

**Phytochemical, Proximate Composition and Antimicrobial Potentials of *Pleurotus tuber-regium* Sclerotium**Ngozi Goodluck Anyanwu<sup>1\*</sup>, Clement Ibi Mboto<sup>2</sup>, Leera Solomon<sup>3</sup> and Nnenna Frank-Peterside<sup>1</sup><sup>1</sup>Department of Microbiology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.<sup>2</sup>Department of Microbiology, Faculty of Science, University of Calabar, P.M.B.1115, Calabar, Cross River State, Nigeria<sup>3</sup>Department of Science Laboratory Technology, School of Science and Technology, Rivers State College of Arts and Science, P.M.B. 5936, Rumuola, Port Harcourt, Nigeria.[anyanwungozi91@yahoo.com](mailto:anyanwungozi91@yahoo.com), [sololeera@yahoo.com](mailto:sololeera@yahoo.com)

**Abstract:** A comparative study of the phytochemical properties, proximate analysis and antimicrobial potentials of ethanolic and aqueous extract of *Pleurotus tuber-regium* sclerotium was carried out. The activities of the extracts were compared with activities of standard antibiotics (Streptomycin (30µg), Ampicillin (30µg), Ciprofloxacin (10µg), Tetracycline (30µg), Penicillin (30µg) and Erythromycin (30µg) and antifungal (Fluconazole (30µg), Nystatin (30µg) and Amphotericin (30µg) discs. The test organisms were *S. aureus*, *Escherichia coli*, *Streptococcus* sp., and *P. aeruginosa* and fungal (*A. niger* and *C. albicans*). Phytochemicals such as alkaloids, flavonoids, saponins, polyphenols and glycosides were in moderate amount with traces of tannins which were more in ethanolic than in aqueous extracts. Proximate analysis revealed moisture (0.25±0.02), ash (2.40±0.1), protein (7.87±0.02), fat (20.80±0.2), fibre (11.88±0.01) and carbohydrate (56.80±0.02). The minimum inhibitory concentration of the ethanolic extract were 12.5mg/ml for *S. aureus*, 50mg/ml for *Streptococcus* sp., 6.25mg/ml for *Escherichia coli*, 3.13mg/ml for *P. aeruginosa* and 25mg/ml for *Aspergillus* sp. whereas the minimum bactericidal concentrations of the aqueous extract gave 50mg/ml, 25mg/ml and 12.5mg/ml for *S. aureus*, *Escherichia coli* and *P. aeruginosa*, respectively. Antimicrobial activity against *P. aeruginosa* was significantly ( $p < 0.05$ ) higher compared to standard antibiotics. The highest zone of inhibition with penicillin was observed on *Staphylococcus aureus* while the lowest was on *P. aeruginosa*. Bacterial isolates were all susceptible to the antibiotics except *Streptococcus* sp. and *S. aureus* which were resistant to ampicillin and tetracycline, whereas *C. albicans* and *Aspergillus* sp. were sensitive to all antifungals. Extracts of *Pleurotus tuber-regium* sclerotium can be used as substitute for conventional antibiotics. [N. G. Anyanwu, C. I. Mboto, L. Solomon and N. Frank-Peterside. **Phytochemical, Proximate Composition and Antimicrobial Potentials of *Pleurotus tuber-regium* Sclerotium**. *N Y Sci J* 2016;9(1):35-42]. ISSN 1554-0200 (print); ISSN 2375-723X (online). <http://www.sciencepub.net/newyork>. 6. doi: [10.7537/marsnys09011606](https://doi.org/10.7537/marsnys09011606).

**Key words:** Proximate analysis, antimicrobial potentials, *Pleurotus tuber-regium* sclerotium.**1. Introduction**

*Pleurotus tuber-regium* (the tiger milk mushroom or the sclerotia-producing mushroom) is a tropical edible mushroom that produces the edible sclerotium or an underground tuber as well as the mushroom fruiting body (Akindahunsi and Oyetayo, 2006; Andrew *et al.*, 2007; Hu *et al.*, 2006; Iwuagwu and Onyekweli, 2012). This mushroom grows naturally in Nigeria during the early and late rainy seasons (Gbolagade *et al.*, 2006) and is usually found in the forests, grasslands and damp rotten logs. *Pleurotus tuber-regium* is useful as food, as well as being medicinal. In traditional medicine practice in Nigeria, *Pleurotus tuber-regium* has been useful in the preparation of cures for headaches, stomach ailments, colds and fever, asthma, smallpox and a high blood pressure.

Other benefits attributable to this mushroom's oligosaccharides include its immuno-modulatory effects, which have been shown to be beneficial to individuals living with HIV (Jong *et al.*, 1991;

Isikhuemhen and LeBauer, 2004; Lekgari, 2010; Oso, 1977).

In recent years, multi drug resistance in pathogenic-microorganisms has developed due to the misuse of antimicrobial drugs commonly used in the treatment of infectious diseases (Karaman *et al.*, 2003). This drastic increase in the resistance of most microorganisms to antimicrobials has necessitated the quest for alternative antimicrobial or chemotherapeutic agents that may suppress these microorganisms.

The scientific community, in search of new therapeutic alternatives has studied many mushrooms and has found variable therapeutic activities such as anti-carcinogenic, anti-inflammatory, immune-suppressors and antibiotics among others (Stamets, 2000; Landequist *et al.*, 2005; Fasidi *et al.*, 1993; Fassatiava, 2005; Gezar *et al.*, 2006). Many ethno-medicinal values have been reported of *P. tuber-regium* (Okhuova and Okogobo, 1990; Singer, 1986; Osemwegie *et al.*, 2002).

Several other medicinal properties have also been reported of *P. tuber-regium* and such include; anti-tumor properties attributed to their polysaccharides (Ijeh *et al.*, 2009; Solax *et al.*, 2006), antigenotoxic and bioantimutagenic activities (Hibbett and Thorn, 1994; Fillipie and Umeke, 2002; Miles and Chang, 1997; Elegbede, 1998; Honda *et al.*, 2008; Remington, 2010).

Anti-inflammatory activities, anti-lipidaemic, antihypertensive and antihyperglycaemic activities (Hu *et al.*, 2006) has also been reported by *Pleurotus tuber-regium*. It has been reported also that *P. tuber-regium* produces a variety of products for the production of feeds and enzymes (Andrew *et al.*, 2007). Conflicting Nutritional/proximate values of this edible fungi and its chemical profiles has been reported and unfortunately, information on the “*in-vitro*” antimicrobial activities of this fungi is very scanty and not really available on the literature as of now especially its sclerotium.

The present research study therefore, was designed to examine the phytochemicals, proximate values and antimicrobial potentials of *Pleurotustuber-regium* sclerotium. This study was therefore aimed at determining the phytochemical properties, proximate analysis and antimicrobial potentials of ethanolic and aqueous extracts of edible mushroom (*Pleurotus tuber-regium* sclerotium).

## 2. Materials and Methods

### 2.1 Sample collection

Dried samples of *P. tuber-regium* was purchased from Watt Market in Calabar, properly cleaned and transported to the laboratory for use (Olasupo *et al.*, 2012).

### 2.2 Extraction of Sclerotium

The method of Nweze and Okafor (2010) was adopted. Sample was cut and ground into powder using Binatone blender. 100g of the powdered sample was separately extracted with 200ml of 95% ethanol and water for 48hr and shaken periodically. The process was repeated 3 times and the liquid extracts filtered and the filtrate evaporated using a rotary evaporator to obtain both aqueous and ethanolic extracts in a paste form. Each extract was stored in a sterile container and preserved in a refrigerator (Ncube *et al.*, 2008; Onuoha and Obi-Adumanya, 2010).

### 2.3 Phytochemical analysis

The freshly prepared liquid extracts of the sample were chemically tested for the presence of bioactive chemical constituents using standard procedures (Culei, 1982; Trease and Evans, 1989 and Sofowara, 1999). The extracts were then screened for alkaloids, flavonoids, tannins, saponins, glycosides, reducing compounds, polyphenol, phlobabtannins,

hydroxymethylantraquinons and anthraquinones (Essien and Odoemena, 1995; Adejumo and Awosanya, 2004).

### 2.4 Proximate analysis

Proximate analysis was done to determine the moisture content, crude protein, fat, ash, fiber and total carbohydrate content (AOAC, 2006; Olasupo *et al.*, 2013).

### 2.5 Antimicrobial susceptibility assay

The Kirby-Bauer method was applied based on zones of inhibition of agar plates (Prescott *et al.*, 2005). Exactly 1.0g of the ethanolic and aqueous extracts was dissolved in 5ml each of Dimethyl Sulfoxide (DMSO) and water respectively to obtain 200mg/ml of each extract. The concentration was diluted two folds to get 100mg/ml and 50mg/ml of each extract. Mueller-Hinton agar was prepared and plates inoculated with the different isolates. The punched circular discs from Whatman No. 2 filter paper were impregnated with 0.1ml of different concentrations of each extract and allowed to air-dry for few minutes.

Each of the discs was pressed onto the surface of inoculated medium to ensure contact with medium. Negative control was prepared by impregnating some discs with water and DMSO which was transferred and pressed unto the inoculated plates while standard antibiotics discs (ciprofloxacin, penicillin, streptomycin, tetracycline, erythromycin and ampicillin) and antifungal discs (Nystatin and Amphotericin B and Fluconazole) served as positive controls. All the tests were carried out in duplicates. Incubation was done at 37°C for 24 hr and plates observed for zones of inhibition. The degree of sensitivity was expressed as a measure of the diameter of zones of inhibition in millimeters (mm).

A diameter of 10 mm or higher was considered as an indication of sensitivity of the test organism to the extracts. The mean inhibition zones (mm) were calculated as the difference between the disc diameter (6 mm) and the diameter of inhibition zones (Akpaja *et al.*, 2003; Solomon *et al.*, 2013).

### 2.6 Minimum inhibitory concentration

The inoculums of the microbial isolates used for the test was prepared by comparing with a standard of MacFaland reagent in order to reduce microbial load or count. The method described by Prescott *et al.* (2005) was adopted. Exactly 1.0g each of both ethanolic and aqueous extracts were dissolved separately in 5 ml each of DMSO and sterile water respectively to get a concentration of 200mg/ml and labeled as solution 1. 2 ml of solution 1. This was serially diluted into 8 folds to get corresponding concentrations of 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.75mg/ml, 3.13mg/ml, 1.56mg/ml and

0.78mg/ml each for both aqueous and ethanolic extract and labeled solutions 3 to 9.

A previously prepared Mueller-Hinton broth containing various concentration of the extract was inoculated with the standard inoculums of each test organisms followed by incubation of the tube at 37°C for 16-20 hr. Thereafter, the tubes were observed for the presence or absence of growth in each tubes determined by turbidity of the test tubes. The lowest concentration of the extracts resulting in no growth after incubation was taken as the minimum inhibitory concentration (MIC) of the melon fungus extracts (Ene-Obong and Camovale, 1992; Aloba, 2003; Essien *et al.*, 2004; Essien and Odoemena, 1995).

### 3. Results

Result of the phytochemical analysis of ethanolic and aqueous extract of *P. tuber-regium* reveals the

presence of alkaloids, glycosides, saponins, flavonoids, tannins and polyphenols. The reducing compounds, phlobatannins, anthraquinones and hydroxymethylanthraquinones were also determined and found to be totally absent in the sample. Alkaloids, saponins and polyphenol compounds were present in excess for ethanolic extract unlike the other components that were moderately present whereas tannin compound was only present in a trace, amount since they did not show very strong and explicit reaction with ethanolic extract. Results of ethanolic extract showed more strong reaction with the phytochemical reagents than that of aqueous extracts. This is because ethanol is a well-known organic solvent and dissolves phytochemical metabolites more readily than water. The phytochemical analysis of *Sclerotium* of *P. tuber-regium* is as presented in Table 1.

**Table 1: Phytochemical Analysis of *Sclerotium* of *P. tuber-regium***

Chemical constituent	Name of test	observation	Ethanolic extract	Aqueous extract
Alkaloids	Mayer's test/dragendorff's	Cream precipitate turbidity	++	++
Anthraquinones	Nitrobenzene test	No coloration	-	-
Flavonoids	Aluminum chloride test	Orang/yellow coloration	++	++
Glycosides	Salkowski test	Brown interface/violet ring	++	++
Hydroxymethylphlobatannins	Ammonia test	Non violet coloration	-	-
Polyphenols	Ferrocyanide test	Green-blue coloration	+++	++
Reducing compounds			-	-
Saponins	Frothing test	Persistent foam	+++	++
Tannins	Ferric chloride test	Coloration	+	+

Key: +++: strongly present, ++: moderately present, +: present in trace amount, -: not present

Table 2 summarized the result obtained from the proximate analysis of the dry sample of sclerotium of *P. tuber-regium* used. The result revealed percentage compositions as 67.52±0.02 for moisture, 2.40 ± 0.1 for ash and 7.87 ± 0.02 for protein. Furthermore, the fat, fibre and carbohydrate contents contained 20.80 ± 0.1, 11.87 ± 0.01 and 56.80 ± 0.02 respectively. This result showed that the sample had high moisture, carbohydrate and fat content but very low in ash content. Presented in Table 3 is the summary of the susceptibility assay of the bacterial and fungal isolates to the ethanolic and aqueous extract of sclerotium of *P. tuber-regium*. The result shows that *Staphylococcus aureus*, *E. coli* and *Pseudomonas* spp are susceptible to both extracts while *Streptococcus* species shows susceptibility to only ethanolic extract. In the case of

fungal isolates, *Aspergillus* sp. was only susceptible to ethanolic extract whereas *C. albicans* was completely resistant to both the ethanolic and aqueous extract of the *Sclerotium* tested.

**Table 2: Proximate composition of *Pleurotus tuber-regium* (*Sclerotium*)**

Content	Composition (g/100g dry mass)
Moisture	0.25±0.02
Ash	2.40±0.1
Protein	7.87±0.02
Fat	20.80±0.2
Fibre	11.88±0.01
Carbohydrate	56.80±0.02

**Table 3: Susceptibility assay of the bacterial and fungal isolates to ethanolic and aqueous extracts of *P. tuber-regium* (sclerotium).**

Isolate	Sensitivity	
	Ethanolic extract	Aqueous extract
<i>Staphylococcus aureus</i>	+	+
<i>Streptococcus spp.</i>	+	-
<i>Escherichia coli</i>	+	+
<i>Pseudomonas spp.</i>	+	+
<i>Candida albicans</i>	-	-
<i>Aspergillus spp.</i>	+	-

Key: + : sensitive, -: resistant

The result of minimum inhibitory concentration (MIC) of ethanolic extract against the test microbial isolates revealed 12.5 mg/ml for *Staphylococcus aureus*, 50mg/ml for *Streptococcus* species, 6.75mg/ml for *Escherichia coli*, 3.13mg/ml for *Pseudomonas* spp. and 25mg/ml for *Aspergillus* sp.

For aqueous extract, the minimum inhibitory concentration (MIC) against *Staphylococcus aureus* was 50mg/ml while that of *Escherichia coli* and *Pseudomonas* sp. were 25mg/ml and 12.5mg/ml respectively. The minimum inhibitory concentration (MIC) results obtained for ethanolic and aqueous extract are as presented in Tables 4 and Table 5. The alkaloids, flavonoids and glycosides which could be toxic in high amount (Ikewuchi and Ikewuchi, 2008) were found to occur in non-toxic doses. The study reported by Sofowara (1999), revealed that these

bioactive compounds contained in this edible melon fungus sclerotium have antimicrobial potency (Holt *et al.*, 1994; Kanika, 2011).

This is in agreement with Moore (2003) who stated that the phytochemicals found in the sclerotium of *P. tuber-regium* promote the function of the immune system, act directly against bacteria and viruses reduce inflammation and it is associated with the treatment and prevention of cancer, cardiovascular diseases and many other maladies affecting the health of individuals. However, this mushroom lacks anthraquinones, phlobatannins, reducing compound and hydroxymethylathraquinones. The absence of these compounds is in agreement with reports by Ikewuchi and Ikewuchi (2008) and Onuoha *et al.* (2010).

**Table 4: Minimum inhibitory concentration of ethanolic extract of *P. tuber-regium* (sclerotium) against selected microbial isolates**

Concentration (mg/ml)	Dilution	<i>Staphylococcus aureus</i>	<i>Streptococcus sp.</i>	<i>E.coli</i>	<i>Pseudomonas sp.</i>	<i>Aspergillus sp.</i>
200	2 <sup>0</sup>	N.T	N.T	N.T	N.T	N.T
100	2 <sup>-1</sup>	N.T	N.T	N.T	N.T	N.T
50	2 <sup>-2</sup>	N.T	N.T	N.T	N.T	N.T
25	2 <sup>-3</sup>	N.T	T	N.T	N.T	N.T
12.5	2 <sup>-4</sup>	N.T	T	N.T	N.T	T
6.25	2 <sup>-5</sup>	T	T	N.T	N.T	T
3.13	2 <sup>-6</sup>	T	T	T	N.T	T
1.56	2 <sup>-7</sup>	T	T	T	T	T
0.78	2 <sup>-8</sup>	T	T	T	T	T

Key: N.T: not turbid, T: turbid

**Table 5: Minimum inhibitory concentration of aqueous extract of *P. tuber-regium* (sclerotium) against selected microbial isolates**

Concentration (mg/ml)	Dilution	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Pseudomonas sp.</i>
200	2 <sup>0</sup>	N.T	N.T	N.T
100	2 <sup>-1</sup>	N.T	N.T	N.T
50	2 <sup>-2</sup>	N.T	N.T	N.T
25	2 <sup>-3</sup>	T	N.T	N.T
12.5	2 <sup>-4</sup>	T	T	N.T
6.25	2 <sup>-5</sup>	T	T	T

Key: N.T: not turbid, T: turbid

The study indicates much lower moisture ( $0.25\pm 0.02$ ) content than that reported by Ikewuchi and Ikewuchi (2008) and its protein content ( $7.87\pm 0.02$ ) was also lower than that reported in the same study. The protein content observed here was higher than the values earlier reported by Onyeike and Ehirim (2001) for *P. tuber-regium* sclerotia and those reported for cowpea, groundnut, pigeon pea, soybeans (Elegbede, 1998), *T. triangulare*, *T. occidentalis*, *P. purpureum* (Okaraonye and Ikewuchi, 2009) and wheat (Singh, 2004).

The results of antibiotics and antifungal susceptibility test against the bacterial and fungal isolates are as present in Table 6. The highest zone of

inhibition diameter with penicillin (30 $\mu$ g) was observed on *Staphylococcus aureus* (26mm) while the lowest was observed on *Pseudomonas* sp. (18mm). All bacterial isolates were susceptible to the standard antibiotics (Solomon *et al.*, 2013) except *Streptococcus* sp. that was resistant to ampicillin (30 $\mu$ g) and *S. aureus* which was resistant to tetracycline (30 $\mu$ g). *Candida albicans* and *Aspergillus* sp. were sensitive to Fluconazole, Nystatin and Amphotericin and were in conformity with the the results obtained by other researchrs (Courvalin and Weber, 2005; Domsch *et al.*, 1993; Adebayo-Tayo and Friday, 2004).

**Table 6: Result of standard antibiotics and antifungal susceptibility test against test microbial isolates with their zones of inhibition**

Standard antimicrobial (mg/ml)	Zones of inhibition (mm)						
	Concentrations ( $\mu$ g)	<i>S. aureus</i>	<i>Streptococcus</i> sp.	<i>Escherichia coli</i>	<i>Pseudomonas</i> sp.	<i>Aspergillus</i> sp.	<i>Candida albicans</i>
Streptomycin	30	14	16	13	16	N.A	N.A
Ampicillin	30	N.A	R	20	R	N.A	N.A
Ciprofloxacin	10	13	14	15	15	N.A	N.A
Tetracycline	30	R	7	10	12	N.A	N.A
Penicillin	30	26	19	20	18	N.A	N.A
Erythromycin	30	23	18	18	14	N.A	N.A
Fluconazole	30	N.A	N.A	N.A	N.A	16	12
Nystatin	30	N.A	N.A	N.A	N.A	20	18
Amphotericin	30	N.A	N.A	N.A	N.A	19	20

Key: N.A: Not applicable, R: Resistant

### Conclusion

The extract of *P. tuber-regium* sclerotium reveals a high level of phytochemicals which contribute to its antimicrobial activities. However, it lacks some chemicals such as anthraquinones, phlobatannins and reducing compounds. *P. tuber-regium* has a high fibre and carbohydrate content in addition to moderate protein and fat content which indicated that this mushroom holds tremendous promise in completing carbohydrate, protein and lipid supply deficits in developing countries like Nigeria.

However, its water and ash content was relatively low, indicating that it is low in mineral compositions. The extract of *P. tuber-*

*regium* sclerotium exhibited significant levels of antimicrobial activities against *S. aureus*, *Streptococcus*, *Aspergillus* spp and most especially *E. coli* and *Pseudomonas* spp in that order. The extract was however resistant to *C. albicans*. The study reveals that the extract compares well with standard antibiotics and antifungals, although in some cases, the antibiotics showed higher activities than the extract while in the other cases such as *Pseudomonas* sp., the extract showed high activity to the standard antibiotics. Therefore, it can be used for the treatment of infections caused by the test pathogens.

### Recommendations

The nutritional, phytochemical and antimicrobial potentials of this edible mushroom sclerotium cannot be over-emphasized. With the abundance of lignocellulosic waste, and favourable environmental conditions, we recommend that private and government farms could be encouraged to go into mass production of this species and other edible species of mushroom. We also recommend that intensive efforts be directed towards the training of farmers by the Forestry Research Institute of Nigeria (FRIN) in modern method of its propagation, to produce affordable mushroom in order to fully maximize the appreciable potentials and benefits derived from the consumption of this mushroom sclerotium and its economic values. Finally, we recommend that pharmacists look into the modern ways of purifying the extract of *P. tuber-regium* sclerotium into standard antibiotic doses.

#### Correspondence to:

Ngozi Goodluck Anyanwu, M.Sc., mNSM.

Department of Microbiology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria.

Tel.: +2348064615300

Email: [anyanwungozi91@yahoo.com](mailto:anyanwungozi91@yahoo.com)

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