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The Phytochemical and Antimicrobial Properties of the Extracts Obtained from *Trametes elegans* Collected from Osengere in Ibadan, Nigeria

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Abstract

The phytochemical and antimicrobial properties of the extracts of the wild macrofungus, *Trametes elegans* (Spreng: Fr.) Fr. (fam.: Polyporaceae) obtained from a farm in Osengere, Egbeda local government area of Ibadan, Oyo State was investigated. The identity of the macrofungus was confirmed by amplifying and sequencing the Internal Transcribed Spacer (ITS 4 and ITS 5) of its nuclear ribosomal DNA (nrDNA). Extracts from the powdered fruiting bodies of the macrofungus were obtained by soaking in methanol and acetone. Antimicrobial effect of the macrofungus extracts was performed on clinical and referenced microbial cultures using standard microbiological techniques. In addition, phytochemical analysis of the *Trametes* species extracts was assessed qualitatively and quantitatively. The level of relatedness of *Trametes* species collected in Osengere and the already existing gene sequences of *Trametes* species in NCBI GenBank was 98%. Its closest relative is *T. elegans*. Phytochemical analysis revealed the presence of saponin, tannins, flavonoid, steroid, terpenoid, and cardiac glycosides in all the extracts with values ranging from 6.726 to 23.682 mg/g. The extracts of *T. elegans* displayed varying antimicrobial activities with zones of inhibition ranging from 1.50 to 25.50mm. The methanol extract of the macrofungus exhibited a better antibacterial activity against *Bacillus cereus* NCIB 6344 (20.5 mm), while the acetone extract of the macrofungus exhibited a higher antifungal activity against *Aspergillus fumigates* (25.5 mm). In light of this finding, *T. elegans*, collected from Ibadan, Oyo State, Nigeria, could be considered as potential sources of natural antimicrobial and could be of a great importance for the treatment of infectious diseases caused by the test organisms.

Keywords: Macrofungus, Trametes elegans, antimicrobial, phytochemicals, extracts.

1. Introduction

The need to explore natural sources for novel bioactive agents has increased in the last three decades. Fungi are among the most creative groups of eukaryotic organisms capable of producing many novel natural products that are directly used as drugs or serve as structural backbone for synthetic modifications (Mitchell *et al*., 2008; Stadler and Keller, 2008).

Fungi have been reported to be the second most diverse organisms, with a diversity (up to 3 to 5 million species) postulated to exceed that of terrestrial plants by an order of magnitude (Dai, 2010; Blackwell, 2011). Only a fraction of all fungal species has been described so far (about 100,000), and those explored for the production of important pharmacological metabolites are even less. Yet, some of the most successful drugs and agrochemical fungicides on the market have been developed from fungal secondary metabolites (De Silva *et al*., 2013). These include antibiotics (penicillins, cephalosporins and fusidic acid), antifungal agents (griseofulvin, strobilurins and echinocandins), cholesterol-lowering agents such as statin derivatives (mevinolin, lovastatin and simvastatin), and immunosuppressive drugs (cyclosporin) (Kozlovskii *et al*., 2013). Hence, the percentage of economically valuable fungal metabolites is still at large small.

Mushrooms are macrofungi with distinctive fruiting body, which can be hypogeous or epigeous, large enough to be seen with the naked eye and to be picked by hand (Chang and Miles, 1992). They are traditionally believed to be remedies for many diseases (De Silva *et al*., 2012) and are known to be prolific producers of bioactive metabolites (Wasser, 2011). Mushrooms have been used for centuries in Asia as popular medicines to prevent or treat different diseases (Xu *et al*., 2011; De Silva *et al*., 2012). The first record of the use of mushroom as

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hallucinogenic agent was credited to the Yoruba tribe of Nigeria in Africa (Griensven, 2009).

Macrofungi of the genus *Trametes* are polyporoid white rot fungi widely distributed in various biotopes and have been the subject of many physiological and biochemical studies (Iimura *et al*., 2002; Koroleva *et al*., 2002). They are capable of expressing a biological and a metabolic diversity as a result of their ability to utilize several substrates, decomposing dead organic matters, and, thus, to explore different habitat for colonization (Thatoi and Singdevsachan, 2014). Nigeria has unique climatic conditions and serves as home to diverse species of mushrooms. Yet, there is a dearth of information about these mushrooms and their medicinal potentials. According to Oyetayo (2011), factors such as mycophobia, trade secret by local herbalist, seasonal nature of mushrooms, and social stigma contribute to the underutilization of mushrooms in Nigeria.

Several authors have reported on the antimicrobial activities of different mushrooms, and have associated these activities to the presence of varieties of secondary metabolites such as peptides, tannins, terpenoids, phenols, and flavonoids (Alves *et al*., 2013; Lavanya and Subhashini, 2013; Avcı *et al*., 2014). The present study, therefore, aims to identify a *Trametes* species collected from Osengere, Egbeda Local government area of Ibadan, Oyo, Nigeria, using molecular tools, and to assess the phytochemical and antimicrobial properties of the macrofungus.

2. Materials and Methods

2.1. Collection of Macrofungus

Fresh fruit bodies of macrofungus suspected to be *Trametes* species were collected from rotten cocoa woods from farms in Osengere, Egbeda Local government area of Ibadan, Oyo State (Latitude:7.39814N, Longitude: 4.00051E) in August, 2013. The fruiting bodies were kept dry by wrapping in tissue paper and kept in a polythene paper containing silica gel. The polythene bags containing the samples were well labelled for easy identification and taken to the Department of Microbiology Laboratory of the Federal University of Technology, Akure for further morphological examination.

2.2. Molecular Identification of Macrofungus

2.2.1. Extraction of DNA of Macrofungus

Standard DNA isolation methods employing CTAB lysis buffer was used (Zolan and Pukkila, 1986). For DNA extraction, dried portions of the macrofungus fruiting bodies (2g) were ground with a mortar and pestle. The grounded materials were transferred into well labelled microtubes. Prewarmed (60^{o}C) extraction buffer (CTAB) was added and the tubes were incubated at 65^{o}C for 30 to 60 minutes. Equal volume of chloroform and alcohol (24:1) was added and mixed by inverting tubes for 15 minutes. The tubes were centrifuged for 10 minutes at 10,000g (13000rpm). The process was repeated but the time of mixing was 3 minutes and time of centrifugation was 5 minutes at the same speed as above. Upper aqueous layers were removed into clean tubes and 40µl 2M sodium acetate (NaAc) was added followed by 260µl of cold isopropanol. This was gently mixed by inverting tubes. The tubes were incubated at -20^{o}C overnight in a freezer (Haier HTF319 Freezer, 99405-0811, China). The second day, the mixture was centrifuged at 10,000g (13000rpm) for 10 minutes. The supernatant was discarded and pellets rinsed with 70% alcohol and mixed for sometimes. This procedure was repeated three times. After discarding the supernatant, the sample was dried in a dryer for 20 minutes at room temperature. Pellets were resuspended in 30µl Tris EDTA (TE) buffer (10 mM Tris/HCl + 1 mM EDTA pH 8). DNA concentration and quality was checked by observing the band on an ethidium-stained agarose gel (0.7%) using 0.2µl of each sample.

2.2.2. PCR Amplification of the ITS Region of macrofungus

The entire region of the rDNA of the macrofungus was amplified using the primers ITS4 and ITS5. The reaction mix was made up to a total volume of 25µl, composed of 23µl of *Taq*polymerase "Ready to Go" (Pharmacia, Sweden) with 0.2 µl of each primer (100 pM) and 2µl of DNA solution. The amplification reactions were performed in a DNA Thermal Cycler (GenAmp OPCR System 2400; Perkin–Elmer, USA) and programed as follows: 1st cycle of 5 min at 95°C (initial denaturation) followed by 30 cycles of 45 sec at 95°C (denaturation), 30 sec at 50°C (annealing), 1 min at 72°C (extension), and 1 cycle of 10 min at 72°C (final extension). The amplification products were purified using a PCR Purification Kit (USA) and electrophoresed on ethidium stained agarose gel (0.7%) to check the purity. DNA sequence was performed using the same primer pair used in the PCR reactions (ITS4 and ITS5) in an Applied Biosystem DNA Analyser (USA).

2.2.3. Sequencing of DNA and Alignment of Sequence

Alignments were performed with the Clustal W package (Thompson *et al*., 1997). The aligned sequences were corrected manually, focusing on gap positions. DNA sequence data were analysed to provide pairwise percentage sequence divergence. The data obtained from the sequence alignment were used to plot a tree diagram (MEGA 4 Software). Extraction, amplification and sequencing of rDNA were carried out at the Key Laboratory of Mycology and Lichenology, Institute of Microbiology, Beijing.

2.3. Collection of Test Organisms

Typed cultures (*Escherichia coli* ATCC 23718, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 29853, *Bacillus cereus* NCIB 6344, *Staphylococcus aureus* NCIB 950, *Salmonella typhi* ATCC 33458) were collected from the Medical Microbiology Laboratory of the University College Hospital (UCH) Ibadan, Oyo state. Clinical isolates of human origin (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Candida albicans* and Methicillin Resistant *Staphylococcus aureus* (MRSA) were collected from Obafemi Awolowo University

Teaching Hospital (OAUTH), Ile-Ife, Osun state. *Aspergillus fumigatus*, *Aspergillus niger* were obtained from the culture collection of the Department of Microbiology, FUTA. The isolates were tested for viability by resuscitating them in buffered peptone water after which they were subcultured on nutrient agar medium for bacteria and potato dextrose agar for fungi and incubated at 37°C for 24 hours and 27°C for 48-72 hours, respectively. The organisms were then stored at 4°C until needed.

2.4. Preparation of Mushrooms Extracts

The collected fresh mushrooms were brush-cleaned of attached soil and humus and then air-dried in an oven at 40°C for 48 hours. They were then cut into bits, pulverised by an electrical mill, and stored in an air-tight container for further use. The powdered mushroom sample (100g) was extracted by maceration in 2000ml of 95% acetone and methanol separately in an Erlenmeyer flask. The flasks were covered with aluminium foil and allowed to stand for 3 days for extraction with occasional stirring. The extracts were then filtered through Whatman filter paper (0.45µm) using vacuum pump. The filtrates were evaporated to dryness at 50°C in a rotary evaporator (RE-52A; Union Laboratory, England) with 90 rpm under reduced pressure. The obtained concentrated extracts were stored in dark at 4°C until further analysis. The percentage yield of extracts was calculated based on dry weight as follows:

$$\text{Yield } (\%) = \frac{\text{W1 x 100}}{\text{W2}}$$

where,

W1= weight of extract after solvent evaporation

W2 = Weight of the grounded mushroom powder

2.5. Phytochemical screening of Trametes species extracts

A qualitative and quantitative phytochemical analysis of the crude mushroom extracts was performed through standard protocols described by Odebiyi and Sofowora (1978), Trease and Evans (2005), and Harborne (2005).

2.5.1. Qualitative phytochemical analysis of Trametes species extracts

2.5.1.1. Test for alkaloids

The extracts (0.5g each) were stirred with 5ml of 1% aqueous hydrochloric acid (HCl) for two minutes on a steam water bath. The mixtures were filtered and few drops of Dragendorff's reagent were added. The samples were then observed for color changes or turbidity to draw inference.

2.5.1.2. Test for saponins

The persistent frothing test for saponin described by Odebiyi and Sofowora (1978) was used. Distilled water (30ml) was added to 1g of each of the mushroom extracts. The mixture was vigorously shaken and heated on a steam water bath. The samples were observed for the formation of froth to draw inference.

2.5.1.3. Test for phlobatannins

The mushroom extracts (0.2g) were dissolved in 10ml of distilled water each and filtered. The filtrates were boiled with 2% HCl solution and observed for deposition of red precipitate which indicates the presence of phlobatannin.

2.5.1.4. Test for tannins

The method of Trease and Evans (2005) was adopted. Each sample (0.5g) was dissolved in 5ml of distilled water, then, boiled gently and cooled. One ml of each solution was dispensed in a test tube and 3 drops of 0.1% ferric chloride solution were added and observed for brownish green or blue black coloration which indicates the presence of tannins.

2.5.1.5. Test for terpenoids

The Salkowski test was used. Five ml of each extract weres mixed in 2 ml of chloroform, and 3 ml concentrated sulphuric acid (H_2SO_4) were carefully added to form a layer. Each solution was then observed for reddish brown coloration which confirms the presence of terpenoids.

2.5.1.6. Test for steroids

Acetic anhydride (2ml) was added to 0.5g of each extract and filtered. Sulphuric acid (2ml) was added to the filtrate and observed for color change from violet to blue or green, which indicates the presence of steroid.

2.5.1.7. Test for flavonoids

Diluted ammonia solution (5ml) was added to portions of aqueous filtrate of each mushroom extracts. This was then followed by the addition of a concentrated sulphuric acid. The solutions were observed for yellow coloration that disappears on standing to confirm the presence of flavonoids.

2.5.1.8. Test for anthraquinones

Borntrager's test was used for the detection of anthraquinone. The extract (0.5g) was shaken with 10ml of benzene, filtered and 5ml of 10% ammonia solution added to the filtrate. The mixture was shaken and observed for the presences of pink red or violet color in the ammonia layer which indicates the presence of free anthraquinones.

2.5.1.9. Test for cardiac glycosides

The following was carried out to test the cardiac glycosides of each extract.

2.5.1.9.1. Legal's test

Each extract was dissolved in pyridine and a few drops of 2% sodium nitroprusside with a few drops of 20% NaOH were added. They were observed for a deep red coloration which fades to a brownish yellow indicating the presence of cardenolides.

2.5.1.9.2. Salkowski's test

Each extract was mixed with 20ml of chloroform and filtered. This was followed by the addition of 3ml of conc. H_2SO_4 to the filtrate to form a layer. A reddish brown color at the interface was observed which indicates the presence of steroidal ring.

2.5.1.9.3. Keller- Killiani's test

Each of the extracts (0.5g) was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1ml of conc. H_2SO_4. It was observed for a brown coloration at the interface indicating the presence of a deoxy sugar which is a characteristic of cardenolides. It was also observed for violet ring which may appear below the brown ring while in the acetic acid layer. The presence of a green ring formed just above the brown ring which can gradually spread throughout this layer, also indicates the presence of cardiac glycosides.

2.5.2. Quantitative phytochemical screening of Trametes species extracts

2.5.2.1. Determination of Tannins

Tannins determination was done according to the method of Association of Official Analytical Chemists [AOAC] (1990), with some modifications. The sample (0.20 g) was mixed with 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80°C for 1 hour to ensure uniform mixing. The mixture was filtered into a 100ml volumetric flask, followed by the addition of 20ml of distilled water, 2.5 ml of Folin-Denis reagent and 10 ml of 17% aq. Na_2CO_3 (Sodium carbonate) and was thoroughly mixed. The mixture was made up to 100 ml with distilled water, mixed and allowed to stand for 20 min. The absorbance of the tannic acid standard solutions as well as sample was measured after color development at 760 nm using the spectrophotometer (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215). Results were expressed as mg/g of tannic acid equivalent using the calibration curve: $Y = 0.0593x - 0.0485$, $R = 0.9826$, where x is the absorbance and Y is the tannic acid equivalent.

2.5.2.2. Determination of saponins

Quantitative determination of saponins was done using the method of Obadoni and Ochuko (2001). The powdered sample (20 g) was added to 100 ml of 20% aqueous ethanol and kept in a shaker for 30 min. The samples were heated over a water bath for 4 hours at 55°C. The mixture was then filtered and the residue re-extracted with another 200 ml of 20% aqueous ethanol. The combined extracts were reduced to approximately 40 ml over the water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel and extracted twice with 20 ml diethyl ether. The ether layer was discarded while the aqueous layer was retained and to which 60 ml n-butanol was added. The n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated on a water bath. After evaporation, the samples were dried in the oven at 40°C to a constant weight.

2.5.2.3. Determination of alkaloids

Alkaloids were quantitatively determined according to the method of Harborne (2005). Two hundred milliliters of 10% acetic acid in ethanol were added to 5g powdered extract, covered and allowed to stand for 4 hours. The filtrate was then concentrated on a water bath to one-fourth of its original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was completed and the whole solution was allowed to settle. The collected precipitates were washed with dilute ammonium hydroxide and then filtered. The residue was dried and weighed. The alkaloid content was determined using the formula:

$$\text{Alkaloid (\%)} = \frac{\text{final weight of sample}}{\text{initial weight of extracts}} \text{ x 100}$$

2.5.2.4. Determination of steroid

The steroid content of the plant sample was determined using the method described by Trease and Evans (2005). A portion of 2ml was taken from a solution of 2.5g of powdered plant material prepared in 50mL of distilled water after vigorous shaking for 1 hour. The extract solution was washed with 3mL of 0.1M NaOH (pH 9) and later mixed with 2mL of chloroform and 3mL 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 (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 420 nm.

2.5.2.5. Determination of cardiac glycosides

Cardiac glycoside content in the samples was evaluated using Buljet's reagent as described by El-Olemy *et al.* (1994). The samples were then purified using lead acetate and disodium hydrogen phosphate (Na_2HPO_4) solution before the addition of freshly prepared Buljet's reagent (containing 95ml aqueous picric acid + 5ml 10% aqueous NaOH). The difference between the intensity of colors of the experimental and blank (distilled water and Buljet's reagent) samples gives the absorbance and is proportional to the concentration of the glycosides.

2.6. Determination of antimicrobial activity of Trametes species extracts

Antimicrobial activity of extracts was determined by the agar well diffusion method described by Schinor *et al.* (2007). Stock cultures were maintained at 4°C on nutrient agar slope. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrient Broth (NB) for bacteria and Sabouraud Dextrose Broth (SDB) for fungi and were incubated without agitation for 24 hours at 37°C and 25°C, respectively. To 5ml of NB and SDB, 0.2 ml of culture was inoculated and incubated till it reached the turbidity equal to that of the standard 0.5 McFarland solution at 600nm which is equivalent to 10^6– 10^8 CFU/ml.

Suspensions of fungal spores were prepared from fresh mature (5 days) cultures that grew at 26 ± 1ºC on a Sabouraud dextrose agar. Spores were rinsed with sterile distilled water. The suspensions were then adjusted to 10^6 spores per ml by microscopic enumeration with a cell counting hematocytometer. Molten Mueller Hinton agar (20ml) cooled to 45°C was poured into sterilized petri dishes and left to solidify. An aliquot of culture (100µl) was evenly spread on the surface of the solidified Mueller Hinton agar plates. Wells of 8 mm were bored in the agar

with sterile cork borers (6 and 7mm). The crude extracts (100µl) were dissolved in 30% dimethylsulfoxide (DMSO) to a concentration of 50 mg/ml and filtered through 0.22µm membrane filter and then introduced into each wells with the aid of a micropipette.

Clotrimazole was used as a positive control for fungi and reference antibiotic disc (gentamicin (10µg), nalidixic acid (30µg), nitrofurantoin (200µg), cotrimoxazole (25µg), amoxicillin (25µg), tetracycline (25µg), augmentin® (30µg), and ofloxacin (5µg) for bacteria. DMSO (30%) was used as negative control. The plates were allowed to stand for one hour at room temperature (26 ± 2ºC) to allow a proper diffusion of the extracts. The plates were then incubated at 37 ºC for 24 hour for bacteria while the fungi were incubated at 26 ± 1ºC for 48 to 72 hour. Inhibition zones were measured with a ruler in triplicates (three plates per indicator organism).

2.6.1. Determination of minimum inhibitory concentration

The agar diffusion method, described in section 2.6, was used to screen the antimicrobial effect of the different concentrations of extracts (6.25 to 50mg/ml). The MIC was determined by establishing a visible growth of microorganisms. The boundary dilution without any visible growth was defined as the MIC for the tested microorganism at the given concentration. DMSO solution (30%) was used as a negative control. The tests were performed in triplicates.

2.7. Statistical analysis

All experiments were carried out in triplicates. Data obtained were analyzed by One way analysis of variance (ANOVA) and means were compared by New Duncan's Multiple Range Test (SPSS version 16). Differences were considered significant at $p<0.05$.

3. Results

The Internal Transcribed Spacer (ITS) region of the macrofungus discriminated between the sequence of *Trametes* species collected from Osengere, Oyo State and the existing genes of *Trametes* species obtained from National Center for Biotechnology Information (NCBI) GenBank. *Trametes elegans* (Spreng: Fr.) Fr. (fam.: Polyporaceae) (Plate 1) with the ascession number (JN048766.1) was seen to be the closest relative to the *Trametes* species collected from Osengere with a percentage relatedness of 98 (Table 1).

Methanol produced a higher yield than acetone when used for extraction (Table 2). Results from the phytochemical analysis revealed the presence of saponins, tannins, steroids, terpenoids, flavonoids and cardiac glycosides in all the *Trametes* species extracts, while anthraquinones, alkaloids and phlobatannins were absent (Table 3).

Plate 1. A- Fruiting body of *Trametes* species collected from rotten woods forest in Osengere, Egbeda Local government area of Ibadan, Oyo State

Table 1.Genomic identification based on nrDNA Internal Transcribed Spacer (ITS) sequence of wild macrofungus collected from Ibadan, Nigeria.

Sample	Source	Tentative phenotypic identity	Closest relative from NCBI GeneBank	NCBIAccession numberOf closest relative	% identity
1	Ibadan, Oyo State	*Trametes* sp.	*Trametes elegans*	JN0 48766.1	98

Keys: NCBI-: National Center for Biotechnology Information**; nrDNA-:** Nuclear Ribosomal Deoxyribonucliec Acid

Table 2. Yield of *Trametes* species extracts

Mushroom	Solvent	Total yield (mg/g)
Trametes elegans	Methanol	3.1
	Acetone	0.9

Table 3. Qualitative phytochemical screening of *Trametes elegans* extracts

Phytochemical	Extracts	
	TEA	TEM
Saponin	+	+
Tannin	+	+
Steriod	+	+
Alkaloid	-	-
Terpenoid	+	+
Flavonoids	+	+
Anthraquinone	-	-
Phlobatannin	-	-
Cardic Glycoside		
1. Legal Test	+	+
2. Keller Killiani Test	+	+
3. Salkwoski Test	+	+

Keys: TEA: Acetone extract of *Trametes elegans***; TEM:** Methanol extract of *Trametes elegans***; +:** Positive**; - :** Negative.

The values of the various phytochemicals present in the extracts ranged from 6.726 to 23.682 mg/g (Table 4). Table 4 also shows that the methanol extract of the macrofungus has the highest amount of cardiac glycosides (23.682mg/g), flavonoids (7.290mg/g), saponins (18.000mg/g) and tannins (11.943mg/g), while the acetone extract of the macrofungus has the highest amount of steroids (14.546mg/g) and terpenoids (22.285mg/g).

Table 4. Quantitative phytochemical components of *Trametes elegans* extracts

Phytochemical	Amount (mg/g)	
	TEA	TEM
Tannin	9.665 ± 0.004^{b}	11.943 ± 0.0199^{c}
Saponin	16.163 ± 0.107^{d}	18.000 ± 0.105^{e}
Flavonoid	6.726 ± 0.004^{a}	7.290 ± 0.023^{a}
Steriod	14.546 ± 0.008^{c}	14.290 ± 0.031^{d}
Terpenoid	22.285 ± 0.008^{f}	10.545 ± 0.016^{b}
Glycoside	20.583 ± 0.056^{e}	23.682 ± 0.018^{f}

Keys: TEA: Acetone extract of *Trametes elegans***; TEM:** Methanol extract of *Trametes elegans***.**

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a column are significantly different at ($p < 0.05$).

Table 5 shows the antimicrobial activities of the extracts of *T. elegans* (50mg/ml) and commercial drugs against the test organisms. For the clinical bacterial isolates, the extracts could only inhibit *E. coli*, *P. aeruginosa* and *E. faecalis*. Methanol extract of *T. elegans* was able to inhibit all the referenced bacteria except for *P. aeruginosa* ATCC 29853, while the acetone extract was found to inhibit *E. coli* ATCC 23718 and *S. typhi* ATCC 33458. The methanol extract had no inhibitory effect on the test fungi, while the acetone extract inhibited all the test fungi except for *A. flavus*. Of the commercial antibiotics used, ofloxacin (5µg) gave the highest antibacterial activity, which was closely followed by nalidixic acid (30µg). The commercial antifungal drug, clotrimazole (1mg/ml) also inhibited all the test fungi. The activities of the commercial drugs when compared to those of the extracts were slightly higher and significantly different ($P< 0.05$). The Minimum Inhibitory Concentration (MIC) of the extracts ranged from 12.5 to 50mg/ml, while with the referenced bacteria exhibiting lesser MIC values (Table 6).

Table 5: Antimicrobial activities of extracts of *Trametes elegans* and commercial drugs against test organisms

Test organisms	Zones of inhibition (mm)						
	TEM (50mg/ml)	TEA (50mg/ml)	Gentamicin (10µg)	Nalidixic acid (30µg)	Nitrofurantoin (200µg)	Ofloxacin (5µg)	Clotrimazole (1mg/ml)
*Escherichia coli**	6.33 ± 0.17 ^{b}	6.00± 0.00 ^{b}	10.00 ± 0.58 ^{c}	12.33 ± 1.20 ^{d}	0.00 ± 0.00 ^{a}	21.33 ± 0.88 ^{e}	ND
*Pseudomonas aeruginosa**	9.50 ± 0 .29 ^{b}	4.17 ± 0.17 ^{a}	12.00 ± 0.29 ^{c}	15.83 ± 0.17 ^{d}	16.17 ± 0.44 ^{d}	20.00 ± 0.00 ^{e}	ND
*Bacillus cereus**	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	12.33 ± 1.20 ^{b}	18.67 ± 1.76 ^{d}	17.00 ± 0.58 ^{cd}	14.33 ± 0.88 ^{bc}	ND
*Staphylococcus aureus**	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	12.00 ± 2.00 ^{b}	16.00 ± 2.00 ^{c}	11.00 ± 1.00 ^{b}	20.67 ± 2.08 ^{d}	ND
MRSA*	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	11.33 ± 0.88 ^{b}	19.00 ± 0.58 ^{c}	11.33 ± 0.88 ^{b}	25.00 ± 1.16 ^{d}	ND
*Salmonella typhi**	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	12.33 ± 1.20 ^{b}	12.00 ± 0.58 ^{b}	11.33 ± 0.88 ^{b}	11.00 ± 0.58 ^{b}	ND
*Enterococcus faecalis**	1.50 ± 0.00 ^{a}	4.17 ± 0.17 ^{b}	11.33 ± 01.53 ^{c}	20.33 ± 0.58 ^{e}	15.33 ± 0.67 ^{d}	19.00 ± 1.53 ^{e}	ND
Escherichia coli ATCC 23718	6.00 ± 0.00 ^{b}	10.17 ± 0.17 ^{c}	10.33 ± 0.33 ^{c}	13.00 ± 0.58 ^{d}	0.00 ± 0.00 ^{a}	26.50 ± 0.29 ^{e}	ND
Escherichia coli ATCC 35218	7.33 ± 0.17 ^{b}	0.00 ± 0.00 ^{a}	15.00 ± 0.58 ^{c}	20.00 ± 0.58 ^{d}	24.00 ± 0.58 ^{e}	32.33 ± 0.88 ^{f}	ND
Pseudomonas aeruginosa ATCC 29853	0.00 ± 0.00 ^{a}	0.00 ± 0.00^{a}	11.00 ± 0.58 ^{b}	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	21.33 ± 0.88 ^{c}	ND
Bacillus cereus NCIB 6344	20.50 ± 0.29 ^{c}	0.00 ± 0.00 ^{a}	12.67 ± 0.88 ^{b}	14.33 ± 2.19 ^{b}	0.00 ± 0.00 ^{a}	25.50 ± 0.29 ^{d}	ND
Staphylococcus aureus NCIB 950	6.33 ± 0.17 ^{b}	0.00 ± 0.00 ^{a}	12.33 ± 1.20 ^{c}	11.33 ± 0.88 ^{c}	0.00 ± 0.00 ^{a}	14.83 ± 0.60 ^{d}	ND
Salmonella typhi ATCC 33458	12.17 ± 0.17 ^{a}	16.17 ± 0.17 ^{b}	12.67 ± 0.88 ^{a}	21.33 ± 0.88 ^{c}	21.00 ± 0.58 ^{c}	24.50 ± 0.29 ^{d}	ND
*Candida albicans**	0.00 ± 0.00 ^{a}	17.17 ± 0.17 ^{b}	ND	ND	ND	ND	15.35 ± 0.05 ^{c}
Aspergillus flavus	0.00 ± 0.00 ^{a}	0.00 ± 0.00 ^{a}	ND	ND	ND	ND	23.98 ± 0.017 ^{b}
Aspergillus fumigatus	0.00 ± 0.00 ^{a}	25.50 ± 0.29 ^{b}	ND	ND	ND	ND	32.33 ± 1.45 ^{c}

Keys: **TEA:** Acetone extract of *Trametes elegans*; **TEM**: Methanol extract of *Trametes elegans;* **MRSA**: Methicillin resistant *Staphylococcus aureus*; **ATCC**: American Type Culture Collection; **NCIB**: National Collection for Industrial Bacteria; *****: Clinical isolate; **ND**: Not determined.

Table 6. Minimum inhibitory concentration (mg/ml) of methanol and acetone extracts of *Trametes elegans* against test organisms.

Test Organism	TEM	TEA
Escherichia coli *	25	25
Pseudomonas aeruginosa *	50	25
*Enterococcus faecalis**	50	25
Escherichia coli ATCC 23718	50	12.5
Escherichia coli ATCC 35218	25	ND
Bacillus cereus NCIB 6344	12.5	ND
Staphylococcus aureus NCIB 950	12.5	ND
Salmonella typhi ATCC 33458	12.5	12.5
Candida albicans *	ND	12.5

Each value is mean of triplicate results.

Keys: **TEA:** Acetone extract of *Trametes elegans*; **TEM**: Methanol extract of *Trametes elegans*; *: Clinical strain; **ATCC**: American Type Culture Collection; **NCIB**: National Collection for Industrial Bacteria; **ND**: Not determined.

4. Discussion

Mushrooms are economically important since they serve as food, medicine, biocontrol agents, and a source of bioactive compounds used in the pharmaceutical and many other industries (Duarte *et al.*, 2006). Medicinal mushrooms have shown therapeutic benefits, primarily because they contain a number of biologically active compounds (Lee and Hong, 2011). The phytochemical properties and the antimicrobial potential of extracts of *T. elegans* indigenous to Nigeria were assessed in this study.

Species constituting the genus *Trametes* are similar in morphology; hence, the identification and separation of these species based on tradition taxonomy are difficult (Zhang *et al*., 2006). DNA sequences have been used in recent years to resolve the taxonomic problems in *Trametes* and in the related genera (Miettinen and Larsson, 2010; Cui *et al*., 2011). ITS sequences of the nuclear ribosomal DNA served as a useful molecular marker in distinguishing the species with similar morphological characteristics (Zhang *et al*., 2006; Cui *et al*., 2011).

In the present study, analysis of the ITS region of the nuclear rDNA revealed the genetic difference between the gene sequence of *Trametes* species collected from forest in Osengere, Ibadan Nigeria, and *Trametes* species sequences obtained from NCBI GenBank. The genes in DNA molecule are known to carry information that determines the characteristics of an organism. One of the implications in the difference between the genetic make-up of the indigenous *Trametes* species collected from Nigeria and the genes of their close relatives is that the type(s) and effectiveness of bioactives they produce may differ.

The differences in the ecological zones where these fungi exist and the influence of time might account for the difference in the gene sequence of *Trametes* species from Nigeria and its counterpart from other parts of the world. Wu *et al*. (2013), in their report, described the geographic distance as the dominant factor driving variation in fungal diversity at a regional scale (1000-4000 km), while environmental factors (total potassium and total nitrogen) account for the variation in fungal diversity at a local scale (<1000 km).

Results from the extraction process showed methanol giving a better yield than acetone. The high percentage yield of the methanol extract in this study may be due to the ability of the solvent to dissolve endogenous compounds (Anokwuru *et al*., 2011). Campos *et al*. (2002) in their findings also reported polar solvents to be more effective in extracting organic and inorganic materials from plants. Factors, such as the chemical nature of the compounds (simple and complex compounds), the extraction method employed, the extraction solvent, the extraction time and conditions such as temperature and pH of solvent, and the presence of interfering substances, can influence the extraction process (Brahmi *et al*., 2012). According to López *et al*. (2011), the nature of the solvent and the chemical properties of the sample are the two most important factors when extraction is subjected to similar conditions of time and temperature. This makes the processing efficiency quantitatively related to extraction yield (De Campos *et al*., 2008).

Results from the qualitative phytochemical analysis revealed the presence of saponins, tannins, steroids, terpenoids, flavonoids, and cardiac glycosides in all the *Trametes* species extracts, while alkaloids, anthraquinones and phlobatannins were absent. These phytochemicals are known to be biologically active, and serve as a defence mechanism for plants against predation by many microorganisms, insects and other herbivores (Bonjar *et al.*, 2004). This suggests that the mushroom can be used in the treatment of infectious diseases. For instance, Lim *et al*. (2006) reported on the antimicrobial activity of tannin extracted from *Rhizophora apiculata* bark. Saponins have been shown to demonstrate antimicrobial properties particularly against fungi, bacteria and protozoa (Sahelian, 2014). Taleb-Contini *et al*. (2003) reported the antibacterial activity of steroids isolated from *Chromolaena* species against *Streptococcus mutans* and *Streptococcus sobrinus* strains. Neumann *et al*. (2004) also confirmed the antiviral property of steroids.

Terpenoids have been reported to have anticarcinogenic (e.g. perilla alcohol), antimalarial (e.g., artemisinin), anti-ulcer, hepaticidal, antimicrobial or diuretic (e.g., glycyrrhizin) effects (Dudareva *et al*., 2004). Extracts of various medicinal plants containing phenolics and flavonoids have been reported to possess antimicrobial properties (Rahman and Moon, 2007; Ayaz *et al*., 2008). The variation observed in the amount of phytochemicals present could be as a result of the differences in the extraction capacity of the solvents used and the differences in the solubility of the different phytochemicals.

The extracts of *T. elegans* used in the present study displayed varying antimicrobial activities. This might be a result of a number of factors, as studies suggest that the antimicrobial activities of all mushroom extracts are changeable, depending upon the test organisms, nature of environment and media in which the test organism grows, genetic nature of the mushroom species, solvent used for extraction, and differences in physical and biochemical nature of the antimicrobial components of the mushroom extracts (Iwalokun *et al*., 2007; Ramesh *et al*., 2010). The differences in the antimicrobial activities of different species of mushrooms have been mainly attributed to the differences in the antimicrobial components found in them (Kosanic *et al*., 2013). Mushrooms require antibacterial and antifungal compounds in order to survive in their natural habitat (Lindequist *et al*., 2005). These attributes might make them a rich sources of natural antibiotics.

The variation observed between the Gram negative and Gram positive bacteria might be due to differences in their cell wall. Gram-negative bacteria have generally been reported to be more resistant to antimicrobials than Gram-positive bacteria. The outer membrane of Gram-negative bacteria plays an important role related to resistance to many antibiotics that are highly effective against Gram-positive bacteria, e.g., macrolides, novobiocin, rifamycin, lincomycin, clindamycin and fusidic acid (Sperandio *et al*., 2013). Gram-positive bacteria possess a porous layer of peptidoglycan and a single lipid bilayer, while Gram-negative bacteria have a double lipid bilayer sandwiching the peptidoglycan layer plus an outer layer of lipopolysaccharide, which results in a low degree of permeability for lipophilic small molecules (Sharma *et al*., 2011).

Results from the present study are, however, in contrast with the findings of several authors, who have reported the higher susceptibility of Gram positive bacteria than Gram negative bacteria. This implies that the antimicrobial activities of the extract of *T. elegans*, might not be cell wall related. The observed result in the present study is in line with the findings of Rakholiya *et al*. (2013) that observed higher susceptibility of Gram negative bacteria while studying the antimicrobial activity of decoction extracts of residual parts (seed and peels) of *Mangifera indica* L. var. Kesar against pathogenic and food spoilage microorganism.

The observed variation in the antibacterial activities of extracts of *T. elegans* to organisms with the same Gram reaction might be connected with their ability to produce capsule and slime. For instance, *E. coli*, *P. aeruginosa*

and *S. typhi* displayed different zones of inhibition. Being gram negative, the cell wall compositions are similar and the variation in their susceptibility might be capsular related. Another reason for the variation observed in organism with the same Gram reaction may be due to the presence of resistance factors like plasmids, transposons and insertion sequence (Clewell and Dunny, 2002; Nikaido, 2009). Plasmids are most commonly found in Gram negative bacteria and they often provide a selective advantage: many confer resistance to one or more antibiotics (Murray *et al*., 2013).

It was also observed that the clinical bacterial isolates and referenced bacterial cultures showed a marked difference in the zone of inhibition. The clinical bacterial isolates were found to be more resistant to the extracts than the typed bacterial cultures in most cases except for *P. aeruginosa* in which the referenced culture was more resistant than the clinical isolate. The resistance of the clinical isolates might be connected to the indiscriminate exposure of the clinical isolates to various antibiotics. Several clinical isolates have developed effective ways to deal with antibiotics through acquisition of resistant gene, production of enzymes such as the β-lactamases, changes in outer membrane porins that block the entry of the drug; and active pumping of the drug out of the cell using complex efflux pumps (Taiwo, 2011).

In contrast with the general trends that have reported a higher sensitivity of bacteria to antimicrobials than fungi, it was observed, in the present study, that the acetone extract of *T. elegans* exhibited higher antifungal than antibacterial activities. An earlier report by Pepeljnjak *et al*. (2005) also showed that fungi were more susceptible than bacteria while studying the antimicrobial activity of juniper berry essential oil. There has been an increase in the level of resistance of fungi to antifungal drug; hence, there is limited number of drugs available for the treatment of mycotic infections. Results from the present study, however, show that extracts of *T. elegans* may be an excellent source of antifungal drugs.

Although a number of natural/synthetic antimicrobial agents have been isolated/developed to control pathogenic microorganisms effectively, global antimicrobial resistance is still an increasing public health problem. Therefore, novel antimicrobial agents from different biological sources are continuously sought. Extracts of *T. elegans* used in the present study exhibited a varying degree of antibacterial and antifungal activities, and could be considered as potential sources of natural antimicrobials.

5. Conclusion

The present study was able to identify indigenous mushroom using molecular data, thus enriching and providing additional information on mushroom biodiversity in Nigeria. The study also showed the antimicrobial potentials of *T. elegans* collected in Osengere, Ibadan, thus giving credence to the therapeutic use of the mushrooms in folkloric medicine. Findings from this study are encouraging; however, further studies are required to isolate and characterize the specific biologically active agents responsible for the antimicrobial properties of the indigenous *T. elegans*. Also, preclinical and clinical studies are needed to establish the usefulness of the natural extracts of these mushrooms in the treatment or prevention of many human diseases.

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