sp., and Vibrio sp.*, shows the diameter of inhibition zone of 9.0±1.0mm, 9.0±1.0mm 9.2±0.8mm and, 19±1.0mm, 9.6±0.2mm, 7.3±0.3 mm for aqueous and methanol respectively. While antifungal sensitivity shows *Penicillium sp*, Yeast and Moulds with diameter of inhibition zone for aqueous extract 9.0±1.0, 7.8± 0.8, and 8±1.0 mm and methanol extract 6.23±0.25, 7.2±0.3, and 6.4±0.2 mm, respectively (Tables 4 and 6).

Table 3: Biochemical Characteristics of some Bacteria

Microorganisms		*Shigella sp.*	*E. coli*	*Staphylococcus sp.*	*Vibrio sp.*
Cell morphology (cell shape)		Rod	Rod	Coccus	Comma
Colony (cell shape)		Round	Spindle	Circular	Curved
Gram reaction		-	-	+	-
Biochemical test	Nitrate reductive	+	+	+	+
	Oxidase	-	-	-	+
	Catalase	+	+	+	-
	Methyl red	+	+	+	-
	V.P.	-	-	+	+
	Indole	-	+	-	+
	Citrate	-	-	+	+
	H_2S reduction	-	-	-	-
	Ureas activity	-	-	+	-

Key: + = Positive; − = Negative; VP = Voges Proskauer; H_2S = Hydrogen sulfide

Table 4: Antibacterial Potency of extracts of edible mushroom obtained from Yenagoa (Diameter of inhibition zone in mm) (Means ± SD)

Name of organism	Methanol extract	Aqueous extract
Shigella sp.	9.6±0.2	9.0±1.0
E. coli	19±1.0	9±1.0
Staphylococcus sp.	9.3±0.3	10±1.0
Vibrio sp.	7.3±0.3	9.2±0.8

Table 5: Identification of fungi with Cultural Morphology

Organisms	Microscopic observation (Medium)	Microscopic observation (gram reaction)
Yeast sp.	White colour, creamy growth on the media surface	Pink colour large cells obtained by gram's staining, oval, budding cells obtained by LPCB staining.
Penicillium sp.	Greyish-green colour colonies, smooth colonies.	Bruish like conidiophores and branched mycelium spores arranged on conidiophores
Mould	Black huge colonial growth	Heavy mycelial growth arranged in filamentous form.

Table 6: Antifungal Potency of extracts of edible mushroom obtained from Yenagoa (Diameter of inhibition zone in mm) (Means ± SD)

Name of organism	Methanol extracts	Aqueous extracts
Penicillium sp.	6.23±0.25	9.0±1.0
Yeast sp.	7.2±0.3	7.8±0.8
Mould	6.4±0.2	8±1.0

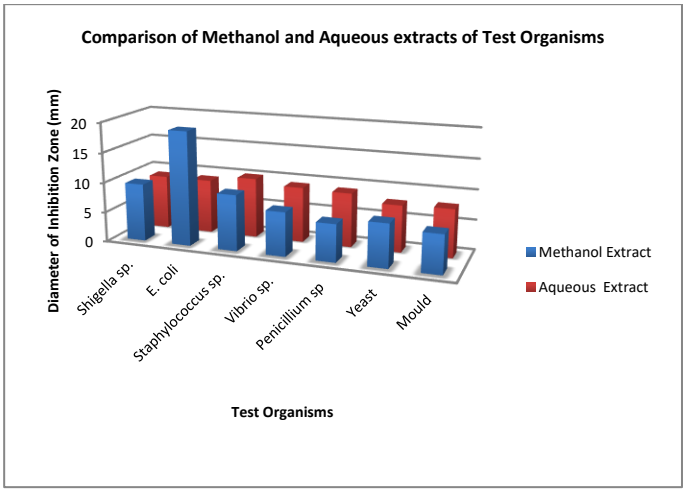

Figure 1: Comparison of Methanol and Aqueous extracts of Test Organisms

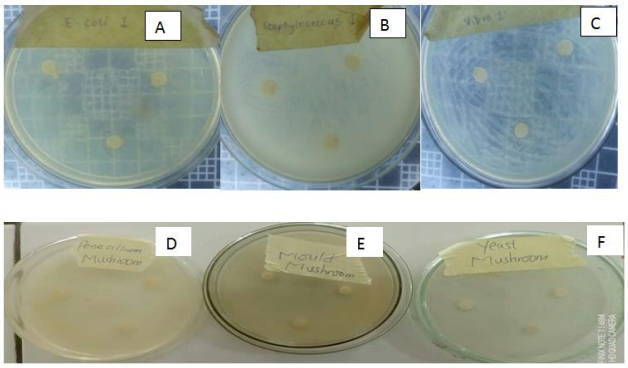

Figure 2: Antimicrobial Activities of Edible Mushroom Samples (A) *E. coli, (*B) *Staphylococcus sp,* (C) *Vibrio sp,* (D) *Penicillium sp,* (E) Mould (F) Yeast

DISCUSSION

Phytochemical constituents

Mushrooms are important macro-fungus that serves as food, medicine, bio-control agents, and a source of bioactive compounds used in pharmaceutical industry, due to their therapeutic benefits [13, 14]. Phytoconstituents and microbial sensitivity of *Pleurotus ostreatus* extracts were assessed in this study. Qualitative phytochemical screening of *Pleurotus ostreatus* shows the presence of flavonoid, saponin, glycosides, tannin, terpenoids, alkaloid, and steroid as shown in Table 1. This result was in accordance with previously reported literatures [11]. These reported phytoconstituents are known to be biologically active, and serve as a defense mechanism for plants against many microorganisms [15].

Quantitative phytochemical constituents, alkaloids content was highest (0.44±0.01%) in the mushroom samples. One of the most effective therapeutically important bioactive compounds in plants is alkaloids. Purely isolated or synthetic derived alkaloids are used as medicinal agents because of their analgesic, antispasmodic and bactericidal properties [16]. Steroid content was observed on the mushroom samples as 0.13±0.012 (Table 2). Taleb-Contini *et al*. [17] and Neumann *et al*. [18] reported and confirmed antibacterial and antiviral properties of steroids in *Chromolaena* plant species against *Streptococcus mutans* and *S. sobrinus* strains. Saponin (0.15 ± 0.01%) and Terpenoid (0.06±0.0) contents suggests the usefulness of the plant as a potential fertility agent and has demonstrated antimicrobial, anticarcinogenic (perilla alcohol), antimalarial (artemisinin), anti-ulcer and hepaticidal effects [19, 20].

Tannin contents were 0.04 ± 0.01% which is a bioactive substance quickens the healing of wounds, useful for the prevention of cancer and treatment of inflamed mucous membranes and ulcerated tissues [21, 22]. The presence of flavonoid (0.05± 0.01%) in the mushroom samples explains their biological functions such as protection against allergies, inflammation, free radical, platelet aggregation, microbes, ulcers, hepatoxins, viruses and anti-oxidation [23]. Lim *et al*. [24] also reported antimicrobial activity of tannin extracted. The phytochemical results in this study are in agreements with similar works [25, 6, 26].

Antimicrobial potency

This study shows antimicrobial efficacy of the macro-fungus extracted with methanol and aqueous. These extracts were observed to be sensitive to both bacteria and fungi tested which suggests that *Pleurotus ostreatus* has broad-spectrum antibacterial and antifungal effects. In the present work, the antibiotic potential of aqueous extracts of mushroom shows higher zone of inhibition in *Staphlococcus sp* (10±1.0mm), *Vibrio sp* (9.2±0.8mm), *penicillium sp* (9.0±1.0mm), yeast (7.8±0.8mm) and mould (8±1.0mm) but lowest on *Escherichia coli* (9±1.0mm) and *Shigella sp* (9±1.0mm) and this result is in agreements with reports of Sanjay *et al* [27]. For methanol extract, the zone of inhibition was highest only in *Escherichia coli* (19±1.0mm) and Shigella sp (9.6±0.2) but lowest in other test organisms as shown in Table 4 and 6. This finding is in agreement with the findings of Samuel and Victor, [28] and Adeoyo and Oluborode, [29].

In comparison with both extracts used in the antimicrobial activities, aqueous extracts were observed to be more potent than methanol extract as shown in Figure 1. This result is in contrary with earlier reports by Aarti and Astha, [25], Odangowei *et al.* [30, 31] and Madhavi *et al.* [32] that observed methanol extract with higher sensitivity of bacteria and fungi than aqueous extract.

CONCLUSION

In this study the phytochemical screening of *Pleurotus ostreatus* shows the presence of Saponins, Tannins, Flavonoids, Alkaloids, Steroids, Glycosides and Terpenoids. From the antimicrobial results obtained in this study, it can be concluded that both aqueous and methanol extracts of the wild edible mushroom can be applied in the development of more efficient and potent antimicrobial agents. However, aqueous extract of mushroom was observed to be more effective on the broad-spectrum antibacterial and antifungal activity, thereby showing more evidence on the therapeutic use of mushrooms in traditional medicine.

Conflict of Interest

Authors declare no conflict of interest

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