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Anti-microbial Activity and Biochemical Constituents of Two Edible and Medicinal Mushrooms of Mid-Western, Uganda

Immaculate Nakalembe and John David Kabasa
School of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

Abstract: As a contribution to a search of alternative drugs against emerging resistant pathogenic strains, this study aimed at determination of anti-microbial activity and identification of the potential bioactive compounds in two medicinal mushrooms (*Termitomyces* sp. (Bunyanaka) and *Termitomyces microcarpus*) used for treating coughs, boils, sore throat or tonsillitis. To achieve this target, the fractions were obtained from petroleum ether and methanol crude extracts and tested *in vitro* for anti-microbial activities against selected bacterial strains and a fungus. The bioactive compounds were identified using GC-MS. Gram positive bacteria were more sensitive than gram negative ones. Isolated fractions had activity at a MIC value of 3.13-200 μg mL^{-1}. Similarly, petroleum ether fractions had high activity than methanol ones. Interestingly, *Pseudomonas aeruginosa* was more sensitive to *Termitomyces* sp. fractions with a MIC value of 50-75 μg mL^{-1}. Several bioactive compounds were identified in the mushrooms. Further studies should be done to elucidate these compounds as a contribution towards drug development.

Key words: Fractions, anti-microbial compounds, *Termitomyces* sp., bioactive, bacteria, Uganda

INTRODUCTION

Anti-microbial activity including anti-bacterial, anti-fungal, anti-parasitic and anti-viral agents is widespread therapeutic effect reported in mushrooms (Wasser and Weis, 1999; Kettering *et al*., 2005). About >200 higher fungal species demonstrate anti-microbial properties (Kettering *et al*., 2005). Combined biological activity is reported for some mushroom species (Wasser and Weis, 1999). Since, the discovery of the traditional anti-microbials (e.g., penicillin), a lot of micro-organisms are now resistant to one or more anti-microbial drugs (Guillot, 1989). Anti-microbial resistance is proving fatal for thousands of people each year resulting in high medical costs and heavy economic losses (Barton, 1998). About 25% of the Ugandan budget (100 billion Uganda Shillings) allocated to health is used for the procurement of new modern medicines (MOH, 2007).

Despite this investment, poor communities have limited access to these drugs because of their poor economic status. This together with poor nutrition prevailing in the societies may result in more deaths and heavy economic losses if no intervention is done. One of the strategic intervention approaches to anti-microbial resistance is the research into new classes of anti-microbials that are cheap and non-toxic to the host. Therefore, this calls for more research into inexpensive, safe natural resources of high nutritional and medicinal value. Mushrooms with all the good attributes of nutritional, medicinal and gastronomical superlative have gained increased attention in the search of safe and superior natural drugs. Basing on these facts, researchers undertook a study to determine the anti-microbial activities of two known medicinal wild mushrooms and their bioactive compounds identified using GC-MS analyses.

MATERIALS AND METHODS

Mushrooms collection, identification and preparation of extract: The wild medicinal mushrooms, *Termitomyces* sp. (Obunyanaka) and *T. microcarpus* were collected from Mid-Western, Uganda between March and June, 2009. Scientific identification was done by Dr. Wong T., Institute of Microbiology, Chinese Academy of Sciences, China. Herbarium specimens were prepared and deposited in the Makerere University Herbarium. The fruiting bodies were carefully air-dried and finely ground to obtain homogeneity of the samples before extraction. About 0.5-1 kg of the sample was soaked in 99.5% Methanol (MEOH) (BDH, UK) and Petroleum Ether (PE) (40-60°C, BDH, UK) at room temperature. The flasks containing the sample were covered with aluminum foil and allowed to

Corresponding Author: Immaculate Nakalembe, School of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda

stand for 3 days with intermittent agitation on a shaker (Mitamura Model-s102) for extraction. The samples were then strained and further filtered using Whatman filter paper No. 4. The solvents were concentrated under vacuum at 40-60°C. The crude extracts were weighed and the percentage yield calculated.

The methanol extract were further purified by adding ethyl acetate and vortexed to mix. About 30 mL of sodium bicarbonate was then added to remove weak acids, filtered and the filtrate evaporated under a reduced pressure at 40-60°C to obtain pure (methanol free) extract. This was repeated 3 times before sterilization. PE and MEOH extracts were sterilized by filtration through a 0.22 μm membrane filter and tested for sterility by exposure to ultraviolet light for 24 h and then streaking out on the nutrient agar plates and incubating for 24 h. The crude extract was divided into small portions of 1-10 g and to each, a known amount of Dimethylsulfoxide (2-20 mL) (DMSO, Sigma Chemical Co. Sternheim, England) was added to a make a stock solution of 0.5 g mL^{-1}. The crude extracts were kept in the dark at 4°C to prevent degradation of the bioactive compounds until used.

Micro-organisms, culture conditions and standard bacterial suspensions: Standard micro-organisms from the American Type Culture Collection (ATCC) and wild Multidrug resistant strains (MD) isolated from different biological fluids and identified in the Department of Microbiology and Parasitology, Faculty of Veterinary Medicine, Makerere University were used. Gram negative organisms comprised *E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 27853, *K. pneumoniae* MD (isolated from urine); the gram positive bacteria comprised *Bacillus subtilis* (ATCC 6633), *S. aureus* ATCC 25923 and *S. aureus* MD (isolated from pus) and a fungus *C. albicans* ATCC 10239. These micro-organisms were initially rejuvenated aerobically at 37°C for 24 h on nutrient agar medium for bacteria except *E. coli* which was cultured on chromocult medium. *C. albicans* was cultured on sabouraud dextrose agar for 48 h at room temperature. A colony of each microbial culture was scooped using a wire loop and separately added to 10 mL of peptone water (Oxoid Bacteriological Peptone 1% with 5% NaCl) and agitated with vortex mixer. The mixture was incubated under the same conditions, 24 h for bacteria and 48 h for fungus. Finally, the turbidity of the mixture was adjusted to 0.5 McFarland standards.

Isolation and fractionation of extracts: Methanol extracts of *Termitomyces* sp. were partitioned using chloroform. The aqueous portion was chromatographed on an open column (2.5 cm by 60 cm) previously packed with silica gel (100-230 mesh cat. #80003-20, Fisher) using ethylacetate/methanol/water as a gradient solvent system. Fractions Fr_1 and Fr_3 were further subjected to column chromatography and eluted with xylene/MEOH and ethylacetate/MEOH/water gradient solvent systems, respectively.

Further fractionation of active subfractions (Fr_{1-1}) and Fr_{3-1} was done by preparative normal phase TLC on silica gel 60 (Fisher, silica gel 60 PF_{254} and PF_{366}, 1.5 mm) using xylene/MEOH (2:3) and ethylacetate/methanol/water (10:2:1) as mobile phases, respectively. The active bands were Fr_{1-1-1} (150.11 mg, R_f, 0.64) and Fr_{3-1-1} (84.43 mg, R_f, 0.70). The chloroform and petroleum ether extracts were subjected to preparative normal phase TLC on silica gel 60 (Fisher, silica gel 60 PF_{254} and PF_{366}, 1.5 mm) using xylene/MEOH (4:1) and ethyl acetate/toluene (1:2) as solvent systems, respectively. For chloroform fractions, only Fr_1 was re-spotted on PTLC plates and developed using ethylacetate/tuolene (1:4) to yield one bioactive fraction (Fr_{1-1}). The resultants bioactive fractions were subjected to GC-MS.

The methanol extract of *T. microcarpus* was subjected to partitioning as above. Only chloroform portion had bioactive activity and was column chromatographed using ethyl acetate/toulene as a solvent gradient system. This yielded four fractions of which Fr_2 and Fr_4 had significant activity against tested micro-organisms. Fractions (Fr_2 and Fr_4) were further fractionated on a preparative normal phase TLC (Fisher, silica gel 60 PF_{254} and PF_{366}, 1.5 mm) using ethyl acetate/toluene (1:4) and ethyl acetate/methanol/water (5:1:0.5) as solvent systems, respectively. The resultant bands were located using UV-light and 10% sulphuric acid spray followed by heating in the oven for 5 min at 120°C. Fr_{2-1} (98.04 mg, R_f, 0.91) and (Fr_{4-3-1}, R_f, 0.54) were biologically active and subjected to GC-MS analysis.

Anti-microbial activity of the isolated fractions: *In vitro* anti-bacterial activity of the fractions was studied using an agar well diffusion method described by Okeke *et al.* (2001). The fractions were diluted in 100% Dimethylsulphoxide (DMSO) at the concentrations ranging from 100-200 μg mL^{-1}. Mueller Hinton agar plate (for bacteria) and Sabouraud Dextrose Agar (SDA) were used. A loop full of suspended organisms (1.5×10^8 CFU mL^{-1}, 0.5 McFarland) was uniformly distributed on the surface of the Mueller Hinton agar plate and Sabouraud Dextrose Agar (SDA) by streaking, using a sterile swab. Wells of approximately, 6 mm in diameter and 2.5 mm deep were made on the surface of the solid medium using a sterile borer. The test fraction/extract (100 μL) was poured into the wells using a microtiter-pipette and allowed to

diffuse at room temperature for 2 h. Tetracycline (10 μg mL^{-1}, Sigma-Aldrich) for bacteria and fluconazole (15 μg mL^{-1}, Sigma-Aldrich) for the fungus were used as positive control. DMSO was used as negative control. The inoculated plates were then incubated for 24 h at 36°C (for bacteria) and at ambient temperature (for fungus) for 48 h. After the incubation period, the plates were carefully examined and the results tabulated. Zones of Inhibition (ZOI) greater or equal to 2.5 mm in diameter were regarded as positive. All the tests were carried out in 3 replicates. The results were recorded as differences between the actual diameter of the zones of inhibition and diameter of the hole.

The minimum inhibition concentration was determined using the broth micro dilution assay with brain heart infusion broth in micro-plates (100 wells) marked into 6 rows. Each row of 10 wells corresponded to the test micro-organism used and each column represented the dilutions of the fractions. The broth (100 μL) was dispensed into micro-plate wells 2-7. About 200 μL of the stock solution of extract was dispensed into well 1. From well 1, 1:2 serial dilution was carried out by transferring 100 μL of stock test sample solution to well 2 and mixed by aspiration and dispensing several times. From well 2, transfer 100 μL to well 3, mix as in well 2 through to well 8. A portion of the mixture (100 μL) was discarded from well 8.

About 50 uL of test micro-organism was dispensed into wells 1-7. Controls were set such that 50 μL of stock test sample, test micro-organisms and broth were dispensed into wells 8-10, respectively. The volumes in wells 8-10 were made up to 100 by 50 μL of broth. Well 8 was for test sample sterility, 9 for organism viability and well 10 for media sterility.

The concentrations used to determine the MIC of the fractions ranged from 200-3.13 μg mL^{-1}. The earlier procedure was set in 2 replicates and carried out for each of the fraction per mushroom species. All inoculated micro plates were properly labeled and incubated under the same conditions as in the zone of inhibition assay. The results were compared with the same standard drugs used in determination of zones of inhibition (2.3.1 above). At the end of the incubation period, growth (turbidity in broth) was observed in wells 1-7 and compared with the controls in wells 8-10.

The results were reported in the form of mean, standard deviation and percentage value of three measurements. The statistical analysis was performed by using one way ANOVA.

Gas chromatography-mass spectrometric analytical conditions: Further fractionation of active fractions was carried out in using a gas chromatography apparatus (Model HP 5890, Hewlett-Packard) coupled with a mass spectrometer (Model HP 5989B, Hewlett-Packard) under the following conditions: column, HP-5; the GC-MS instrument was equipped with a split/splitless injector and an HP-5 55% diphenyl, 95% dimethylpolysiloxane fused column (30 m×0.25×0.25 μm), column oven temperature was initially at 60°C for 1.5 min and raised to 300°C at a rate of 14°C min^{-1} and held for 5 min. Splitless injection was carried out at 240°C. The total run time was 23 min and injected volume (3 μL). The carrier gas (helium) flow rate was 1 mL min^{-1}. MS conditions, ionization energy: 70 eV interface temperature: 320°C.

Identification of compounds: The identification of various compounds present in each sample was based on GC retention time relative to computer matching of mass spectra with those of MS database library (Wiley 275 Library). Each peak in the gas chromatography was examined separately.

The relative percentage of the fraction constituents were expressed as percentages by peak area normalization. Mass spectra patterns of the unknown compounds were within narrow mass ranges (50-60 m z^{-1}) and the GC retention times (about ±1-2 sec) with that of the library.

RESULTS AND DISCUSSION

Anti-microbial activity of isolated fractions: The growth of micro-organisms was inhibited by the fractions in concentration dependant manner as shown in Table 1-3. The isolated fractions exhibited higher anti-microbial activity compared to their corresponding crude extracts (not shown) and had lower activities than the positive controls (Table 2). The isolated fraction of *T. microcarpus* fraction had comparable activity to that of the positive control (tetracycline) against *S. aureus* at concentrations ranging between 150 and 200 μg mL^{-1}.

The PE fractions had higher ZOI than the MEOH fractions. Gram positive bacteria were more sensitive to most fractions. Only Tsp(Fr_{1-1-1})aq methanolic-derived fraction had significant activity against *S. aureus*, MD and *P. aeruginosa* (ATCC 27853) in addition to Tsp(Fr_2)pe against *P. aeruginosa*. Chloroform portion of methanol and PE fractions were active against *C. albicans*. From the MEOH extract, the fraction Tsp(Fr_{1-1-1})aq of *Termitomyces* sp. alone exhibited anti-microbial activity against all tested micro-organisms.

Minimum Inhibition Concentrations (MICs) of isolated fractions: Gram positive bacteria were more sensitive to the fractions. Petroleum ether extracts/fractions had more activity than methanol except for *T. microcarpus* against

Table 1: Anti-microbial activity of isolated fractions of methanol extracts

Test organisms	Extracts/fraction	Concentrations (μg mL^{-1}) 100	150	200
S. aureus (ATCC 25923)	Tsp(Fr_{1-1-1})aq	11.5±00^{a}	12.5±0.2^{c}	14.7±0.12^{b}
	Tsp(Fr_{3-1-1})aq	6.7±0.1^{b}	9.0±0.4^{d}	12.7±00^{c}
	Tsp(Fr_{1-1})chl	8.0±0.02^{d}	12.0±0.3^{c}	14.5±0.02^{b}
	Tm(Fr_{2-1})chl	22.3±0.1^{c}	25.25±0.01^{e}	26.5±0.4^{e}
S. aureus (MD)	Tsp(Fr_{1-1-1})aq	6.25±0.1^{b}	9.45±0.1^{d}	11.0±00^{a}
	Tsp(Fr_{3-1-1})aq	NI	NI	NI
	Tsp(Fr_{1-1})chl	NI	NI	NI
	Tm(Fr_{2-1})chl	NT	NT	NT
B. subtilis (ATCC 6633)	Tsp(Fr_{1-1-1})aq	6.5±0.3^{b}	10.9±0.5^{d}	12.9±0.2^{c}
	Tsp(Fr_{3-1-1})aq	9.3±0.01^{d}	12.3±0.1^{c}	15.5±00^{b}
	Tsp(Fr_{1-1})chl	11±0.33^{a}	14.2±0.2^{b}	16±0.01^{b}
	Tm(Fr_{2-1})chl	15.5±0.2^{ab}	17.0±00^{ba}	18.5±0.5^{ba}
P. aeruginosa (ATCC 27853)	Tsp(Fr_{1-1-1})aq	8.0±0.4^{d}	11.7±00^{a}	14.7±0.3^{b}
	Tsp(Fr_{3-1-1})aq	NT	NT	NT
	Tsp(Fr_{1-1})chl	NT	NT	NT
	Tm(Fr_{2-1})chl	NT	NT	NT
C. albicans (ATCC 10239)	Tsp(Fr_{1-1-1})aq	8.5±0.12^{d}	11±0.05^{a}	14.7±0.04^{b}
	Tsp(Fr_{3-1-1})aq	NI	NI	NI
	Tsp(Fr_{1-1})chl	NT	NT	NT
	Tm(Fr_{2-1})chl	7.00±0.01^{b}	8.5±0.04^{d}	10±0.01^{a}

Table 2: Anti-microbial activity of isolated fractions of petroleum ether extracts and positive controls

Test organisms	Extracts/fraction	Extracts/fraction concentrations (μg mL^{-1}) 100	150	200	Positive controls Tetracycline (10 μg μmL^{-1})	Fluconazole (15 μg μL^{-1})
S. aureus (ATCC 25923)	Tsp(Fr_1)pe	13.25±0.1^{a}	14.2±0.1^{b}	15.5±0.3^{b}	26.7±1.3^{e}	NT
	Tsp(Fr_2)pe	15.2±0.5^{b}	17.25±0.5^{c}	19.00±00^{d}		
S. aureus (MD)	Tsp(Fr_1)pe	NT	NT	NT	26.1±0.4^{e}	NT
	Tsp(Fr_2)pe	NT	NT	NT		
B. subtilis (ATCC 6633)	Tsp(Fr_1)pe	13.0±0.4^{a}	15.2±0.2^{b}	16.0±0.01^{b}	28.3±0.1^{f}	NT
	Tsp(Fr_2)pe	9.5±0.02^{ab}	10±0.15^{ab}	11±0.42^{ab}		
P. aeruginosa (ATCC 27853)	Tsp(Fr_1)pe	NI	NI	NI		
	Tsp(Fr_2)pe	8.4±0.05^{c}	10.0 ±00^{ab}	12.34±1.0^{d}	24.17±0.3^{d}	NT
C. albicans (ATCC 10239)	Tsp(Fr_1)pe	9±0.3^{c}	9.8±0.01^{ab}	11±0.02^{ab}		
	Tsp(Fr_2)pe	6.5±0.1^{c}	8.7±0.05^{d}	10.5±0.3^{ab}	NT	22.5±0.1^{d}

Results are differences between the actual diameter of the ZOI and hole diameter, Values are mean±SD (mm) of two separate experiments, NT: Not Tested, NI: Not Inhibited, Tsp: *Termitomyces* sp., Tm: *T. microcarpus*, T: Tetracycline, FL: Fluconazole, $^{**-d}$Values with the same superscripts along the rows and long the vertical column are not significantly different ($p<0.05$)

Table 3: Minimum inhibition concentration of fractions

	Extract concentration range (200-3.13) μg mL^{-1} (Mean of two replicates)							
	Petroleum ether		Methanol					
	Termitomyces sp.		*Termitomyces* sp.				Positive controls	
Test organisms	Tsp (Fr_1)pe	Tsp (Fr_2)pe	Tsp (Fr_{1-1-1})aq	Tsp (Fr_{3-1-1})aq	Tsp (Fr_{1-1})chl	*T. microcarpus* Tm(Fr_{2-1})chl	Tetracycline (10 μg μL^{-1})	Fluconazole (15 μg μL^{-1})
S. aureus (ATCC 25923)	37.5	12.5	25.0	75	50	12.5	1.17	NT
S. aureus (MD)	NT	NT	62.5	NT	NI	NT	NI	NT
B. subtilis (ATCC 6633)	50	100	25.0	50	37.5	37.5	1.56	NT
P. aeruginosa (ATCC 27853)	NT	75	50.0	NT	NT	NT	18.75	NT
C. albicans (ATCC 10239)	125	100	75.0	NI	100	75	NT	9.38

NT: Not Tested because they were disregarded in the preliminary anti-microbial sensitivity screening, NI: Not Inhibited

S. aureus. The MIC for PE fractions ranged between 12.5-100 μg mL^{-1} for the bacteria and 100-125 μg mL^{-1} for the fungus and for ME fraction, 25-100 μg mL^{-1} and 75-100 μg mL^{-1}, respectively. $Tsp_2(Fr_2)$pe and $Tsp_2(Fr_{1-1-1})$aq exhibited fairly good MICs (50-75 μg mL^{-1}) against *P. aeruginosa*, respectively. Tm(Fr_{2-1})chl from *T. microcarpus* exhibited moderate MICs on the tested micro-organisms (12.5-37.5 μg m^{-1}) for the bacteria and 75 μg mL^{-1} for the fungus.

GC-MS identification of bioactive compounds: The lists of compounds are shown in Table 4-9, respectively. The

Table 4: Chemical constituents of ethanol extract of *T. microcarpus*, fraction Tm(Fr_{2-1})chl

Compounds	Area (%)*	RT (min)	MQ (%)
Fatty acids/esters			
Myristic acid	1.87	12.22	94
Methyl palmitate	1.54	13.41	96
Palmitic acid[a]	34.68	13.71	99
Stearic acid[a]	16.49	15.01	97
Aromatic hydrocarbon/esters			
2H-Pyran-3-ol	1.10	14.41	82
Ambrettolide	3.86	15.19	94
Aromatic N-containing compound			
D-Camphor oxime	2.77	15.44	82
Aromatic S-N -containing compound			
2-Methylsulfonyl-5-dimethylaminomethyl eneaminopyrimidine	0.81	13.05	93
Aliphatic hydrocarbon/alcohol			
2-Pentanone, 4-hydroxy-4-methyl-[a]	24.36	3.16	84
3-Methyl butan-l-al	0.18	11.16	43
1-Hexadecene[a]	9.37	14.88	93

Table 5: Chemical constituents of methanol extract of *Termitomyces* sp. fraction Tsp(Fr_{1-1-1})aq

Compounds	Area (%)*	RT (Min)	MQ (%)
Aromatic N-containing compounds			
2-Methyl-7-phenylindole	2.01	6.80	88
3-Pyridinecarboxylic acid	2.21	7.93	90
N-Methyl-L-prolinol[a]	5.83	9.14	81
Aromatic S-N-containing			
2-Methyl-5- (4'-methylphenyl) sulfonyl-4-nitroimidazole	2.07	8.26	40
Aromatic HC/esters/ketones			
2 (3H)-Furanone, dihydro-3-hydroxy-4, 4-dimethyl- (R)[a]	7.14	5.46	88
Nona-6-ene-8-one	1.06	6.65	83
2-N-Butyltetrahydrofuran	1.31	10.93	49
1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester[a]	4.69	13.06	83
1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester[a]	6.18	13.40	90
1, 2-Benzenedicarboxylic acid, di butyl ester[a]	5.35	13.74	94
Hexadec-7-en-16-olide	2.29	15.18	96
1, 2-Benzenedicarboxylic acid, bis (2-ethylhexyl) ester[a]	35.97	17.36	91
Fatty acids			
Palmitic acid	2.54	13.67	99
Linoleic acid	1.77	15.42	80

Compounds are listed in the order of their elusion from the GC capillary column, [a]Major compounds in the fraction, *Area percentage as calculated from peak areas of the gas chromatogram, MQ: Match Quality of identified compounds with Wiley library standards, RT: Retention Time in minutes of the compound on the GC column

chloroform fraction of *T. microcarpus*, Tm(Fr_{2-1})chl was dominated by fatty acids (44.58%) and a ketone (24.36%). Eleven different compounds were identified, palmitic acid; 4-hydroxy-4-methyl stearic acid, 2-pentanone and 1-hexadecene being the major components as shown in Table 4. The *Termitomyces* sp. methanol fractions, Tsp(Fr_{1-1-1})aq was dominated by aromatic hydrocarbons/ esters (63.99%). The major components were 1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester; 2 (3H)-furanone, dihydro-3-hydroxy-4, 4-dimethyl-(R)-; 1, 2-benzenedicarboxylic acid, bis (2-methylpropyl) ester; N-

Table 6: Chemical constituents of methanol extract of *Termitomyces* sp., fraction Tsp(Fr_{3-1-1})aq

Compounds	Area (%)	RT (Min)	MQ (%)
Aromatic N-containing compounds			
1, 3-dimethyl-4-azaphenanthrenea	6.65	3.03	88
1H-Indene-l, 3 (2H)-dione, 2-(2-pyridinyl)[a]	7.35	3.99	81
1-Methyl-2- (nitrophenyl) benzimidazole[a]	7.79	5.47	80
Isoxazolo (4, 3-a] phenazine, 1-phenyl[a]	3.84	5.76	83
N-[(5-Bromo-6-uracily) acetyl)-D, L	4.44	7.41	87
Morphinan-6-one, 7, 8-didehydro-4, 5 Alpha.-epoxy-14-hydroxyl-3-methoxy l-17-methyl, cyclic ethylene acetal[a]	6.90	9.09	84
1-Decanamine	3.53	9.97	27
Acetamide, N-((8. Alpha., 9R)-9-hydro-	3.36	10.75	28
5-Amino-4-cyano-3-(3-methylaminopy)-	2.66	11.42	9
Phenol, 4-(2-aminopropyl)-, (.$^{+}$.)	0.42	15.41	90
Ketone			
2-Pentanone, 4-hydroxy-4-methyl-[a]	36.46	3.14	80

Table 7: Chemical constituents of methanol extract *Termitomyces* sp., fraction Tsp(Fr_{1-1})chl

Compounds	Area (%)*	RT (min)	MQ (%)
Fatty acids/esters			
Hexanoic acid	0.51	4.89	80
Monomethyl succinate	1.08	6.45	86
Nonanoic acid	0.22	8.04	90
2-Pentenoic acid, methyl ester	0.46	9.58	98
Methyl myristate	0.39	11.89	96
Myristic acid[a]	3.17	12.31	99
Pentadecanoic acid	1.30	13.01	99
Methyl palmitate[a]	2.48	13.42	97
Palmitic acid[a]	19.86	13.92	96
Heptadecanoic acid	0.46	14.42	98
8, 11-Octadecadienoic acid, methyl ester[a]	6.29	14.63	95
Linoleic acid[a]	54.56	15.22	93
Aromatic hydrocarbon/esters			
Benzene acetic acid	0.66	7.94	90
Benzoic acid	0.70	7.17	93
Nitrogen-containing compound			
Cyclo hexanone, O-methyloxime	1.66	10.77	53
Furan, 2-butyltetrahydro-	0.25	10.93	43

Table 8: Chemical constituents of petroleum ether extract of *Termitomyces* sp., fraction Tsp(Fr_{1})pe

Compounds	Area (%)*	RT (min)	MQ (%)
Fatty acids/esters			
Propanoic acid, 2-methy l- 2, 2-dimethyl-1-ethyl (2-hydroxy-1-methylethyl) propyl ester	0.09	8.76	59
Methyl myristate	0.44	11.90	97
Myristic acid	1.53	12.25	98
Pentadecanoic acid, methyl ester	0.21	12.67	95
Pentadecanoic acid	0.56	12.98	99
Methyl palmitate[a]	3.19	13.42	95
Palmitic acid[a]	17.33	13.88	99
Stearic acid	0.36	14.11	95
Heptadecanoic acid	0.41	14.41	94
8, 11-Octadecadienoic acid methyl ester[a]	9.22	14.63	95
Linoleic acid[a]	60.82	15.20	93

Compounds are listed in the order of their elusion from the GC capillary column, [a]Major compounds in the fraction, *Area percentage as calculated from peak areas of the gas chromatogram, MQ: Match Quality of identified compounds with Wiley library standards, RT: Retention Time in minutes of the compound on the GC column

Table 9: Chemical constituents of petroleum ether extract of *Termitomyces* sp. fraction Tsp(Fr_2)pe

Compounds	Area (%)*	RT (min)	MQ (%)
Aliphatic hydrocarbons			
Pentadecane	0.96	10.01	90
Hexadecane	0.16	10.87	96
Heptadecane	1.01	11.68	98
Octadecane	0.64	12.45	98
Triacontane[a]	6.45	13.06	86
Nonadecane	0.51	13.19	98
Eicosane	0.96	13.89	97
Docosane	0.12	17.00	93
Hexacosane	0.13	18.09	95
Squalene	0.31	18.80	94
Fatty acids/esters			
Myristic acid	0.15	12.19	98
Palmitic acid	2.01	13.72	68
1-MethylethylPalmitate	0.16	14.09	97
Linoleic acid[a]	16.43	14.91	91
Stearic acid[a]	2.94	15.04	95
Propanoic acid, 2-methyl-, 1-(1,1-dimethylethyl)-2-methyl-1, 3-propanediyl ester	0.92	10.93	60
Aromatic hydrocarbon esters			
2 (3H)-Furanone, dihydro-3-hydroxy-4, 4-dimethyl- (R)-	1.14	5.42	80
Nona-6-ene-8-0ne	0.57	6.65	43
1, 2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester[a]	6.43	13.41	84
(5Z, 7Z)-4-methoxy-5-(1-methoxy-3-phenyl-2-propenyidene) -2 (5H)- Furanone[a]	36.90	17.92	88
Aromatic S-N-containing compound			
12H-Quinoxalino (2, 3-B)(1,4) Benzothiazane	0.36	16.75	64
Ketones/alcohol			
Ethanol, 2-(trimethylsilyl)-, acet	2.08	4.73	37

Compounds are listed in the order of their elusion from the GC capillary column, [a]Major compounds in the fraction, *Area percentage as calculated from peak areas of the gas chromatogram, MQ: Match Quality of identified compounds with Wiley library standards, RT: Retention Time in minutes of the compound on the GC column

methyl-L-prolinol and 1, 2-benzenedicarboxylic acid, di butyl ester (Table 5). Tsp(Fr_{3-1-1})aq was dominated by aromatic N-containing compound (28.27%) and ketones (36.46%) of which 2-pentanone, 4-hydroxy-4-methyl; 1H-Indene-l, 3 (2H)-dione, 2-(2-pyridinyl); morphinan-6-one, 7, 8-didehydro-4, 5. Alpha-epoxy-14-hydroxyl-3-methoxyl-17-methyl, cyclic ethylene acetal; 1, 3-dimethyl-4-azaphenanthrene; isoxazolo (4, 3-a) phenazine, 1-phenyl and 1-methyl-2- (nitrophenyl) benzimidazole were the main components (Table 6). Tsp(Fr_{1-1})chl was high in fatty acids (90.78%) with linoleic acid; palmitic acid; 8, 11-octadecadienoic acid, methyl ester; methyl palmitate and myristic acid being the major compounds (Table 7). Tsp(Fr_1)pe revealed 12 compounds, linoleic acid; palmitic acid; 8, 11-octadecadienoic acid methyl ester and methyl palmitate were the main components (Table 8). Tsp(Fr_2)pe had 22 different components of which (5Z, 7Z) -4-methoxy-5- (1-methoxy-3-phenyl-2-propenyidene) -2 (5H)- furanone; linoleic acid; 1, 2-benzenedicarboxylic acid (2-methylpropyl) ester and triacontane were dominant (Table 9).

The chloroform fraction of *T. microcarpus*, Tm(Fr_{2-1})chl exhibited comparable anti-bacterial activity as tetracycline against *S. aureus* (ATCC 25923) (Table 1) and had moderate activity against *C. albicans*. The anti-microbial effects of this fraction may have been contributed mainly by the dominant fatty acids. However, it is also possible that the minor components in the fraction such as 2-pyra-no-l, D-camphor oxime and 2-methylsulfonyl-5-dimethylaminomethyl eneaminopyrimidine might have been involved in some type of anti-microbial synergism with other active components. The biological activities of fatty acids in relation to anti-microbial activity have been established (Seidel and Taylor, 2004; Agoramoorthy *et al.*, 2007; Manivachagam *et al*., 2008). Saturated fatty acids (palmitic saturatedc fatty acids (plamitic and stearic acid) are known to cause modifications (i.e., decreasing the intracellular pH) of the intracellular pH of the microbe below the survival level, thus killing the organisms instantly 2-Pentanone, 4-hydroxy-4-methyl has been isolated from natural sources. For instance, it was a major component of essential oil of *Nandina domestica* which had complete growth inhibition of plant fungi (Bajpai *et al*., 2008). Furthermore, an ambrettolide (synonym: omega-6-hexadecenlactone) which is a natural essential oil with powerful smelling scent was also identified in this mushroom. This essential oil is used in perfumes as a scent and fixative (http://chemicalland21.com/specialtychem/NH/AMBRETTOLIDE.htm). Its use as an anti-microbial has not been reported in literature. It is presumed that this component produces the powerful

aroma of this mushroom. As far as literature is concerned, the occurrence of 2-pentanone, 4-hydroxy-4-methyl and ambrettolide has never been reported in mushrooms.

The methanol extract of *Termitomyces* sp., Tsp(Fr_{1-1-1})aq, exhibited promising anti-microbial activity against bacteria but had low activity against the fungus (Table 1 and 3). The anti-microbial effects observed were believed to be due to phthalic acid derivatives (1, 2-benzenedicarboxylic acid, bis (2-ethylhexyl) ester, 1, 2-benzenedicarboxylic acid, di butyl ester and 1, 2-benzenedicarboxylic acid, bis (2-methylpropyl) ester), 2-pentanone, 4-hydroxy-4-methyl and alkaloids (2 (3H)-furanone, dihydro-3-hydroxy-4, 4-dimethyl-(R) and N-methyl-L-prolinol.

This fraction is unique in that its exhibited some activity against *S. aureus*, MD and *P. aeruginosa*, probably due to the synergistic action of phthalic acid derivatives and alkaloids such as 2-methyl-7-phenylindole, 3-pyridinecarboxylic acid and 2-methyl-5-(4'-methylphenyl) sulfonyl-4-nitroimidazole. Anti-microbial activity of phthalic acid derivatives against several micro-organisms including *P. aeruginosa* have been reported (Chen, 2004; Al-Bari *et al*., 2006; Sani and Pateh, 2009). Other biological activities of phthalates include anti-leukaemic and anti-mutagenic properties (Lee *et al*., 2000). Phthalic acid derivatives occur naturally in other organisms (Kavitha *et al*., 2009; Srinivasan *et al*., 2009, Lyutskanova *et al*., 2009; Gohar *et al*., 2010). Chemically synthesized phthalates are produced by organic synthesis from vacuum oils and are used as plasticizers (Graham, 1973). *Termitomyces* sp. contained azaphenanthrene among other compounds and has been reported to have anti-microbial activity (Gupta *et al*., 1970). The chloroform fraction of *Termitomyces* sp., Tsp(Fr_{1-1})chl had significant anti-microbial activity against all tested micro-organisms due to high amounts of fatty acids. Linoleic acid is a known anti-bacterial agent (Seidel and Taylor, 2004; Zheng *et al*., 2005) and it acts through inhibition of the bacterial enoyl-acyl carrier protein reductase (FabI), a protein necessary for fatty acid synthesis in the target organism (Zheng *et al*., 2005). The lack of activity of this fraction against gram negative may be derived from the impermeability nature of their outer cell membrane against hydrophobic substances. The Candida species were also found to be inhibited in addition to other organisms by the fatty acid methyl esters comprising mainly of palmitic acid (Sheu and Freese, 1973). However, the anti-microbial activities of the minor components such as benzoic acid and bis (4-ethylhexyl) phthalate in the fraction should not be underestimated. Compound (5Z, 7Z)-4-methoxy-5-(1-methoxy-3-phenyl-2-propenyidene)-2 (5H) -furanone (36.90%) in the petroleum ether fraction of *Termitomyces* sp. Tsp(Fr_2)pe, together with linoleic acid may have contributed to the strong anti-microbial activity against *P. aeruginosa*. The anti-microbial activity of furanones is documented (Lattmann *et al*., 2005; Viresh and Bharti, 2009).

CONCLUSION

The mushrooms under study have a diversity of bioactive compounds which may be very useful in the management of common human pathogenic organisms. This diversity highlights these mushrooms as good candidates for prospecting for novel bioactive compounds against many emerging multi-resistant strains particularly, those of *S. aureus* and *P. aeruginosa*. Researchers recommend further studies to test the pure compounds that have been isolated against microbes especially, the *P. aeruginosa* and the multi-resistant strain of *S. aureus*.

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