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Evaluation of the mycochemical composition and antimicrobial potency of wild macrofungus, *Rigidoporus microporus* (Sw).

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ABSTRACT

This study examined the myochemical and antimicrobial properties of extracts obtained from *Rigidoporus microporus* (Sw), a wild, non- edible mushroom. Methanol and acetone extracts of *R. microporus* were screened qualitatively and quantitatively. Also the antimicrobial effect of the crude and partially purified fractions of the extracts of *R. microporus* was evaluated on clinical and referenced microbial cultures. The probable mechanism of action of the extract was assessed by measuring the amount of sodium and potassium ions leaked from the bacterial and fungal cells. In addition, the chemical compositions of mushroom extracts were analyzed using GC-MS and fragmentation analyses. Results of the phytochemical analysis revealed the presence of tannins, saponins, terpenoids, flavonoids, steroids and cardiac glycosides in the extracts with values ranging between 2.692 to 23.435 mg/g. Among the phytochemicals, terpenoids (23.65 mg/g) and cardiac glycosides (22.57 mg/g) were the most abundant while flavonoid (2.54 mg/g) was the least abundant in the mushroom extracts. The extracts produced varying antimicrobial activities with zones of inhibition ranging from 4.03 to 18.00mm. The methanol extract displayed better antimicrobial activities. *Pseudomonas aeruginosa* ATCC 29853 and *Aspergillus fumigatus* were the most susceptible bacterial (16.77mm) and fungal isolate (18.00mm) respectively. Lesser minimum inhibitory concentration values were expressed by the extracts against the fungal isolates (6.25 mg/mL). The partially purified fractions of *Rigidoporus microporus* extracts exhibited better antimicrobial activities against the selected test organisms with zones of inhibition ranging from 10.17mm to 32.32mm. *Candida albicans* had the highest leakage of sodium (390 mg/L) and potassium (455 mg/L) when exposed to the mushroom extracts, whereas *Pseudomonas aeruginosa* (80mg/L) and *Klebsiella pnuemoniae* (225 mg/L) produced the least amount of leaked sodium and potassium ion respectively. The bioactive compounds found in the extracts were hexadecane, n-hexadecanoic acid, pentadecanoic acid, octadecane, hexadecanoic acid, n-octadecenoic acid, di-n-octyl phthalate (1, 2-benzenedicarboxylic acid, dioctyl ester) and n-decanoic acid. These findings has shown that *Rigidoporus microporus* (Sw) collected from Ondo State, Nigeria could be considered a potential source of natural antimicrobials and could be exploited in the pharmaceutical industry.

Keywords: *Rigidoporus microporus*, Mycochemicals, Antimicrobial, Ondo state.

INTRODUCTION

The phenomenon of resistance of pathogenic organisms to conventional antimicrobials had increased greatly globally. According to the World Health Organization [1], the rise in antimicrobial resistance is an impediment to the treatment and prevention of diseases of microbial origin. Resistant microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* pose a real threat to the society [2]. This significant increase in emergence of antibiotic resistant microorganism has accelerated research into natural sources for novel and effective antimicrobial substances.

Macrofungi have been exploited as functional foods and sourced for the development of drugs in recent time [3]. In order to survive the extreme environment in which they grow, wild mushrooms accumulate a wild variety of pharmacological agents which are mostly left unexploited [4]. These pharmacological agents have been reported to possess anticancer, immunostimulatory, hypotensive, hypocholesterolaemic and antibacterial effects [5, 6].

The aim of this research was to evaluate the antimicrobial potentials of crude and partially purified fractions of *Rigidoporus microporus* extracts against some selected microorganisms in order to ascertain their utilization as possible alternatives to synthetic antimicrobials.

MATERIALS AND METHODS

Collection, Identification and Preservation of mushroom

Samples of the wild mushroom, *Rigidoporus microporus*, were collected on rotten woods from the wild around Aba-Oyo Community, Akure (Latitude: N 7°17'49.5" and longitude: E5° 07'20.5") in July, 2013. The mushroom was identified and authenticated in the Department of Microbiology, Federal University of Technology, Akure (FUTA). Afterwards the mushroom samples were cleansed with clean water and soil debris removed. The mushroom samples were thereafter dried in an oven at 60°C for 72 hours, cut into bits and then pulverized with the aid of an electric mill at the Department of Animal Production and Health, FUTA. The powdered mushroom sample was afterwards transferred into air tight container to avoid absorption of moisture.

Preparation of extracts

Mushroom samples (400 g) were weighed into clean containers, into which 4 liters of 100% methanol and acetone were added respectively. The mixtures was left covered in the laboratory for 72 hours with frequent stirring after which each mixture was sieved using three fold sterile muslin cloth and filtered further using No 1 Whatman filter paper (0.45µm). The filtrates were concentrated *in vacuo* using rotary evaporator (RE-52A; Union Laboratory, England). The extracts obtained were stored in refrigerator at 4°C.

Phytochemical screening of mushroom extracts

The methanol and acetone extracts of *Rigidoporus microporus* were subjected to qualitative phytochemical screening for the presence of anthraquinones, alkaloids, tannins, saponins, phlobatannins, steroids, flavonoids, terpenoids and cardiac glycosides using the methods of Sofowora [7], Harbone [8] and Trease and Evans [9]. Quantitative phytochemical screening of the mushroom extract was also evaluated using spectrophotometric methods described by Brunner [10], Sofowora [7], Harbone [8] and Trease and Evans [9].

Antimicrobial activity of crude mushroom extracts

Antimicrobial activity of the extracts was performed against clinical and referenced microbial cultures collected from the Department of Microbiology, Obafemi Awolowo University Teaching Hospital (OAUTH), Ile-Ife, Osun State, Nigeria and the University College Hospital (UCH), Medical Microbiology Laboratory, Ibadan, Oyo state, Nigeria. The agar well diffusion method as described by Esimore *et al*. [11] and Toki *et al.* [12] was employed in evaluating the antimicrobial efficacy of the extracts. The bacterial isolates suspensions were standardized spectrophotometerically to 10^6–10^8 CFU/ml using 0.5 McFarland solution, while the fungal spore suspensions were adjusted to 10^6 spores per/ml by microscopic enumeration with a cell counting hematocytometer.

Mueller Hinton agar plates were prepared, allowed to solidify and seeded with 100µL of the bacterial and fungal suspension. Wells of 7mm in diameter were subsequently bored into the agar using a sterile cork borer. Exactly 0.1 ml of 100 mg/mL of the extract was then introduced into the wells and appropriately labeled. The plates were allowed to stand on the laboratory bench for 15 minutes to allow diffusion of the solution into the medium. The plates were then incubated at 37°C for 24 hours for bacteria and 27°C for 48 hours for fungi. Clear zones of inhibition were read after the incubation period with a ruler. To determine the minimum inhibitory concentration (MIC), varying concentrations of the extracts (50, 25 12.5 and 6.25mg/mL) were prepared and used against the test organisms as indicated above. The least concentration of the mushroom extracts without any visible growth was regarded as the MIC.

Determination of Sodium and Potassium ion leakage

The sodium and potassium ion leakage was determined using the method described by Oladunmoye *et al*. [13]. An aliquot of 0.5mL each of the standardized organisms was added to 4.5mL of the prepared concentration of the mushroom extracts. Afterwards the supernatant solution obtained after been centrifuged at 7000rpm was analysed for the presence of sodium and potassium ion using a flame photometer at 589nm and 766nm respectively.

Antimicrobial activity of partially purified fractions against selected clinical isolates.

Freshly prepared inoculum (100µL) of selected clinical isolates as described above was spread evenly on the surface of the plates and allowed to stand for about 5 minutes. Afterwards sterile paper discs impregnated with the partially purified fractions of the extracts were aseptically placed on the surface of the plate using a sterile forceps. The plates were then incubated at 37°C for 24 hours for bacteria and 27°C for 48 hours for fungi. Antibacterial and antifungal activities were determined by measuring clear zones of inhibition around each paper disc with a ruler.

Partial purification of mushroom extracts

The mushroom extracts were partially purified using column chromatography as described by Philip [14]. The column was packed using a simple dry-pack method. A 250ml burette was plugged with a small piece of glass wool with the aid of applicator stick to tamp it down lightly. Exactly 60 grams of column chromatography silica gel was poured into the plugged burette using a 100ml beaker. The filled column was clamped securely to a retort stand using a small three prolonged clamp and placed on the bench top. Petroleum ether was added to the top of the silica gel to flow slowly down the column. One gram of the sample to be purified was thoroughly mixed with a small amount of the column chromatography silica gel and then loaded onto the column. Petroleum ether, ethyl acetate, methanol and acetone: ethyl acetate in ratio 1:4 respectively was used to elude the packed column until there were no more bands. About 100ml fractions were collected in round bottom flasks and distillated. Afterwards, thin layer chromatography plates were activated by heating at 110°C for 15 minutes. Fractions obtained from column chromatography were spotted on thin layer chromatography plates using 2µl micropipettes. Ethyl acetate, chloroform and methanol: ethyl acetate in ratio 1:3 was employed as the mobile phase. The thin layer chromatography plates were allowed to develop over a path of 8cm, and air-dried at room temperature for one hour before viewing in the iodine chamber. The refractive fraction (R_f) was measured and calculated as:

Refractive fraction (R_f) = Distance travelled by solute ÷ Distance travelled by solvent.

Gas Chromatography Mass Spectrometry (GC-MS) analysis of partially purified fractions

GC-MS analysis of the partially purified mushroom fractions was performed using a Perkin Elmer GC Claurus 500 system and Gas

Chromatograph interfaced to a mass spectrometer (GC/MS) equipped with an Elite-fused silica capillary column (30m × 0.25mm, coating thickness 0.25, and composed of 100% Dimethyl poly siloxane). For GC-MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1ml/min and an injection volume of 2µL was employed (at split ratio of 10:1). Injector temperature and ion source temperature were set at 250°C and 280°C respectively. The oven temperature was programmed from 110°C (isothermal for 2mins), with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, and ending with 9 minutes isothermal at 280°C. The molecular weight of decomposition products were performed at the scan range of 60-200g/mol at an interval of 0.5secs. Diluted samples (1/100 in chloroform, v/v) of 1.0µL were injected manually. Mass spectra were taken at 70eV. Total GC running time was 36minutes. The identification of components was conducted according to the comparison of their mass spectra with those of Wiley 7N which (contains 392.086 compounds spectra) and Nist 2002 which (contains 174.948 compounds spectra) libraries and as well as by comparison of their retention times [15]. The relative percentage of each component was calculated by comparing its average peak area to the total area. Software adopted to run the mass spectra and chromatograms was TurbiMass Version 5.2.0.

Statistical analysis

The experiments were carried out in triplicates where applicable. Data obtained were subjected to One Way Analysis of Variance (ANOVA) and the means were compared using the New Duncan's Multiple Range Test (SPSS version 16). P values less than 0.05 were considered to be significant.

RESULTS

Tannin, saponin, terpenoid, alkaloid, steroid, phlobatannin, anthraquinone and cardiac glycoside, were found in the mushroom extracts while anthraquinone, phlobatannin and alkaloids were absent as presented in Table 1. Quantitatively, terpenoid concentration (23.65 mg/g) in methanol extract was the highest followed by cardiac glycoside concentration (22.57 mg/g) found in acetone extract (Fig. 1). As displayed in Figure 1, flavonoid had the lowest concentration in both methanol extract (2.54 mg/g) and acetone extract (2.63 mg/g).

All the test organisms were susceptible to the methanol and acetone extract of *R. microporus* at a concentration of 100mg/mL except for the clinical isolates of *Bacillus cereus* and *Staphylococcus aureus* (Table 2). The extracts produced slightly better antifungal activity than antibacterial activity as depicted by the zones of inhibition produced. Also, the methanol extract was mostly effective against the test organisms. The highest zone of inhibition (18.00mm) was exhibited by the methanol extract against *A. fumigatus* while the lowest zone of inhibition (4.03mm) was displayed by the acetone extract against the clinical strain of *Escherichia coli*. The commercial drugs were more effective in their antimicrobial action. The minimum inhibitory concentration (MIC) values for ranged from 6.25 to 25 mg/mL for the methanol extract and 6.25 to 100 mg/mL for the acetone extract (Table 3).

Table 1: Qualitative phytochemical screening of *extract of R. microporus*

Phytochemical	Extracts	
	RMM	**RMA**
Saponin	-	+
Tannin	+	+
Flavonoid	+	+
Steroids	+	+
Terpenoids	+	+
Alkaloids	-	-
Phlobatannin	-	-
Anthraquinone	-	-
Cardiac Glycosides		
Legal test	+	+
Keller kiliani	+	+
Salkowski	+	+
Liberman test	+	+

Keys: RMM: Methanol extract of R. microporus; RMA: Acetone extract of R. microporus; +: Present; -: Absent

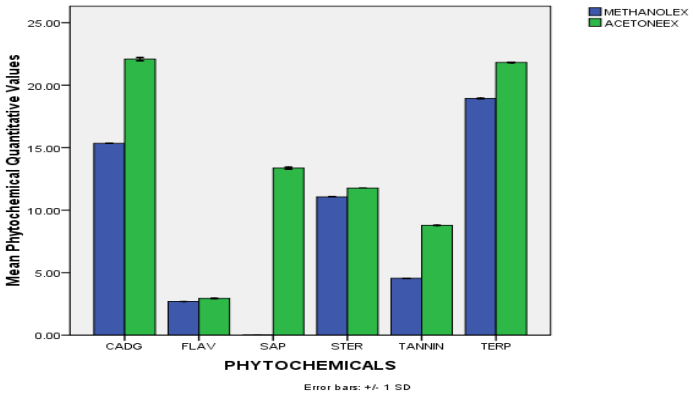


Figure 1: Quantitative phytochemical composition (mg/g) of the extracts of *R. microporus*

Keys: CADG: Cardiac glycosides; FLAV: Flavonoid; SAP: Saponin; STER: Steroid; TERP: Terpenoid; METHANOLEX: Methanol extract; ACETONEEX; Acetone extract.

Table 3: The Mean Minimum Inhibitory Concentration (mg/ml) of mushroom extracts against clinical bacterial isolates

Test Organisms	RMM	RMA
Escherichia coli *	12.5	100
Pseudomonas aeruginosa *	12.5	25
Bacillus cereus *	ND	ND
Staphylococcus aureus *	ND	ND
Methicillin Resistant *Staphylococcus aureus* *	25	50
Salmonella typhi *	25	50
Klebsiella pneumonia *	12.5	12.5
Enterococcus faecalis *	25	50
Escherichia coli ATCC 23718	12.5	25
Escherichia coli ATCC 35218	25	12.5
Pseudomonas aeruginosa ATCC 29853	12.5	6.25
Bacillus cereus NCIB 6344	12.5	50
Staphylococcus aureus NCIB 950	12.5	100
Salmonella typhi ATCC 33458	12.5	25
Klebsiella pneumoniae ATCC 25922	12.5	6.25
Candida albicans *	6.25	12.5
Aspergillus fumigatus	6.25	12.5
Aspergillus flavus	6.25	12.5

Keys: RMA: Acetone extract of R. microporus; RMM: Methanol extract of R. microporus; ATCC: American Type Culture Collection; NCIB: National Collection for Industrial Bacteria; *: Clinical isolate; ND: Not determined.

Table 2: Antimicrobial activities of extracts of *R. microporus* and commercial drugs against test organisms

Test organisms	Zones of inhibition (mm)							
	RMM (100mg/ml)	**RMA (100mg/ml)**	**Gentamicin (10µg)**	**Nalidixic acid (30µg)**	**Ofloxacin (5µg)**	**Nystatin (1mg/ml)**	**Ketoconazole (1mg/ml)**	**Griseofulvin (1mg/ml)**
Escherichia coli *	6.73±0.14^{b}	4.03±0.14^{a}	10.00±0.58^{c}	12.33±1.20^{d}	21.33±0.88^{e}	ND	ND	ND
Pseudomonas aeruginosa *	6.67±0.09^{a}	6.73±0.15^{a}	12.00±0.29^{b}	15.83±0.17^{c}	20.00±0.00^{d}	ND	ND	ND
Bacillus cereus *	0.00±0.00^{a}	0.00±0.00^{a}	12.67±0.88^{b}	14.33±2.18^{b}	25.50±0.28^{c}	ND	ND	ND
Staphylococcus aureus *	0.00±0.00^{a}	0.00±0.00^{a}	12.00±1.16^{b}	16.00±1.16^{c}	20.67±1.20^{d}	ND	ND	ND
MRSA *	6.37±0.07^{a}	5.00±0.29^{a}	11.33±0.88^{b}	19.00±0.58^{c}	25.00±1.16^{d}	ND	ND	ND
Salmonella typhi *	9.37±0.12^{b}	5.00±0.29^{a}	12.50±0.29^{c}	12.00±0.29^{c}	14.80±0.15^{d}	ND	ND	ND
Klebsiella pneumoniae	8.37±0.07^{a}	9.40±0.06^{b}	10.50±0.29^{c}	17.50±0.29^{d}	24.83±0.17^{e}	ND	ND	ND
Enterococcus faecalis *	7.10±0.17^{a}	9.07±0.12^{ab}	11.33±0.88^{c}	20.33±0.33^{d}	19.00±1.53^{d}	ND	ND	ND
Escherichia coli ATCC 23718	8.50±0.12^{b}	6.27±0.15^{a}	10.33±0.33^{c}	13.00±0.58^{d}	26.50±0.29^{e}	ND	ND	ND
Escherichia coli ATCC 35218	7.43±0.07^{a}	6.37±0.07^{a}	15.00±0.58^{b}	20.00±0.58^{c}	32.33±0.88^{d}	ND	ND	ND
Pseudomonas aeruginosa ATCC 29853	6.37±0.07^{b}	16.70±0.11^{b}	11.00±0.57^{c}	0.00±0.00^{a}	21.33±0.88^{e}	ND	ND	ND
Bacillus cereus NCIB 6344	6.70±0.00^{a}	6.77±0.07^{a}	12.67±0.88^{b}	14.33±2.19^{b}	25.50±0.29^{c}	ND	ND	ND
Staphylococcus aureus NCIB 950	6.63±0.07^{a}	4.70±0.06^{a}	12.33±1.20^{b}	11.33±0.88^{b}	14.83±0.60^{c}	ND	ND	ND
Salmonella typhi ATCC 33458	7.00±0.29^{b}	5.00±0.29^{a}	12.67±0.88^{c}	21.33±0.88^{d}	24.50±0.29^{e}	ND	ND	ND
Klebsiella pneumoniae ATCC 25922	10.10±0.10^{a}	10.10±0.10^{a}	17.17±0.17^{b}	22.50±0.29^{c}	25.83±0.44^{d}	ND	ND	ND
Candida albicans *	7.36±0.11^{c}	12.50±0.29^{d}	ND	ND	ND	6.17±0.17^{b}	0.00±0.00^{a}	20.50±0.29^{e}
Aspergillus fumigatus	18.00±0.58	9.17±0.44^{a}	ND	ND	ND	20.00±0.29^{c}	18.70±0.12^{c}	18.00±0.29^{c}
Aspergillus flavus	15.50±0.50^{c}	11.65±0.33^{b}	ND	ND	ND	18.50±0.28^{d}	8.88±0.09^{a}	9.37±0.18^{a}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a column are significantly different at (P=.05).
Keys: **RMA**: Acetone extract of *R. microporus*; **RMM**: Methanol extract of *R. microporus*; **MRSA**: Methicillin resistant *Staphylococcus aureus*; **ATCC**: American Type Culture Collection; **NCIB**: National Collection for Industrial Bacteria; *****: Clinical isolate; **ND**: Not determined.

The partially purified fractions of the mushroom extracts produced higher zones of inhibition against the selected clinical test organisms when compared with the activity of the crude extracts. The zones of inhibition produced ranged from 10.17 to 32.32mm (Table 4). The ethyl acetate acetone fraction at ratio 1 to 3 was the most effective and produced the highest zone of inhibition (32.20mm) against *Candida albicans*.

Table 4: Antimicrobial activity of purified fractions of mushroom extract against selected clinical bacterial and fungal isolates

Test Organisms	P1	P2	E1	E2	EA	A2	AM	MI
Pseudomonas aeruginosa	0.00±0.00^{a}	0.00±0.00^{a}	20.50±0.29^{c}	22.17±0.17^{d}	28.33±0.33^{e}	0.00±0.00^{a}	10.17±0.17^{b}	0.00±0.00^{a}
Salmonella typhi	0.00±0.00^{a}	0.00±0.00^{a}	18.17±0.17^{b}	18.17±0.17^{b}	25.17±0.41^{c}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Escherichia coli	0.00±0.00^{a}	0.00±0.00^{a}	23.33±0.33^{d}	20.50±0.29^{c}	30.50±0.29^{e}	0.00±0.00^{a}	14.17±0.17^{b}	0.00±0.00^{a}
Klebsiella pneumonia	0.00±0.00^{a}	0.00±0.00^{a}	18.17±0.17^{b}	20.50±0.29^{c}	23.33±0.33^{d}	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}
Candida albicans	0.00±0.00^{a}	0.00±0.00^{a}	27.50±0.29^{d}	23.33±0.33^{c}	32.32±0.32^{e}	0.00±0.00^{a}	19.17±0.44^{b}	0.00±0.00^{a}

Each value is expressed as mean ± standard error (n = 3). Values with different superscript within a column are significantly different at (P=.05).
Keys: P1, P2: Petroleum ether, E1, E2: Ethyl acetate, EA: Ethyl acetate acetone (1: 3), A2: Acetone, AM: Acetone methanol (1: 3), M1: Methanol, NI: No inhibition

Table 5: Quantities of sodium and potassium ion leakage from selected clinical bacterial and fungal isolates by extracts of *R. microporus*

Test organisms	Acetone extract		Methanol extract	
	Na^{+} (mg/L)	K^{2+} ion (mg/L)	Na^{+} (mg/L)	K^{2+} ion (mg/L)
Candida albicans	380.00	427.50	390.00	455.00
Klebsiella pnuemoniae	315.00	400.00	180.00	225.00
Pseudomonas aeruginosa	80.00	405.00	358.00	450.00
Escherichia coli	355.00	430.00	375.00	420.00
Salmonella typhi	275.00	300.00	370.00	415.00

The quantities of sodium and potassium ions leaked from the bacterial and fungal cells by the methanol and acetone mushroom extracts are presented in Table 5. *Candida albicans* had the highest leakage of sodium and potassium ion (390 mg/L and 455 mg/L respectively) when exposed to the methanol extract of the mushroom, whereas *Klebsiella pnuemoniae*, had the least amount of sodium and potassium ion (180 mg/L and 225 mg/L respectively) leaked from the cell. Similarly, the highest quantity of sodium and potassium ion leaked by the acetone extract of *R. microporus* was from *Candida albicans* (380 mg/L) and *Escherichia coli* (430 mg/L). Whereas, the lowest quantity of sodium and potassium ions leaked from *Pseudomonas aeruginosa* (80 mg/L) and *Salmonella typhi* (300 mg/L) in the presence of the acetone extract. Generally, more potassium ions were leaked from the bacterial and fungal cells in comparison to sodium ion.

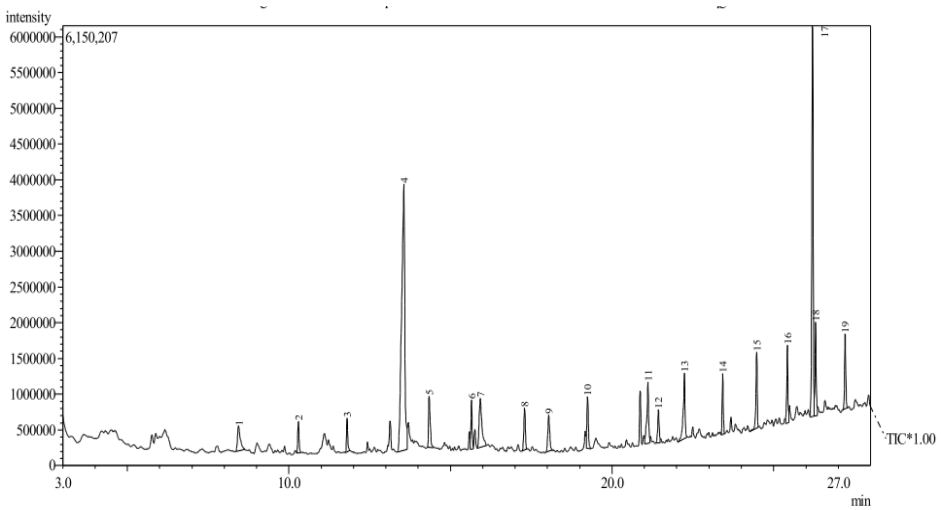


Figure 1: The reproducible volatile chromatogram of the E1 (Ethylacetate 1) fraction of *R microporus* extract by HS-SPME. The peak numbers correspond to the typical volatile compounds that are continuously studied.

Chemical compositions of mushroom extracts analysed using GC-MS technique and fragmentation analysis is presented in Figures 1, 2 and 3. Twenty-five compounds were identified in *R. microporus* extracts by GC-MS analysis (Tables 6, 7 and 8). The prevailing compounds were hexadecane, n-hexadecanoic acid, pentadecanoic acid, octadecane, hexadecanoic acid, n-octadecenoic acid, di-n-octyl phthalate (1, 2-benzenedicarboxylic acid, dioctyl ester) and n-decanoic acid.

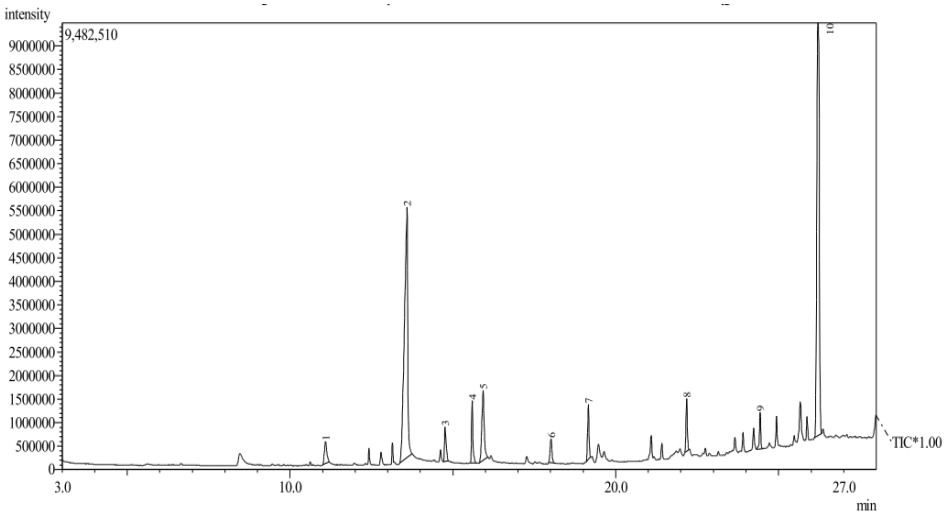


Figure 2: The reproducible volatile chromatogram of the E2 (Ethylacetate 2) fraction of *R. microporus* extract by HS-SPME. The peak numbers correspond to the typical volatile compounds that are continuously studied.

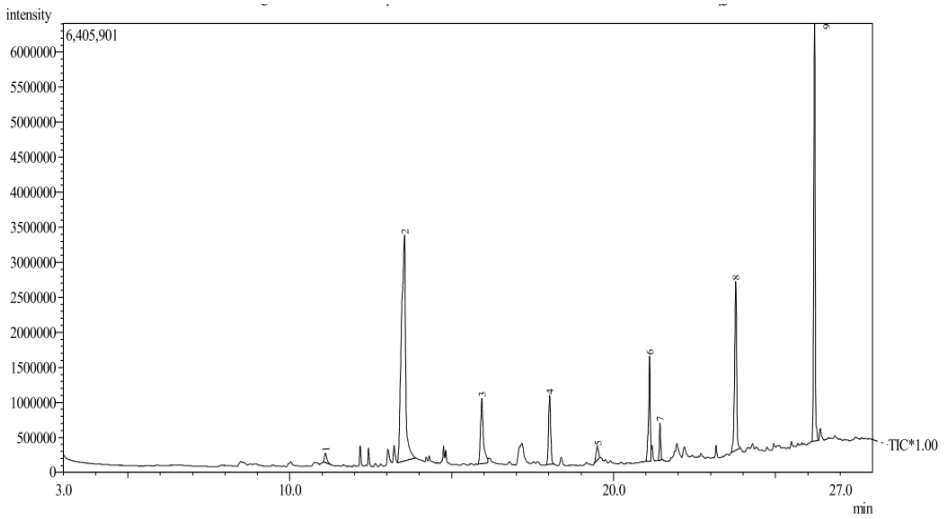
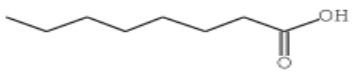


Figure 3: The reproducible volatile chromatogram of the EA (Ethylacetate acetone in ratio 1:3) fraction of *R. microporus* extract by HS-SPME. The peak numbers correspond to the typical volatile compounds that are continuously studied.

Table 6: GC-MS Identified components of the E1 fraction of *R. microporus* mushroom extract (Compounds are listed in ascending order of Retention Time)

Peak/No	Retention Time (Minutes)	Name of Compounds	Functional Group of compounds
1	08.442	Octanoic acid	
2	10.293	Hexadecane	
3	11.801	Tridecane	

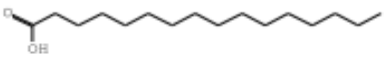
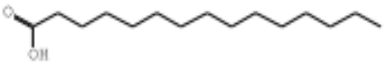
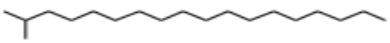
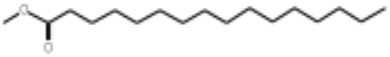
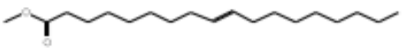
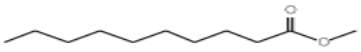
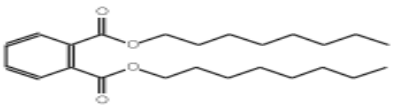
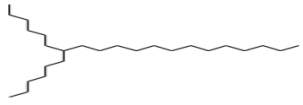
4	13.558	n-Hexadecanoic acid	
5	14.339	n-Hexadecane	
6	15.650	n-Tridecane	
7	15.923	Pentadecanoic acid	
8	17.293	Octadecane	
9	18.039	Hexadecanoic acid	
10	19.242	Nonadecane	
11	21.110	n-Octadecenoic acid	
12	21.435	Decanoic acid	
13	22.238	Nonadecane	
14	23.424	Pentadecane	
15	23.473	Octadecane	
16	25.424	Hexadecane	
17	26.204	Di-n-Octyl phthalate	
18	26.299	Eicosane	
19	27.212	7-n-Hexyleicosane	

Table 7: GC-MS Identified components of the E2 fraction of *R. microporus* mushroom extract (Compounds are listed in ascending order of Retention Time)

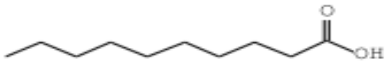
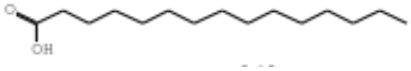
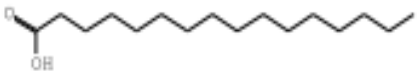
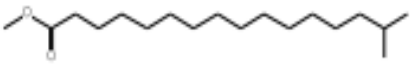
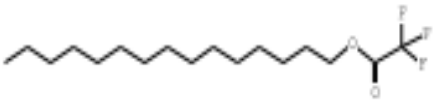
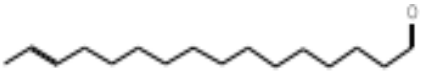
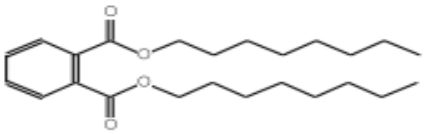
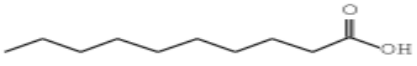
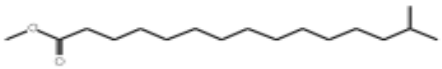
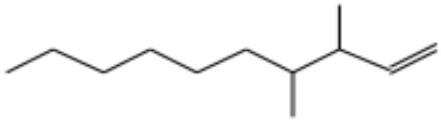
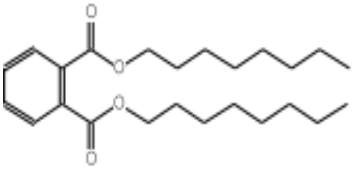
Peak/No	Retention Time (Minutes)	Name of Compounds	Functional Group of compounds
1	11.089	n-Decanoic acid	
2	13.591	Pentadecanoic acid	
3	14.760	Hexadecanal	
4	15.593	1-Tetradecene	
5	15.930	n-Hexadecanoic acid	
6	18.014	Hexadecanoic acid	
7	19.159	1-Pentadecene	
8	22.179	Trifluoroacetic acid	
9	24.434	E-14-Hexadecenal	
10	26.210	Di-n-Octyl phthalate	

Table 8: GC-MS Identified components of the EA fraction of *R. microporus* mushroom extract (Compounds are listed in ascending order of Retention Time)

Peak/No	Retention Time (Minutes)	Name of Compounds	Functional group of compounds
1	11.099	n-Decanoic acid	
2	13.545	n-Hexadecanoic acid	

3	15.931	Tetradecanoic acid	
4	18.027	Pentadecanoic acid	
5	19.499	n-Hexadecanoic acid	
6	21.113	n-Octadecanoic acid	
7	21.434	Octadecanoic acid	
8	23.774	3,4-Dimethyl-1-decane	
9	26.208	Di-n-Octyl phthalate	

DISCUSSION

The resistance of microorganisms to commonly used agents has been on the increase in the last few decades. Scientists have been looking for the solution to this problem by exploring macrofungi growing in the wild with potential antimicrobial properties. The present study reports the antimicrobial properties of *R. microporus*, a wild mushroom collected from Akure.

The results of the phytochemical screening of *R. microporus* extract showed that the methanol and acetone extracts of the mushroom contained almost similar phytochemical constituents but at different quantities. This variation could be explained by the difference in the solubility of the macrofungus constituents in the solvents. This agrees with the suggestion of Oloke and Kolawole [16] that bioactive components of any medicinal plant may differ in their solubility depending on the extraction solvents used. The presence of alkaloids, saponin, terpenoids and phenolic compounds such as tannins and flavonoids in the mushroom extracts is an indication of the antimicrobial potential of the mushroom. These phytochemicals have been reported to produce varying degree of antimicrobial effects [17-20].

The results of the antimicrobial activity of the mushroom extracts revealed variation in zones of inhibition produced against the test organisms. This variation may be dependent on the nature and genetic makeup of the test organisms such as cell wall components, spore formation and capsulation which have been reported to confer resistance on microorganisms [21, 22]. For instance, *Bacillus cereus* is a spore producing Gram positive organism with capsulated cell wall. These attributes might have led to the resistance of this organism to the mushroom extracts. This variation might also be dependent on the phytochemical composition of the mushroom extracts, the type and nature of solvent used for extraction as well as the microbial strain used for the assay. Variation in antimicrobial activities of plant extracts have been demonstrated in several studies. For instance, Mostafa *et al*. [23] observed similar result while studying the antimicrobial activity of some plant extracts on bacterial strains casing food borne diseases.. Likewise, Barros *et al*. [24] associated the antimicrobial activities of macrofungi to diversities of secondary metabolites they contain. Findings from the study suggests that the mushroom, *R. microporus*, may serve as an important source of antimicrobial compounds with broad spectrum activities as it was able to successfully inhibit the growth of both bacterial and fungal isolates.

The antimicrobial activity of the partially purified column fractions of the mushroom extract on the selected test organisms showed higher zones of inhibition compared to that of the crude extracts. A study by Merawie *et al*. [25] on crude and semi-purified fractions of *Warburgia ugandensis* against some pathogens also showed the semi purified fraction of the ethyl ether extract displaying better antimicrobial

activity. The removal of non-active compounds from the crude extracts might have contributed to the higher efficacy of the partially purified extracts to the test organisms. Also the partially purified extract would most likely have a smaller molecular size. This will aid their solubility into the medium and subsequent penetration into the cells of the test organisms as compared to the crude extracts.

The antimicrobial effects of the mushroom extracts on the test organisms led to a progressive damage on the cell membrane of the organisms based on the rate of release of potassium and sodium ion from their cells. The release of these ions might be responsible for the bactericidal and bacteriostatic activities of the macrofungus extract. Bacteria biosynthetic process is mediated by enzymes activated by sodium and potassium ions [26]. Hence, the inhibitory effect of the extract might have resulted from the release of these ions. According to the report of Mailard [27], antimicrobial agents can cause the release of cytoplasmic materials such as sodium and potassium ions from microorganisms. The rate of leakage of these ions also varies from one organism to the other. This variation might be due to the difference in the nature of the cell wall composition [28]. This may also be due to the variations in their genetic coding, which may be chromosomal or plasmid [29]. This affects some physiological properties of organisms like solute uptake, active transport and facilitated diffusion of molecules across the cell membrane. The differences observed in the amount of leached sodium and potassium ion might have resulted from the difference in the molecular size of these two ions. Based on the report of Oladunmoye *et al*. [13], the amounts of intracellular constituents released have been found to be dependent on the size of pores on the microbial cell wall and membrane as well as the molecular size of ions. The observed antimicrobial activity of the crude extract of *R. microporus* may have resulted from the cell damage and inactivation of enzymes due to their ability to induce the leakage of sodium and potassium ions.

The Gas Chromatography Mass Spectrometry (GC-MS) analysis on the partially purified fractions of *R. microporus* extracts revealed the presence of arrays of important chemical constituents with proven antimicrobial potency [30-32]. The chemical compounds identified in the mushroom extracts are likely responsible for its antimicrobial activities. Among these compounds, Adeleye *et al*. [30] reported hexadecane and octadecane as potent antifungal agents while studying the volatiles compounds of "Epa-ijebu", a natural drug recipe from Southwest Nigeria. Pentadecane isolated from Sea Urchin was reported to be a potent antibacterial agent [33]. Hsouna *et al*. [31] reported the antimicrobial potency of n-decanoic acid isolated from essential oil of *Ceratonia silisqua*. Hexadecanoic acid, tetradecanoic acid, octadecanoic acid obtained from non-polar components of two leguminoceae species *Albizia adianthifolia* and *Pterocarpus angolensis* have also been proven to be potent antimicrobial [32]. Therefore, the antimicrobial attributes observed in *R. microporus* extract may be due to the actions of the identified bioactives.

CONCLUSION

This study has provided information on the antibacterial and antifungal potentials of crude and partially purified extracts of *R. microporus* against common human pathogens as well as the chemical composition of the wild mushroom. The study revealed the potentials of mushroom extracts as a broad spectrum antimicrobial agent due to their inhibitory effect against Gram positive and Gram negative organisms as well as fungal isolates that have been implicated to causative agents of human diseases. The antimicrobial activities demonstrated by the extracts of *R. microporus* is a promising development that may help to discover new chemical classes of antibiotics and antifungal agents that could be used in the treatment of infection caused by microorganisms.

Competing of Interests

The authors declare no competing conflict of interests.

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