Journal of Advances in Medical and Pharmaceutical Sciences
4(3): 1-14, 2015, Article no.JAMPS.20059
ISSN: 2394-1111

SCIENCEDOMAIN *international*
www.sciencedomain.org

Molecular Identity and Antimicrobial Profile of *Trametes* species Collected from the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria

S. I. Awala[1*] and V. O. Oyetayo[1]

[1]*Department of Microbiology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.*

Authors' contributions

This work was carried out in collaboration among both authors. Authors SIA and VOO designed the study and wrote the protocol. Author SIA collected all data, performed the statistical analysis, and wrote the first draft of the manuscript. Author SIA did the literature search and author VOO wrote part of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JAMPS/2015/20059
Editor(s):
(1) Palmiro Poltronieri, National Research Council of Italy, CNR-ISPA, Italy and Institute of Sciences of Food Productions, Operative Unit in Lecce, Italy.
Reviewers:
(1) Mutemi Muthangya, South Eastern Kenya University, Kenya.
(2) Eduardo Bernardi, Universidade Federal de Pelotas, Brazil.
Complete Peer review History: http://sciencedomain.org/review-history/11211

Original Research Article

Received 8th July 2015
Accepted 28th July 2015
Published 1st September 2015

ABSTRACT

Aims: The present study investigated the identity, antimicrobial, and secondary metabolites profile of *Trametes* species collected from forest near the Teaching and Research farm of the Federal University of Technology, Akure (FUTA), Ondo State.
Methodology: The Internal Transcribed Spacer (ITS 4 and ITS 5) of the mushroom nuclear ribosomal DNA (nrDNA) was used in its identification. Extracts of the mushrooms were prepared using methanol and acetone as extracting solvents. The phytochemical profile of the extracts was assessed quantitatively and qualitatively. The antimicrobial potential of the extracts on clinical and typed microbial cultures was assessed using standard microbiological techniques.
Results: The gene sequence of the *Trametes* species collected was observed to be 99% close to the gene sequence of *Trametes lactinea* from the National Center for Biotechnology Information (NCBI) GenBank. Phytochemical analysis revealed the presence of saponin, tannins, flavonoid,

*Corresponding author: E-mail: imisioluwaawala@gmail.com;

steroid, terpenoid, and cardiac glycosides in all the extracts. Cardiac glycoside was the most abundant in the extracts (23.183 and 19.138 mg/g in acetone and methanol extracts respectively), while flavonoid had the least content (7.008 and 7.854 mg/g in acetone and methanol extracts respectively). The extracts exhibited varying antibacterial and antifungal properties. Methanol extract of *T. lactinea* at concentration of 50 mg/ml exhibited a better antibacterial (28.17 mm against *Staphylococcus aureus*) and antifungal activity (35.33 mm against *Aspergillus flavus*).

Conclusion: Findings from this study show that extracts of *Trametes lactinea* indigenous to Akure is a potential source of natural antibacterial and antifungal agents which could be very useful in the treatment of infectious disease.

Keywords: Mushrooms; antimicrobial; phytochemical; sequence; Trametes lactinea.

1. INTRODUCTION

The rapid emergence of resistance to prevalent antibiotics by microbial agents has caused an increased interest towards natural antimicrobial sources. Hence, there has been an upsurge in the exploration of natural sources for novel bioactive compounds in recent years. This has led to searching new ecological niches for potential sources of natural bioactive agents for different pharmaceutical, agriculture, and industrial applications [1]. Since nature is the master chemist, bioactive compounds of natural origin have been the most consistent successful source for new drugs [2].

Fungi are the second most diverse group of organisms, yet only a fraction of all fungal species have been described so far and an even smaller number explored for the production of pharmacologically important metabolites [2]. Less intensively investigated organisms such as the macrofungi seem to have great promise in terms of compounds with potential biological activities.

In order to endure in their natural milieu, mushrooms need antibacterial and antifungal compounds [3]. Hence, they may be rich sources of natural antibiotics. This renewed interest on exploration for natural antimicrobial compounds from mushrooms has resulted in numerous mushroom extracts being tested with considerable positive activities reported [4-6]. Several authors have attributed these activities to the presence of diverse groups of secondary matabolites metabolites such as peptides, tannins, terpenoids, phenols, and flavonoids [7-9].

To effectively explore the untapped medicinal benefits in macrofungi, it is imperative that proper identification of the macrofungus is carried out. Identification of mushrooms is mainly by morphological description of the fruiting bodies, host specificity and geographical distribution [10]. In most cases morphological characteristics have their limitation in allowing a reliable distinction of intraspecific characteristics. Species constituting the genus *Trametes* are similar in morphology, hence it is difficult to identify and separate these species based on traditional taxonomy [11]. Molecular tools provide more accurate methods for identification than the few characters afforded by traditional morphological features.

It has been impossible to distinguish between genetically related species by these phenotypic characteristics [12]. Morphologically, mushrooms belonging to the same and even different genera may look similar. The implication of the above statement is that some mushrooms reported in this area might have been erroneously identified. It is therefore imperative that a combination of morphological and molecular identification based on gene sequence be employed for correct identification. The present study is therefore aimed at characterizing the indigenous *Trametes* species collected from FUTA using molecular tools, and assessing the antioxidant and antimicrobial potentials 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 woods of *Gmelina arborea* in forest near the Teaching and Research farm of the Federal University of Technology, Akure (FUTA), Ondo State (Latitude:7.3064N, Longitude: 5.12227E) in the month of June 2013. The fruit 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 examination.

2.2 Molecular Identification of Macro-fungus

2.2.1 Extraction of DNA of macrofungus

Standard DNA isolation methods employing CTAB lysis buffer was used [13]. For DNA extraction, dried portions of the macrofungus fruiting bodies (2 g) were ground with a mortar and pestle. The grounded materials were transferred into well labelled microtubes. Prewarmed (60℃) extraction buffer (CTAB) was added and the tubes were incubated at 65℃ 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 10,000 g (13000 rpm).

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 40 µl Sodium acetate (NaAc) was added followed by 260 µl of cold isopropanol. This was gently mixed by inverting tubes. The tubes were incubated at -20℃ overnight in a freezer (Haier HTF319 Freezer, 99405-0811). On the second day, the mixture was centrifuged at 10,000 g (13000 rpm) 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. DNA concentration and quality was checked by observing the band on an ethidium-stained agarose gel (0.7%) using 0.2 µl of each sample.

2.2.2 PCR amplification of the ITS region of macrofungus

The entire region of the rDNA of the macrofungus were amplified using the primer ITS4 and ITS5. The reaction mix was made up to a total volume of 25 µl, composed of 23 µl of *Taq* polymerase “Ready to Go” (Pharmacia, Sweden) with 0.2 µl of each primer (100 pM) and 2 µl 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 5min at 95℃ (initial denaturation) followed by 30 cycles of 45 sec at 95℃ (denaturation), 30 sec at 50℃ (annealing), 1min at 72℃ (extension), and 1 cycle of 10min at 72℃ (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 [14]. 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). The processes of extraction of DNA, amplification of DNA and sequences were carried out at the Key Laboratory of Mycology and Lichenology, Institute of Microbiology, Beijing.

2.3 Collection of Test Organisms

Clinical isolates of human origin (*Bacillus cereus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis, Candida albicans*, and Methicillin Resistant *Staphylococcus aureus*) were obtained from Obafemi Awolowo University Teaching Hospital (OAUTH), Ile-Ife, Osun state. 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. *Aspergillus fumigatus, Aspergillus niger* were obtained from the culture collection of the Department Microbiology, FUTA. The isolates were tested for viability by resuscitating them in buffered peptone water after which they were subcultured into nutrient agar medium for bacteria and potato dextrose agar for fungi and incubated at 37℃ for 24 hours and 27℃ for 48-72hours respectively. The organisms were then stored at 4℃ until needed.

2.4 Preparation of Mushrooms Extracts

The fresh mushrooms collected were brush-cleaned of attached soil and humus and then air-dried in an oven at 40℃ 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 (100 g)

was extracted with 2000 ml 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 (0.45 μm) using vacuum pump. The filtrates were evaporated to dryness at 50℃ 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℃ until further analysis. The percentage yield extracts were calculated based on dry weight as:

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

Where;

W1= weight of extract after solvent evaporation
W2 = Weight of the grounded mushroom powder

2.5 Phytochemical Screening of *Trametes* Species Extracts

Qualitative and quantitative phytochemical analysis of the crude extracts from the mushroom was determined through standard protocols described by Odebiyi and Sofowora [15], Trease and Evans [16], and Harborne [17].

2.5.1 Qualitative phytochemical analysis of *Trametes* species extracts

2.5.1.1 Test for alkaloid

The extracts (0.5 g 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 colour changes or turbidity to draw inference.

2.5.1.2 Test for saponin

The persistent frothing test for saponin described by Odebiyi and Sofowora [15] was used. Distilled water (30 ml) was added to 1 g 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 phlobatannin

The mushroom extracts (0.2 g) were dissolved in 10 ml 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 [16] was adopted. Each the samples (0.5g) were dissolved in 5 ml of distilled water, then boiled gently and cooled. One ml of each solutions was dispensed in test tubes and 3 drops of 0.1% ferric chloride solution were added and observed for brownish green or blue black colouration.

2.5.1.5 Test for terpenoids

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

2.5.1.6 Test for steriod

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

2.5.1.7 Test for flavonoids

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

2.5.1.8 Test for anthraquinone

Borntrager's test was used for the detection of anthraquinone. The extract (0.5 g) was shaken with 10 ml 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 colour in the ammonia layer which indicates the presence of free anthraquinone.

2.5.1.9 Test for cardiac glycosides

The followings were carried out to test for cardiac glycosides for each extract.

2.5.1.9.1 Legal's test

Each extracts was dissolve in pyridine and a few drops of 2% sodium nitroprusside with 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 extracts was mixed with 20 ml 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 colour 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 (0.5 g) was dissolved in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayed with 1 ml of conc. H_2SO_4. It was observed for a brown colouration at the interface indicating the presence of a deoxy sugar which is 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 tannin

Tannin determination was done according to the method of Association of Official Analytical Chemists [AOAC] [18], with some modifications. The sample (0.20 g) was added 20 ml of 50% methanol. This was shaken thoroughly and placed in a water bath at 80℃ for 1 hour to ensure uniform mixing. The extract 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 bluish-green colour developed at the end of the reaction mixture of different concentrations ranges from 0 to 10 ppm. The absorbance of the tannic acid standard solutions as well as sample was measured after colour 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 saponin

Quantitative determination of saponin was done using the method of Obadoni and Ochuko [19]. 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℃. 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℃. 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℃ to a constant weight. The saponin content was calculated using the formula:

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

2.5.2.3 Determination of alkaloids

Alkaloids were quantitatively determined according to the method of Harborne [17]. Two hundred milliliters of 10% acetic acid in ethanol was 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

Steroid content of the plant sample was determined using the method described by Trease and Evans [16]. 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 (Gulfex Medical and Scientific England, Spectrum Lab 23A, model number 23A08215) at 420 nm.

2.5.2.4.1 Determination of cardiac glycosides

Cardiac glycoside content in the samples was evaluated using Buljet's reagent as described by El-Olemy et al. [20]. 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 + 5 ml 10% aqueous sodium hydroxide [NaOH]).

2.6 Determination of Antimicrobial Activity of *Trametes* Species Extracts

Antimicrobial activity of extracts was determined by the agar well diffusion method [21]. Stock cultures were maintained at 4℃ on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Nutrientr broth (NB) for bacteria and Sabouraud dextrose broth (SDB) for fungi and were incubated without agitation for 24 hours at 37℃ and 25℃ respectively. To 5 ml 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 600 nm 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±1℃ 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 (20 ml) cooled to 45℃ was poured into sterilized Petri plates and left to solidify. An aliquot of culture (100 µl) 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 7 mm). The crude extracts (100 µl) were dissolved in 30% dimethyl sulfoxide (DMSO) to a concentration of 50 mg/ml and filtered through 0.22 µm membrane filter and then introduced into each wells with the aid of a micropipette.

Clotrimazole was used as positive control for fungi and reference antibiotic disc (gentamicin (10 µg), nalidixic acid (30 µg), nitrofurattoin (200 µg), cotrimoxazole (25 µg), amoxicillin (25 µg), tetracycline (25 µg), augmentin (30 µg), and ofloxacin (5 µg)) 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 proper diffusion of the extracts. The plates were then incubated at 37ºC 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 well diffusion method earlier described was used to screen the antimicrobial effect of the different concentrations of extracts (6.25 to 50 mg/ml). The MIC was determined by establishing 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.

2.6.2 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=.05.

3. RESULTS

The molecular identity of the *Trametes* species collected from forest in the Federal University of Technology, Akure is shown in Table 1. Data obtained from the Basic Local Alignment Search

Table 1. Genomic Identification based on nrDNA Internal transcribed Spacer (ITS) sequence of wild macrofungus collected from Forest in FUTA, Nigeria

Sample	Source	Tentative phenotypic identity	Closest relative from NCBI Gene Bank	NCBI accession number	% identity
1	Akure, Ondo State	*Trametes* sp.	*Trametes lactinea*	JN048769.1	99

Tool (BLAST) analysis revealed differences in the sequence of the *Trametes* species collected from FUT Akure, Nigeria and the *Trametes* sequences from National Center for Biotechnology Information (NCBI) GenBank. The result revealed that the *Trametes* species collected from FUT Akure, Nigeria was 99% related to *Trametes lactinea* (Table 1).

Result from the extraction process shows methanol producing better yield than acetone (Table 2). Saponin, tannins, steroid, terpenoid, flavonoids and cardiac glycosides were present in all the *Trametes* species extracts, while anthraquinone alkaloid and phlobatannin were absent (Table 3).

Result from the quantitative phytochemical screening, shows that the acetone extract of Trametes lactinea, has higher amount of saponin (19.952 mg/g) and cardiac glycoside (23.183 mg/g), while the methanol extract has higher amount of tannin (12.699 mg/g), flavonoid (7.854 mg/g), steroid (14.792 mg/g), and terpenoid (15.293 mg/g) (Table 4).

Table 5 shows the antimicrobial activities of the extracts of *Trametes lactinea* at concentration of 50mg/ml and commercial antibiotics against the test organisms. The methanol extract of *Trametes lactinea* inhibited all the clinical bacterial isolates except *Samonella typhi* while acetone extract of *Trametes lactinea* inhibited all the clinical bacterial isolates except from *Staphylococcus aureus* and *Samonella typhi*. Both the methanol and acetone extracts of *Trametes lactinea* exhibited antibacterial effect against all the typed bacteria used. Acetone extract of *Trametes lactinea*, however could not inhibit *Pseudomonas aeruginosa* ATCC 29853. Methanol extract of *Trametes lactinea* exhibited the highest antibacterial activity against the clinical isolate of *Staphylococcus aureus* with a zone of inhibition of 28.17 mm. Table 5 also shows the extract of *Trametes lactinea* exhibiting appreciable antifungal activity. Methanol extract of the macrofungus displayed a higher antifungal activities but did not inhibit *Candida albicans*. However, acetone extracts of *T. lactinea* inhibited *Candida albicans* with a zone of inhibition of 10.17 mm. The commercial antibiotics were slightly more effective in their antimicrobial activities against the test organisms. The minimum inhibitory concentration (MIC) of the extracts ranged between 12.5 to 50 mg/ml (Table 6).

Table 2. Yield of *Trametes lactinea* extracts

Mushroom	Solvent	Total yield (mg/g)
Trametes lactinea	Methanol	2.2
	Acetone	1.2

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

Phytochemicals	Extracts	
	TLA	TLM
Saponin	+	+
Tannin	+	+
Steriod	+	+
Alkaloid	-	-
Terpenoid	+	+
Flavonoids	+	+
Anthraquinone	-	-
Phlobatannin	-	-
Cardic Glycoside		
1. Legal Test	+	+
2. Keller Killiani Test	+	+
3. Salkwoski Test	+	+

Keys: TLA: Acetone extract of Trametes lactinea; TLM: Methanol extract of Trametes lactinea; +: Positive; - : Negative

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

Phytochemicals	Amount (mg/g)	
	TLA	TLM
Tannin	12.504 ± 0.0158^{c}	12.699 ± 0.005^{c}
Saponin	19.952 ± 0.048^{d}	10.400 ± 0.219^{b}
Flavonoid	7.008 ± 0.004^{a}	7.854 ± 0.003^{a}
Steriod	12.804 ± 0.32^{c}	14.792 ± 0.001^{d}
Terpenoid	9.319 ± 0.027^{b}	15.293 ± 0.041^{e}
Glycoside	23.183 ± 0.042^{e}	19.138 ± 0.038^{f}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a column are significantly different at (P=.05).

Keys: TLA: Acetone extract of Trametes lactinea; TLM: Methanol extract of Trametes lactinea

Table 5. Antimicrobial activities of extracts of *Trametes lactinea* and commercial antibiotics against test organisms

Test organisms	Zones of inhibition (mm)						
	TLM	**TLA**	**Gentamicin (10 µg)**	**Nalidixic acid (30 µg)**	**Nitrofurattoin (200 µg)**	**Ofloxacin (5 µg)**	**Clotrimazole (1 mg/ml)**
Escherichia coli *	12.17±0.17 [d]	6.17±0.17 [b]	10.00±0.58 [c]	12.33±1.20 [d]	0.00±0.00 [a]	21.33±0.88 [e]	ND
Pseudomonas aeruginosa *	6.00±0.00 [a]	17.17±0.17 [d]	12.00±0.29 [b]	15.83±0.17 [c]	16.17±0.44 [c]	20.00±0.00 [e]	ND
Bacillus cereus *	14.17±0.17 [bc]	4.33±0.17 [a]	12.33±1.20 [b]	18.67±1.76 [d]	17.00±0.58 [cd]	14.33±0.88 [bc]	ND
Staphylococcus aureus *	28.17±0.29 [e]	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 *	10.17±0.17 [b]	3.00±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 *	5.17±0.17 [a]	5.33±0.17 [a]	11.33±01.53 [b]	20.33±0.58 [d]	15.33±0.67 [c]	19.00±1.53 [d]	ND
Escherichia coli ATCC 23718	16.50±0.29 [e]	7.33±0.17 [b]	10.33±0.33 [c]	13.00±0.58 [d]	0.00 ±0.00 [a]	26.50±0.29 [f]	ND
Escherichia coli ATCC 35218	6.00±0.00 [a]	5.17±0.17 [a]	15.00±0.58 [b]	20.00±0.58 [c]	24.00±0.58 [d]	32.33±0.88 [e]	ND
Pseudomonas aeruginosa ATCC 29853	4.17±0.17 [b]	0.00±0.00 [a]	11.00±0.58 [c]	0.00±0.00 [a]	0.00±0.00 [a]	21.33±0.88 [d]	ND
Bacillus cereus NCIB 6344	6.17±0.17 [b]	13.17±0.17 [c]	12.67±0.88 [c]	14.33±2.19 [c]	0.00±0.00 [a]	25.50±0.29 [d]	ND
Staphylococcus aureus NCIB 950	4.00±0.00 [b]	12.17±0.17 [c]	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	17.00±0.00 [b]	12.17±0.17 [a]	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]	10.17±0.17 [b]	ND	ND	ND	ND	15.35±0.05 [c]
Aspergillus flavus	35.33±0.17 [b]	1.50±0.00 [a]	ND	ND	ND	ND	23.98±0.017 [c]
Aspergillus fumigatus	35.17±0.17 [b]	26.17±0.17 [a]	ND	ND	ND	ND	32.33±1.45 [b]

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a column are significantly different at (P=.05).

***Keys**: **TLA**: Acetone extract of Trametes lactinea; **TLM**: Methanol extract of Trametes lactinea; **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 lactinea* against test organisms

Test organism	TLM	TLA
Escherichia coli *	25	25
Pseudomonas aeruginosa *	25	25
Bacillus cereus *	25	25
Staphylococcus aureus *	12.5	ND
MRSA *	25	50
Enterococcus. faecalis *	25	25
Salmonella typhi *	ND	ND
Escherichia coli ATCC 23718	12.5	50
Escherichia coli ATCC 35218	12.5	50
Pseudomonas aeruginosa ATCC 29853	50	ND
Bacillus cereus NCIB 6344	50	25
Staphylococcus aureus NCIB 950	50	12.5
Salmonella typhi ATCC 33458	12.5	12.5
Candida albicans *	ND	25
Aspergillus flavus	25	25
Aspergillus fumigatus	25	12.5

Each value is mean of triplicate results.
*Keys: TLA: Acetone extract of Trametes lactinea; TLM: Methanol extract of Trametes lactinea; *: Clinical strain; ATCC: American Type Culture Collection; NCIB: National Collection for Industrial Bacteria; MRSA: Methicillin resistant Staphylococcus aureus; ND: Not determined*

4. DISCUSSION

Wild and cultivated mushrooms contain diverse components with nutritional [22] and/or medicinal importance [23,24]. Fruiting bodies, mycelia and spores of the mushrooms accumulate a variety of bioactive metabolites with immunomodulatory, hepatoprotective, antifibrotic, antiinflammatory, antidiabetic, antiviral, antioxidant, antitumor, antimicrobial and cardiotonic properties [23,25]. In this study, the phytochemical and antimicrobial properties of methanol and acetone extract from *Trametes lactinea* were assessed using various *in vitro* methods.

The genus *Trametes* was considered as one of the most complicated genera in the Polyporaceae [26]. In recent years, mycologist have attempted to use sequence data to resolve the taxonomic problems in *Trametes* and in the related genera [27,28]. ITS sequences are useful tools in distinguishing the genera with similar morphological characteristics [11,28].

This study revealed that the gene sequences of *Trametes* species collected from forest in FUTA, are not 100% homologous with existing gene sequence of *Trametes* species found in NCBI GenBank. Several data obtained from the molecular identification of macrofungus have also revealed that the gene sequence of these macrofungus are not 100% homologous to the gene sequences of their closest relative in the NCBI GenBank [11,29-31].

The difference in the gene sequences of *Trametes* species from Nigeria and its counterpart from other parts of the world maybe due to the influence of time, and the different ecological zones where they exist. Most fungi are highly plastic, with individuals displaying substantial spatial and temporal variation in morphology and physiology that can obscure differences in life-history strategy observed between taxa [32].

Result from the extraction process revealed variation in the yields of the two solvents used. This might be attributed to the polarity of the solvents and the different compounds present in the mushroom. Comparison between the two solvents (methanol and acetone) used for extraction, methanol was more efficient in its yield for *T. lactinea*. According to Perva-Uzunalic et al. [33], satisfactory results are always produced when alcoholic solutions are used for extraction. The yield of chemical extraction depends on the type of solvents with varying polarities, pH, extraction time and temperature, as well as on the chemical compositions of the sample [34,35].

Phytochemical analysis in this study revealed the presence of saponin, tannins, steroid, terpenoid, flavonoid, and cardiac glycosides in all the *Trametes* species extracts, while alkaloid, anthraquinone and phlobatannin were absent. Macrofungi are of medicinal importance and they represent an unlimited source of secondary metabolites of high medicinal value [36]. A large number of biologically active molecules have been identified in many species of macrofungi throughout the world [37].

The absence of some of the phytochemicals in the mushrooms extracts could be due to the fact that a single or particular type of solvent may not be selective for a single compound as a result of multicomponent nature of plant materials with complex interactions [38]. The presence of these various phytochemicals suggest that this macrofungus could be of great importance in the treatment of infectious diseases. Saponin for instance has demonstrated antimicrobial properties particularly against fungi and additionally against bacteria and protozoa [39]. Tannin compounds have been reported to have some antibacterial [40], antiviral [41] and antiparasitic effect [42]. 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 [43]. Flavonoids are hydroxylated phenolics, and have been reported to possess strong antimicrobial properties [44]. Figueroa-Valverde et al. [45] reported the antimicrobial activity of steroids. The presence of these phytochemicals suggest that the mushroom used in this study can be a potential source of new antimicrobials.

Result from the phytochemical analysis also displayed variation in the amount of phytoconstituents present. Among the two solvents used for extraction, methanol extract showed more number of phytoconstituents when compared with the acetone extracts. This could be due to the different extracting capacity of the solvents, as the extracting capacity of different solvents to different phytoconstituents has been reported to depend on their solubility and polarity [46].

Trametes lactinea extract exhibited varied antimicrobial activities, with the methanol extract of *T. lactinea* displaying a higher antimicrobial activity on all tested organism. The antimicrobial activities of the extracts may have been as a result of the presence of variety of secondary metabolites, such as tannins, terpenoids, phenols, and flavonoids, which have been found in vitro to have antimicrobial properties. This study thus supports the claims of Rai et al. [47] and Barros et al. [48] who associated the antimicrobial activities of macrofungi to diversities of secondary metabolites they contain.

It was observed that there were variations in the reaction of Gram-negative and Gram-positive bacteria to the extracts of *Trametes lactinea*. In general, Gram-negative bacteria have been reported to be more resistant to antimicrobials than Gram-positive bacteria [49,50]. The differences in the antimicrobial effects between Gram-positive and Gram- negative bacteria are mainly due to their different cell wall structures [51,52]. The cell wall of Gram-positive bacteria consists of a single layer, whereas the Gram-negative cell wall is a multilayered structure bounded by an outer cell membrane [51,52].

A single membrane structure has a poor buffering capacity against the localized protonation effects generated by phytochemicals. Therefore, the bacterial intracellular space can be easily hyper-acidified, causing functional disorder of bacterial energy metabolism [53]. However, an external lipopolysaccharide layer and additional minor membrane components of the cell wall give more buffering capacity to Gram-negative bacteria, functioning as a preventive barrier against hydrophobic compounds [53]. Consequently, these bacteria exhibit less sensitivity to the antimicrobial activities of phytochemicals [49,50].

From the result of this study, it was observed that most of the Gram-negative bacteria were more susceptible to the *Trametes lactinea* extracts than the Gram-positive bacteria. This contradicts the findings of several authors that have reported Gram-negative bacteria to be more resistant to antimicrobials than Gram-positive bacteria [49, 50]. In the antimicrobial activity of the methanol extract of *Trametes lactinea* against typed bacteria, Gram-negative bacteria such as *E. coli*, *Pseudomonas aeruginosa* and *Salmonella typhi* were more susceptible than the Gram-positive bacteria such as *Bacillus cereus* and *Staphylococcus aureus*.

This observation is in line with the findings of Kandhasamy et al. [54] and Rakholiya et al. [55]. According to the result of Kandhasamy et al. [54], Gram negative bacteria such as *E. coli*, *P. aeruginosa* and *S. typhi*, were more susceptible

to the crude extracts of *Drynaria quercifolia* than the Gram-positive bacteria such as *Bacillus substilis* and *Staphylococcus aureus*.

A comparison of the antimicrobial activities of extracts of *Trametes* species to clinical bacterial isolates and typed bacterial cultures showed a marked difference in 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 typed culture was more resistant than the clinical isolate.

The lesser susceptibility of the clinical isolates might be as a result of the indiscriminate exposure of the isolates to antibiotics which has generated resistance. According to Adeleye et al. [56], clinical isolates are known to carry a resistance gene which makes them to be resistant to antimicrobial agents.

Numerous reports have shown that bacteria are more sensitive to antimicrobials than fungi [57,58], and the reason for different sensitivity between the fungi and bacteria can be found in different transparency of the cell wall [59]. However, the result from this study shows extracts of *Trametes lactinea* exhibited better antifungal than antibacterial effect. This is line with the findings of Pepeljnjak et al. [60] who observed that the essential oil of juniper berry possess higher antifungal activity than antibacterial. This is an indicator of the broad spectrum antimicrobial potential of *Trametes lactinea*, which makes it a candidate for bioprospecting for antibiotic and antifungal drugs.

Antibiotics were more effective in inhibiting test organisms than extracts of *Trametes lactinea*. This may be due to purity level of commercial antibiotics. This is in line with several findings that have reported the higher potency of antibiotics to their high degree of purity as compared to the crude state of the extracts [61]. The molecular size of antibiotics which aid their solubility in diluents may be another reason for their better performance [62]. This could enhance their penetration through the cell wall into the cytoplasm of the organism where they act [63]. The extracts of the *Trametes lactinea* are of higher molecular size and thus affecting their solubility. Subsequent penetration through the outer membrane into the cytoplasm will be limited owing to the smaller size of the membrane compared with the size of the extracts.

5. CONCLUSION

This results of this study show that extracts from *Trametes lactinea* exhibited significant antimicrobial activities. In light of this finding, *Trametes lactinea* indigenous to Nigeria could be considered as potential sources of natural antimicrobials and could be of great importance for the treatment of infectious diseases. Further work aimed at the isolation and characterization of the specific biologically active agents responsible for the antimicrobial properties is the next focus.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Selim KA, El-Beih AA, Abdul-Rahman TM, El-Diwany AI. Biology of Endophytic Fungi. Curr Res Environ Appl Mycol J Fungal Biol. 2012;2(1):31–82.
2. De Silva DD, Rapior S, Sudarman E, Stadler M, Xu J, Alias SA, et al. Bioactive metabolites from macrofungi: Ethnopharmacology, biological activities and chemistry. Fungal Divers. 2013;62:1–40.
3. Lindequist U, Niedermeyer THJ, Julich WD. The pharmacological potential of mushrooms-Review. Evid Based Complement Alternat Med. 2005;2(3):285-299.
4. Alves MJ, Ferreira IC, Dias J, Teixeira V, Martins A, Pintado M. A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. Planta Med. 2012a;16:1707–1718.
5. Alves MJ, Ferreira IC, Martins A, Pintado M. Antimicrobial activity of wild mushroom extracts against clinical isolates resistant

to different antibiotics. J Appl Microbiol. 2012b;2:466–475.

6. Schwan WR. Mushrooms: An untapped reservoir for nutraceutical antibacterial applications and antibacterial compounds. Curr Top Nutraceutical Res. 2012;10(1): 75–82.

7. Alves MJ, Ferreira IC, Froufe HJ, Abreu RM, Martins A, Pintado M. Antimicrobial activity of phenolic compounds identified in wild mushrooms, SAR analysis and docking studies. J Appl Microbiol. 2013;115(2):346-57.

8. Lavanya J, Subhashini S. Therapeutic proteins and peptides from edible and medicinal mushrooms-Review. ESJ. 2013;9(4):1857-7881.

9. Avcı E, Avcı GA, Kose DA. Different Solvents and Chemical Composition via GC/MS Analyses. JFNR. 2014;2(8):429-434.

10. Seo GS, Kirk PM. Ganodermataceae: Nomenclature and classification, In J. Flood, P. D. Bridge, and P. Holderness (Eds.). *Ganoderma* Disease of perennial Crops (pp. 3-22). Walling Ford, UK: CABI Publishing; 2000.

11. Zhang XQ, Yuan J, Xiao YZ, Hong YZ, Tang C. A primary studies on molecular taxonomy of *Trametes* species based on the ITS sequences of rDNA. Mycosystema. 2006;25(1):23-30.

12. Oyetayo VO, Yao YJ. Identification of Wild *Genoderma* Species indigenous to Nigeria using ITS region of the DNA. Niger J Econ Soc Stud. 2010;24(1):2140-2144.

13. Zolan ME, Pukkila PJ. Inheritance of DNA methylation in *Coprinus cinereus*. Mol Cell Biol. 1986;6:195-200.

14. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The Clustal_X windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. Nuc. Acids Res. 1997;25:4876–4882.

15. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants. Lloyd. 1978;41(3):234–246.

16. Trease GE, Evans MC. Pharmacognosy (14th ed.). New Delhi, India: Elsevier; 2005.

17. Harborne JB. Phytochemical methods. A guide to modern techniques of plant analysis (3rd ed.). New Delhi: Springer Pvt Ltd; 2005.

18. Association of Official Analytical Chemists [AOAC]. Official Methods of Analysis (15th ed.). Arlington, V.A; 1990.

19. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the extracts of some haemostatic plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci. 2001;8:203–208.

20. El-Olemy MM, Al-Muhtadi FJ, Afifi AFA. Experimental Phytochemistry: A Laboratory Manual. Saudi Arabia: King Saud Univ. Press; 1994.

21. Schinor EC, Salvador MJ, Ito IZ, Dias DA. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. Braz J Microbiol. 2007;38:145-149.

22. Kalač P. Chemical composition and nutritional value of European species of wild-growing mushrooms: A review. Food Chem. 2009;113:9–16.

23. Borchers A, Keen CL, Gershwin ME. Mushrooms, tumors, and immunity: an update. Exp Biol Med. 2005;229:393-406.

24. Poucheret P, Fons F, Rapior S. Biological and pharmacological activity of higher fungi: 20-Year retrospective analysis. Cryptog Mycol. 2006;27:311-333.

25. Gonçalves O, Pereira R, Gonçalves F, Mendo S, Coimbra MA, Rocha SM. Evaluation of the mutagenicity of sesquiterpenic compounds and their influence on the susceptibility towards antibiotics of two clinically relevant bacterial strains. Mutat Res. 2011;723:18–25.

26. Zuo LH. Taxonomy and Phylogeny of Trametes and Related Genera in China. 2013;

Available:http://www.dissertationtopic.net/doc/1794525

(Accessed 03 May 2014)

27. Miettinen O, Larsson KH. *Sidera*, a new genus in *Hymenochaetales* with poroid and hydnoid species. Mycol Prog. 2010;10:131-141.

28. Cui DZ, Zhao M, Yang HY, Wan CI, Dai HB. Molecular phylogeny of *Trametes* and related genera based on internal transcribed spacer (ITS) and nearly complete mitochondrial small subunit ribosomal DNA (mt SSU rDNA) sequences. Afr J Biotechnol. 2011;10(79): 18111-18121.

29. Oyetayo VO. Molecular Characterisation of *Termitomyces* species collected from Ado Ekiti and Akure, Nigeria. N J Microbiol. 2009;23(1):1933-1938.
30. Rajaratnam S, Thiagarajan T. Molecular characterization of wild mushroom. Eur J Exp Biol. 2012;2(2):369-373.
31. Das SK, Mandal A Datta AK, Gupta S, Paul R, Saha A, Sengupta S, Dubey PK. Nucleotide Sequencing and Identification of Some Wild Mushrooms. Scientific World Journal. 2013;2013:7.
32. Aguilar-Trigueros CA, Powell JR, Anderson IC, Antonovics J, Rillig MC. Ecological understanding of root-infecting fungi using trait-based approaches. Trends Plant Sci. 2014;19:432-438.
33. Perva-Uzunalic A, Skerget M, Knez Z, Weinreich B, Otto F, Grunner S. Extraction of active ingredients from green tea (*Camellia sinensis*): Extraction efficiency of major catechins and caffeine. Food Chem. 2006;96(4):597-605.
34. Brahmi F, Madani K, Dahmoune F, Rahmani T, Bousbaa K, Oukmanou S, et al. Optimisation of solvent extraction of antioxidants (phenolic compounds) from Algerian mint (*Mentha spicata* L.). Phcog Commn. 2012;2(4):72-86.
35. Hayouni EA, Abedrabba M, Bouix M, Hamd, M. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts. Food Chem. 2007;105:1126-1134.
36. Kawagishi H. Biologically functional molecules from mushrooms. J Synth Org Chem Jpn. 2010;68(5):514–521.
37. Wasser SP. Current findings, future trends and unsolved problems in studies of medicinal mushrooms. Appl Microbiol Biotechnol. 2011;89:1323-1332.
38. Markom M, Hasan M, Daud WRD, Singh H, Jahim J. Extraction of hydrolysable tannins from *Phyllanthus niruri* Linn: Effects of solvents and extraction methods. Sep Purif Technol. 2007;52:487-496.
39. Sahelian R. Saponin in plants benefit and side effects, glycosides and extraction; 2014. Available:http://www.raysahelian.com/saponin.html (Accessed 27th January 2015)
40. Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001;48(4):487-491.
41. Lu L, Liu SW, Jiang SB, Wu SG. Tannin inhibits HIV- 1 entry by targetting gp41. Acta Pharmacol Sin. 2004;25(2):213-218.
42. Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on Leishmania parasitized RAW 264.7 cells. Phytochemistry. 2005;66(17):2056-2071.
43. Dudareva N, Pichersky E, Gershenzon J. Biochemistry of plant volatiles. Plant Physiol. 2004;135:1893–1902.
44. Orhana DD, Özçelikb B, Özgenb S, Erguna, F. Antibacterial, antifungal, and antiviral activities of some flavonoids. Microbiol Res. 2010;165(6);496–504.
45. Figueroa-Valverde L, Diaz-Cedillo F, Garcia-Cervera E, Pool-Gomez E, Lopez-Ramos M, Sarabia-Alcocer B, et al. Antimicrobial activity induced by a steroid-brucine derivative on *Salmonella typhi*, *Klebsiella pneumonia* and *Escherichia coli*. J Biol Sci. 2014;14:67-72.
46. Marjorie MC. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12(4):564-582.
47. Rai M, Tidke G, Wasser SP. Therapeutic Potential of Mushrooms. NPR. 2005;4(4): 246-257.
48. Barros L, Cruz T, Baptista P, Estevinho, LM, Ferreira ICFR. Wild and commercial mushrooms as source of nutrients and nutraceuticals. Food Chem Toxicol. 2008;46:2742-2747.
49. Kaneria MJ, Chanda, SV. The effect of sequential fractionation technique on the various efficacies of Pomegranate (*Punica granatum* L.). Food Anal Method. 2013;6:164-175.
50. Kim SJ., Cho AR, Han, J. Antioxidant and antimicrobial activities of leafy green vegetable extracts and their applications to meat product preservation. Food Control. 2013;29:112-120.
51. Kavoosi G, Rowshan V. Chemical composition, antioxidant and antimicrobial activities of essential oil obtained from *Ferula assa-foetida* oleo-gum-resin: Effect of collection time. Food Chem. 2013;138:2180-2187.
52. Hosni K, Hassen I, Sebei H, Casabianca, H. Secondary metabolites from *Chrysanthemum coronarium* (Garland) flowerheads: Chemical composition and

biological activities. Ind Crops Prod. 2013;44:263–271.
53. Helander LM, Alakomi HL, Latva-Kala K. Characterization of the action of selected essential oil components on Gram negative bacteria. J Agr Food Chem. 1998;46:3590-3595.
54. Kandhasamy M, Arunachalam KD. Thatheyus AJ. *Drynaria quercifolia* (L.) J. Sm: A potential resource for antibacterial activity. Afr J Microbiol Res. 2008;2:202-205.
55. Rakholiya K, Kaneria M, Desai D, Chanda S. Antimicrobial activity of decoction extracts of residual parts (seed and peels) of *Mangifera indica* L. var. Kesar against pathogenic and food spoilage microorganism. Formatex. 2013;2:850-856.
56. Adeleye IA, Ogunniyi AA, Omonigbehin EA. Antimicrobial activity of some local herbs on common skin pathogens. Biochem Biophys Res Commun. 2003; 15(3):231-239.
57. Kosanic M, Rankovic B, Dasic M. Antioxidant and antimicrobial properties of mushrooms. Bulg J Agric Sci. 2013;19: 1040-1046.
58. Haruna MT, Anokwuru, CP, Akeredolu, AA, Akinsemolu AA, Alabi OA. Antibacterial and Antifungal Activity of *Acalypha wilkesiana*, Eur. J. Med. Plants. 2013;3(1):52-64.
59. Yang Y, Anderson EJ. Antimicrobial activity of a porcine myeloperozidase against plant pathogenic bacteria and fungi. J Appl Microbiol. 1999;86:211-220.
60. Pepeljnjak S, Kosalec I, Kalodera Z, Blazevic Z. Antimicrobial activity of juniper berry essential oil (*Juniperus communis* L. *Cupressaceae*). Acta Pharm Sin. 2005;55:417-422.
61. Makanjuola OO, Dada EO, Ekundayo FO. Antibacterial activities of *Moringa oleifera* (LAM) on coliforms isolated from some surface waters in Akure, Nigeria. FUTA J Res Sci. 2013;1:63-71.
62. Raza MW, Shad A, Pedler SJ, Karamat KA. Penetration and activity of antibiotics in brain abscess. JCPSP. 2005;15(3):165-167.
63. Mailard JY. Bacterial target sites for biocide action. J Appl Microbiol. 2002;9:16-27.

© 2015 Awala and Oyetayo; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/11211