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Bioactivity of Four Nigerian Wild Mushrooms against Some Typed Clinical Isolates

Mobolaji A. Titilawo[1,*], Abidemi O. Faseun[1], Sunday B. Akinde[1], Janet O. Olaitan[1] and Olu Odeyemi[2]

[1]*Department of Microbiology, Osun State University, Osogbo, Osun State, Nigeria ;* [2]*Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria*

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Abstract

This study aimed at investigating the antimicrobial activity of some wild Nigerian mushrooms against selected typed clinical isolates. We collected wild mushrooms from an integrated organic farm in Ilesa, Southwest Nigeria. Crude methanolic extracts of *Lentinus squarrosulus* Mont., *Termitomyces robustus* (Beeli) R. Heim, *Trametes ochracea* (Pers.) Gilb. & Ryvarden and *Xylaria hypoxylon* (L.) Grev. were screened singly and in different combinations for bioactivity against the selected bacterial and yeast isolates. The minimum inhibitory concentration (MIC) and chemical constituents of the extracts were studied following standard procedures. Overall, we obtained a total of 16 mushrooms belonging to 14 genera. The extracts showed varied clearance zones against at least one of the eight bacteria, and one yeast when applied singly with the antimicrobial inhibitory zone ranging from 7.2 mm to 20.0 mm in *Staphylococcus aureus* (*T. ochracea* extract) and *Pseudomonas aeruginosa* (*L. squarrosulus* extract) respectively. Furthermore, the MIC ranged from 2.09 to 16.75 mg/mL. When combined, the blends were active against some Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus mirabilis*) but inactive against the Gram-positive bacteria and yeast. Except for *X. hypoxylon*, other extracts contained saponins, tannins and terpenoids. Our findings revealed that the wild mushrooms are potential antimicrobial agents against the tested isolates.

Keywords: Wild mushrooms; Bioactivity; Clinical isolates; Minimum inhibitory concentration; Bacteria; Yeast

1. Introduction

Mushrooms are valuable food source and nutraceuticals owing to their rich nutrient and preventive capability of various ailments (Valverde *et al*., 2015; Roy *et al*., 2016). In nature, they are found all-year-round but more abundantly during the wet season in the terrestrial or ligneous habitats (Adeniyi *et al*., 2018a). Macrofungi thrive on a variety of substrates, especially those rich in lignin, cellulose and organic matter. Thus, they play a significant role in the terrestrial ecosystem as biodegraders (Adebiyi and Yakubu, 2016; Adeniyi *et al*., 2018a).

In alternative medicine, mushrooms are famous for their therapeutic value against ailments such as rheumatism, kwashiorkor, obesity, diarrhoea, and as a purgative (Apetorgbor *et al*., 2005; Ejelonu *et al*., 2013). Earlier studies revealed the anticholesterol, antitumor, antimicrobial, antiviral, antineoplastic, antimutagenic, antioxidant, antilipidemic, antidiabetic, antihyperglycaemic, antihypotensive, antiparasitic, anti-inflammatory, hepatoprotective, hypocholesterolemic, immunomodulatory and anti-ageing properties of mushrooms (Iwalokun *et al*., 2007; Patel *et al*., 2012; Sevindik, 2019; Mushtaq *et al*., 2020). However, inadequate scientific investigations, the dearth of clinical trials, and lack of data to validate the evidence limited their acceptance as drugs in modern-day medicine (Sullivan *et al*., 2006).

The increasing failure of chemotherapeutics recently mandated the quest for newer and less expensive antimicrobials effective against disease-causing microorganisms (Kotra and Mobashery, 1998; Thomson and Moland, 2000; Saki *et al*., 2020; Sevindik, 2020). The search for inexpensive but potent antimicrobial is essential for the low- and middle-income African countries including Nigeria, that are currently hit by the menace of multidrug resistance and infectious diseases (Okeke *et al*., 2005). Fortunately, mushrooms, which commonly grow in the wild in Nigeria are getting the scientists' attention for possible development into novel drugs (Alves *et al*., 2012; Roy *et al*., 2016; Khatua *et al*., 2017; Krupodorova and Sevindik, 2020).

About 140,000 mushroom species exist globally, but a small percentage has been investigated for their therapeutic property and pharmacological screening (Wasser, 2002). A recent study, however, documented 158 mushroom species identified as potential antibiotic sources (Shen *et al*., 2017).

Unfortunately, out of the 172 wild mushrooms reported so far in Nigeria, the most populous black African country, only 26 have been screened for their antimicrobial and pharmacological activities. These include *Auricularia polytricha* (Mont.) Sacc., *Boletus* sp., *Coprinellus*

* Corresponding author. e-mail: mobolaji.adeniyi@uniosun.edu.ng.

micaceus (Bull.) Vilgalys, Hopple & Jacq. Johnson, *Corilopsis occidentalis* (Klotzsch) Murrill, *Daedalea elegans* Spreng, *D. quercina* (L.) Pers., *Daldinia concentrica* sensu auct. NZ, *Flammulina* sp., *Ganoderma lucidum* sensu auct. asiatic., *Lentinus squarrosulus* Mont., *Lenzite quercina* (L.) P.Karst., *Lycoperdon giganteum* Batsch, *L. pusillum* sensu auct. mult., *Marasmius oreades* (Bolton) Fr., *Pleurotus ostreatus sensu Cooke, P. tuber-regium* (Fr.) Singer, *Psathyrella atroumbonata* Pegler, *Psalliota campestris* (L.) Quél., *Schizophyllum commune* Fr., *Termitomyces robustus* (Beeli) R. Heim, *Trametes elegans* (Spreng.) Fr., *T. versicolor* (L.) Lloyd, *Trichaptum* sp., *Tricholoma lobayensis* R. Heim, *T. nudum* (Bull.) P. Kumm and *Volvariella volvacea* (Bull.) Singer from different regions such as Ebonyi (Udu-Ibiam *et al*., 2014; Udu-Ibiam *et al*., 2015), Ekiti (David *et al*., 2012), Abuja (Etim *et al*., 2014), Kogi (Ayodele and Idoko, 2011), Ondo (Ogidi *et al*., 2015), Oyo (Jonathan and Fasidi, 2003; Gbolagade and Fasidi, 2005; Awala and Oyetayo, 2015) and Uyo (Etim *et al*., 2012).

Our previous investigation on the biodiversity of wild mushrooms in ENPOST integrated organic farm, Ilesa, Osun State Southwest Nigeria, had 151 mushroom species documented (Adeniyi *et al*., 2018a). To our knowledge, none of the species has undergone screening for antimicrobial potential, and it is in this light that the current study aimed at elucidating some wild mushrooms from the farm for possible bioactivity.

2. Materials and Methods

2.1. 2.1 Description of the Study Area

Environmental Pollution Science and Technology (ENPOST) integrated organic farm is located between Latitude 4°42ˈ30ˈE to 4°42ˈ45ˈˈE and longitude 7°36ˈ55ˈˈN to 7°37ˈ10ˈˈN, Ilesa, Osun State, Southwest Nigeria. The farm which is on a large expanse, about 10 hectares of land was established to address the challenges of environmental pollution, food insecurity, agroforestry/biodiversity destruction, and provide research opportunities (Adeniyi *et al*., 2018a).

2.2. Mushroom Collection and Identification

Fresh mushroom fruiting bodies were collected during May and October 2017. Samples were gently placed in paper bags and immediately transported to the laboratory for identification using standard keys (Odeyemi and Adeniyi, 2015).

2.3. Habitat and Substrate Classification of Mushroom Samples

The habitats and substrates of the mushrooms were differentiated alongside sample collection. Samples obtained were either classified as ligneous or terrestrial habitat whereas, substrate classification was based upon woody or soil-like material.

2.4. Preparation of Crude Extracts

The mushroom samples were oven-dried at 40°C for 1 – 5 d, ground into powder using an electric blender, sieved through 160 mesh and preserved in an airtight plastic container prior extraction. After pulverization, four samples were selected for further analysis. Exactly 50 g of the mushroom powder was extracted by soaking in 200 mL of 70 % methanol for 3 d with continuous agitation and thereafter filtered using a muslin cloth and Whatman no. 1 paper. Additionally, the residue was extracted twice using the same solvent, evaporated at 65°C, and the resultant semisolid extract was freeze-dried and kept at 4°C before use.

2.5. Test Organisms

All isolates used in this study were sourced from the National Institute of Medical Research (NIMR), Yaba, Lagos State, Nigeria. They included five Gram-negative bacteria [(*Escherichia coli* (ATCC 25900), *Klebsiella pneumoniae* (ATCC 43816), *Proteus mirabilis* (ATCC 7002), *Pseudomonas aeruginosa* (ATCC 10145), *Salmonella typhimurium* (ATCC 14028)], three Gram-positive bacteria [*Bacillus subtilis* (NCTC 8263), *Corynebacterium diphtheriae* (ATCC 13812), *Staphylococcus aureus* (NCTC 6571)] and a yeast, *Candida albicans* (ATCC 10231).

2.6. Antimicrobial Assay

Twenty-four-hour (24 h) old bacterial and 48 h old yeast broth cultures were washed in physiological saline thrice and standardized to 0.5 McFarland standard having approximately 10^8 CFU/mL for bacteria and 10^7 CFU/mL for *C. albicans*. Lyophilized extracts were dissolved in 3 % dimethylsulfoxide (DMSO) to a concentration of 67 mg/mL, sterilized by passing through a membrane filter (0.22 µm pore size), and kept in amber bottles at 4°C. The antimicrobial assay was carried out using the standardized agar well diffusion method (CLSI, 2018). Exactly 100 µL of 0.5 McFarland standardized culture was spread plated on Mueller Hinton agar (Oxoid, UK) using a sterile swab and allowed to dry. A sterile 7 mm cork borer was used to create wells and 50 µl (67 mg/mL) of mushroom crude extracts were added to the holes. After incubating bacteria at 37°C for 24 h and yeast at 27°C for 48 h, antimicrobial activities were determined by measuring the diameter (in millimetres) of inhibition. Negative control was pure DMSO solvent, whereas positive controls were gentamicin (30 mg) for bacteria and fluconazole (25 µg) for yeast.

2.7. Combination Effect of Extracts on the Test Isolates

The same antimicrobial assay previously described was employed. The synergistic, antagonistic, indifference and additive effects of the extracts were determined in dual, triple and quadruple combinations. Each blend consisted of 67 mg/mL of individual crude extract.

2.8. Determination of Minimum Inhibitory Concentration of the Extracts

The minimum inhibitory concentration (MIC) was determined by macro-broth dilution technique as specified (CLSI, 2018). Double-fold dilution of 67 mg/mL extract was prepared in Muller Hinton broth to obtain 6 different concentrations (34.50, 16.75, 8.38, 4.19, 2.09, 1.05 mg/mL). Each dilution was seeded with 100 µL of the standardized suspension of the test organisms and incubated under standard condition. The lowest concentration that showed no visible growth was considered as MIC.

2.9. Screening of the Mushroom Extracts for Chemical Constituents

The mushroom extracts were qualitatively screened for saponins, tannins, terpenoids and anthraquinones as described (Sofowora, 1993). Briefly, saponins were detected by adding 5 mL distilled water to 5 ml of the extract, with vigorous shaking and warming. The formation of stable foam indicates the presence of saponins. In tannins, 3 mL of the extract was added to 3 mL 10 % $FeCl_3$. A blue/black colouration suggests tannins. Furthermore, 5 mL of the extract mixed with 2 mL chloroform and 3 mL concentrated H_2SO_4 was gently poured into the tube. A reddish-brown colouration at interface indicates the presence of terpenoids. Also, 0.5 g of extract was boiled in 10 % HCl and filtered when hot. To the filtrate, 2 mL of chloroform and 10 % NH_3 solution was added. Development of the pink colour in the aqueous layer indicates anthraquinones.

3. Results and Discussion

3.1. Mushrooms Species Obtained at the Site of Study

Mushrooms are non-timber forest products and have served as food, medicine, enzymes, and are also an important source of earnings for people in different parts of the world (Boa, 2004). However, human activities such as deforestation, bush burning, application of pesticides and herbicides, urbanization and climate change have resulted in their gradual disappearance in the wild (Adeniyi *et al*., 2018a).

In the present investigation, a total of 16 mushrooms belonging to 14 genera were obtained, of which eleven species were collected in May, one species in October and four species during both months (Table 1). Representative pictures are in Figure 1. Previous studies in India (Singha *et al*., 2017), Mexico (Álvarez-Farias *et al*., 2016), Italy (Leonardi *et al*., 2017), Ethiopia (Sitotaw *et al*., 2015) and Nigeria (Adeniyi *et al*., 2018a,b; Buba *et al*., 2018), have recorded related mushroom species, with some even at higher frequencies.

Table 1. Sampling months and mushrooms species collected.

Sampling month	Mushroom
May 2017	*Cantharellus cibarius* Fr.
	Collybia plicatilis (Curtis) Fr.
	Clitopilus prunulus (Scop.) P. Kumm.
	Collybia sp.
	Gloeophyllum sepiarium (Wulfen) P. Karst.
	Hydnellum peckii Banker
	Mycena acicula (Schaeff.) P. Kumm.
	Mycena inclinata (Fr.) Quél.
	Pleurotus lignatilis (Pers.) P. Kumm.
	Stereum hirsutum (Wild.) Pers.
	Tricholoma inocybeoides A. Pearson
October 2017	*Termitomyces robustus* (Beeli) R. Heim
May and October 2017	*Ganoderma resinaceum* Boud.
	Lentinus squarrosulus Mont.
	Trametes ochracea (Pers.) Gilb. & Ryvarden
	Xylaria hypoxylon (L.) Grev.

a b c d e f

Figure 1. Representative pictures of mushrooms obtained from the site of study. (a) *Hydnellum peckii* (b) *Lentinus squarrosulus* (c) *Mycena inclinata* (d) *Termitomyces robustus* (e) *Trametes ochracea* (f) *Xylaria hypoxylon*.

3.2. Habitat and Substrate of Mushroom Samples

Mushrooms have a wide ecological range and can grow in both coniferous and broadleaf forests (Leonardi *et al*., 2017; Sevindik *et al*., 2018). While we obtained eleven of our mushrooms from ligneous habitat, the remaining five came from the terrestrial counterpart. This observation concurs with an earlier report (Buba *et al*., 2018). The number of mushroom species found on decaying ligneous substrates was in the order: 3 (18.75 %) each of mango and palm, 2 (12.5 %) each of kola nut and unidentified trunk logs and bamboo leaves 1 (6.25 %), whereas on terrestrial substrates, were soil debris 4 (25 %) and termite mound 1 (6.25 %) (Figure 2). Additionally, the mushrooms were

found to be habitat- and substrate-specific, as described elsewhere (Adeniyi *et al*., 2018a).

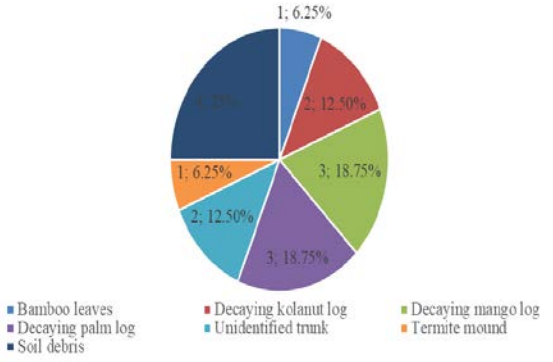

Figure 2. The occurrence of the mushroom species on growth substrates.

3.3. Antibacterial and Antifungal Screening

All the extracts screened had varied clearance zones against at least one of the eight bacteria, and the yeast except *X. hypoxylon*. Generally, the inhibitions of the extracts against the test isolates were in the order *T. ochracea* > *T. robustus* > *X. hypoxylon* > *L. squarrosulus* (Table 2). Among the isolates tested, *P. aeruginosa* (*L. squarrosulus* extract) had the highest inhibition (20 mm), whereas *S. aureus* (*T. ochraceus* extract) had the lowest (7.2 mm) (Table 2). Our observation tallies with Chowdhury *et al*. (2015) whose report ranged between 7.0 ± 0.2 and 30.0 ± 0.3 mm. The production of slime and capsule in microorganisms are responsible for variability in the potency of the extracts (Awala and Oyetayo, 2015; Murray *et al*., 2013).

Generally, not all inhibition zones observed in an *in-vitro* sensitivity test are considered sensitive (CLSI, 2018). According to Chowdhury *et al*. (2015), mushroom crude extracts are highly effective when the clearance diameter is greater than 10 mm. In the current investigation, *P. aeruginosa* and *C. albicans* were sensitive to *L. squarrosulus*; *E. coli*, *K. pneumoniae*, *S. typhimurium* and *C. albicans* to *T. robustus*; *E. coli*, *K. pneumoniae*, *B. subtilis*, *C. diphtheria*, *S. aureus* and *C. albicans* to *T. ochracea*; *E. coli* and *B. subtilis* to *X. hypoxylon* (Table 2). Generally, Gram-positive bacteria are more susceptible to different medicinal compounds than Gram-negative because of the porous peptidoglycan layer and single lipid bilayer in Gram-positive bacteria (Khatua *et al*., 2017). In contrast, the current study observed higher susceptibilities in Gram-negative to the different extracts screened (Table 2). Our finding agrees with Awala and Oyetayo (2015) who also reported low resistance in Gram-positive bacteria in the presence of *Trametes elegans* extract, suggesting that the antimicrobial activities of the extracts may not be cell wall-related.

Broad-spectrum antimicrobials have played an invaluable role in treating bacterial infections and saved lives in situations where early diagnosis and identification of infectious diseases' causative agents is not possible (Melander *et al*., 2018). This current study reveals the broad-spectrum activities of *T. robustus*, *T. ochracea* and *X. hypoxylon* extracts against bacteria and yeast screened (Table 2). A previous study (Sharma *et al*., 2015) reported the broad-spectrum nature of *Agaricus bisporus*. One strategy being highlighted in the fight against bacteria resistance is the development of narrow-spectrum antimicrobials that are either genus or species-specific (Melander *et al*., 2018). Our work reveals the species-specificity of *L. squarrosulus* extract and thus, can be a potential drug for *P. aeruginosa* infections. The range of inhibition by the standard drugs was between 18.2 and 26.0 mm (Table 2).

Table 2. Diameter of inhibition of crude extracts against test microorganisms.

Mushroom extract	Gram-negative bacteria					Gram-positive bacteria			Yeast
	EC (mm)	KP (mm)	PA (mm)	PM (mm)	ST (mm)	BS (mm)	CD (mm)	SA (mm)	CA (mm)
L. squarrosulus	0.0±0.0	0.0±0.0	20.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	14.1±0.1
T. robustus	10.2±0.2	12.0±0.1	0.0±0.0	0.0±0.0	14.0±0.1	0.0±0.0	0.0±0.0	0.0±0.0	19.2±0.1
T. ochracea	10.1±0.1	14.2±0.2	0.0±0.0	8.1±0.1	9.2±0.2	11.1±0.1	10.0±0.0	7.2±0.2	17.0±0.1
X. hypoxylon	15.2±0.1	0.0±0.0	0.0±0.0	0.0±0.0	19.0±0.0	14.0±0.3	0.0±0.0	0.0±0.0	0.0±0.0
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Standard drug	19.0±0.1	21.0±0.1	21.1±0.1	18.2±0.1	26.0±0.0	20.1±0.2	19.0±0.1	18.1±0.0	22.8±0.3

Legend**:** EC – *E. coli*; KP – *K. pneumoniae*; PA – *P. aeruginosa*; PM – *P. mirabilis*; ST – *S. typhimurium*; BS – *B. subtilis*; CD – *C. diphtheria*; SA – *S. aureus*; CA – *C. albicans*.

3.4. Effect of Different Extract Combinations on the Test Isolates

Combination therapy has been envisaged to be an effective strategy in treating complex infections (Xu *et al*., 2018) and are more superior compared to single drug dosage (Vakil and, Trappe, 2019). Generally, all the extract blends were resistant to 2 Gram-negative bacteria, *K. pneumoniae* and *S. typhimurium*, all the Gram-positive bacteria and the fungus (Table 3). It is possible that the effects of active ingredient which may be present in some of the extracts were concealed by other compounds in the mixture and thus suggests antagonism between the individual extracts in combination. Usually, drug antagonism is often undesirable but could be a useful selective factor for drug-resistant mutations (Chait *et al*., 2007). Furthermore, the extract composites were sensitive to at least one of *E. coli*, *P. aeruginosa* and *P. mirabilis* with different relationships. Except for combination *L. squarrosulus* and *X. hypoxylon* which was antagonistic, other active mixtures were indifferent to *E. coli*. Likewise, an indifferent relationship was observed against *P. aeruginosa* albeit synergistic in *T. ochracea* and *T. robustus* blend. Interestingly, the interactions between A, B and G against *P. mirabilis* were synergistic (Table 3). In drug production, synergistic interaction is preferable due to its effectiveness (Xu *et al*., 2018).

Table 3. Sensitivity pattern of the different crude extract combinations.

Extract combinations	Gram-negative					Gram-positive			Fungus
	EC (mm)	KP (mm)	PA (mm)	PM (mm)	ST (mm)	BS (mm)	CD (mm)	SA (mm)	CA (mm)
A	15.1±0.1	0.0±0.0	19.1±0.0	10.2±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
B	14.2±0.2	0.0±0.0	13.3±0.3	10.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
C	12.1±0.0	0.0±0.0	0.0±0.0	11.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
D	13.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
E	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
F	10.2±0.2	0.0±0.0	0.0±0.0	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
G	8.1±0.0	0.0±0.0	12.0±0.1	11.2±0.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
H	11.4±0.2	0.0±0.0	0.0±0.0	12.0±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
I	0.0±0.0	0.0±0.0	0.0±0.0	10.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
J	0.0±0.0	0.0±0.0	0.0±0.0	12.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Negative control	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Standard drug	19.0±0.1	21.0±0.1	21.1±0.1	18.2±0.1	26.0±0.0	20.1±0.2	19.0±0.1	18.1±0.0	22.8±0.3

Legend: EC – *E. coli*; KP – *K. pneumoniae*; PA – *P. aeruginosa*; PM – *P. mirabilis*; ST – *S. typhimurium*; BS – *B. subtilis*; CD – *C. diptheriae*; SA – *S. aureus*; CA – *C. albicans*

A – *T. ochracea* + *T. robustus*; B – *T. ochracea* + *L. squarrosulus*; C – *T. ochracea* + *X. hypoxylon*; D - *T. robustus* + *L. squarrosulus*; E - *T. robustus* + *X. hypoxylon*; F - *L. squarrosulus* + *X. hypoxylon*; G - *T. ochracea* + *T. robustus* + *L. squarrosulus*; H - *T. ochracea* + *T. robustus* + *X. hypoxylon*; I - *T. ochracea* + *L. squarrosulus* + *X. hypoxylon*; J - *T. ochracea* + *T. robustus* + *L. squarrosulus* + *X. hypoxylon*.

3.5. MIC of the Mushroom Extracts

In this investigation, MIC ranged between 2.09 and 16.75 mg/mL (Table 4). While the lowest value (2.09 mg/mL) was recorded in *E. coli* (*T. robustus*), *S. typhimurium* (*T. ochracea*) and *C. diphtheriae* (*T. ochracea*), the highest (16.75 mg/mL) was obtained in *C. albicans* for *L. squarrosulus* and *T. ochracea* extracts (Table 4). Our observation contradicts the previous report (Chowdhury *et al*., 2015) on low MIC for their fungal species investigated.

Table 4. MIC values (mg/mL) of the mushroom extracts against the test isolates.

Mushroom	MIC concentration (mg/mL)								
	EC	KP	PA	PM	ST	BS	CD	SA	CA
L. squarrosulus	ND	ND	8.38	ND	ND	ND	ND	ND	16.75
T. robustus	2.09	4.19	ND	ND	4.19	ND	ND	ND	8.38
T. ochracea	4.19	8.38	ND	4.19	2.09	4.19	2.09	8.38	16.75
Xylaria hypoxylon	4.19	ND	ND	ND	8.38	8.38	ND	ND	ND

Legend**:** EC – *E. coli*; KP – *K. pneumoniae*; PA – *P. aeruginosa*; PM – *P. mirabilis*; ST – *S. typhimurium*; BS – *B. subtilis*; CD – *C. diphtheriae*; SA – *S. aureus*; CA – *C. albicans*; ND – Not determined.

3.6. Chemical Components of the Mushroom Extracts

Mushrooms are rich in phytochemicals such as polyketides, steroids, terpenes, ceramides, glycoproteins, proteoglycans, polysaccharides and phenols (Chowdhury *et al*., 2015). Our findings frequently detected saponins, terpenoids and tannins in *L. squarrosulus*, *T. robustus* and *T. ochracea* extracts (Table 5). This finding tallies with Gbolagade and Fasidi (2005) and Anyanwu *et al*. (2016) who had similar observations for *Trametes elegans* (Spreng.) Fr. and *Pleurotus tuber-regium* (Fr.) Singer sclerotium. The absence of saponins, terpenoids and tannins in *X. hypoxylon* (Table 5) is contrary to the evidence of Jang *et al*. (2009) and Elias *et al*. (2018) that genus *Xylaria* contains a diversity of bioactive substances. Different ecological locations, age of mushroom, time of harvest and extraction protocols might account for the variance. Likewise, anthraquinones were not detected in *T. robustus* and *T. ochracea* mushroom extracts (Table 5). Earlier works (Gbolagade and Fasidi, 2005; Wandati *et al*., 2013) also noted the absence of anthraquinones compounds in mushroom samples.

Table 5. Phytochemical constituents of mushroom extracts.

Mushroom	Saponins	Tannins	Terpenoids	Anthraquinones
L. squarrosulus	+	+	+	ND
T. robustus	+	+	+	-
T. ochraceus	+	+	+	-
X. hypoxylon	-	-	-	ND

Legend: '+' = Present; '-'= Absent; 'ND' – Not determined

4. Conclusion

In this study, 16 wild mushrooms from ligneous and terrestrial habitats were collected from ENPOST farm, Ilesa, Southwest Nigeria and the antimicrobial potential of *L. squarrosulus*, *T. robustus*, *T. ochracea* and *X. hypoxylon* were investigated. The methanolic crude extracts of the mushrooms were active against at least one of the eight bacteria, and the yeast except for *X. hypoxylon*. Generally, the extracts were active against the test isolates: *T. ochracea* > *T. robustus* > *X. hypoxylon* > *L. squarrosulus*. Among the investigated isolates, *P. aeruginosa* exhibited the highest inhibition zone (20 mm) whereas *S. aureus* had the lowest (7.2 mm). Furthermore, all the extract combinations were resistant to *K. pneumoniae* and *S. typhimurium*, all the Gram-positive bacteria and the fungus, *C. albicans*. The MIC range of 2.09 and 16.75 mg ml^{-1} was equally obtained. Also, the extracts except *X. hypoxylon* contained saponins, terpenoids and tannins. Our study reveals the antimicrobial potential of *L. squarrosulus*, *T. robustus*, *T. ochracea* and *X. hypoxylon.* However, this study is limited by the application of thorough-put techniques such as gas chromatography – mass spectrometry and fourier-transform infrared spectrometry for determination of concise constituents of extracts and time-kill assay to assess the *in-vitro* reduction of test organisms after exposure to the extracts. Extensive screening of more native mushrooms for biomedical potential and the domestication of the therapeutic species is advocated. Therefore, we recommend further investigations on isolation, evaluation, and identification of key constituent(s) with antimicrobial prospects and mechanisms of actions.

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