Antimicrobial, Antioxidant Activities *in vitro* and Polyphenol Contents of the Leaf Extract of a Versatile Medicinal Plant

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ABSTRACT-*This study assessed polyphenolic contents, antioxidant and antimicrobial activities of the solvent solutions obtained from Combretum racemosum leaf extract. The crude aqueous methanolic leaf extract of C. racemosum was partitioned into n-hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and n-butanol (n-BuOH) fractions. Antibacterial activities were determined using agar- well diffusion and agar dilution methods. In vitro free radical scavenging activity of the fractions was measured by 2, 2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), 2,2-diphenyl-1-picryl dihydrazyl (DPPH), ferric-reducing antioxidant power (FRAP) and nitric oxide (NO) assays. MIC of the EtOAc fraction ranged from 0.63 mg/ml to 1.25 mg/ml while that of n-BuOH and DCM fractions ranged from 0.31mg/ml to 1.25 mg/ml and 0.31 mg/ml to 0.63 mg/ml respectively. The n-BuOH fraction contains highest phenolic compounds (phenol and flavonoids), followed by EtOAc, crude, hexane and DCM. Total antioxidant capacity of the fractions follows the same order as the antioxidant. NO radical scavenging activities of all the fractions showed stronger activity than that of the reference ascorbic acid (AA). The greatest antiradical activity was found in butanol fraction; IC_{50} for DPPH, NO and FRAP are 38.39±7.20µg/ml; 0.11±0.03 µg/ml and 180.5203±0.86mg/ml respectively. Our findings revealed that the leaf extract of C. racemosum possesses bioactive agents that exhibit antioxidants and antimicrobials properties.*

Keywords--- Antimicrobial, antioxidant, free radicals, *Combretum racemosum*, polyphenols

1. INTRODUCTION

Medicinal plant extracts have been used in folklore remedy against various infectious diseases in developing countries dated back to ancient time [1-3]. These plants have proved to be rich sources of potent bioactive compounds such as terpenes, saponins, carotenoids, anthraglycosides, alkaloids, phytosterols and polyphenols. [4].

Polyphenols are phenolic compounds which are secondary products of plant metabolism of plants and are widely distributed in nature. These naturally occurring polyphenols vary in structures from simple phenolic acids and flavonoids to complex compounds such as tannins, stilbenes, coumarins and lignins. Fruits and vegetables such as apples, grapes, onions and cabbage contain abundant amount of polyphenols, vitamin C, vitamin E, selenium, β-carotene, lycopene, lutein, and other carotenoids. These bioactive compounds play important roles in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides thereby acting as protective agents against a variety of diseases, particularly cardiovascular disease and cancer [4].

Recent problems related to the frequent and indiscriminate use of antibiotics, and development of multiple drug resistant (MDR) strains of a number of pathogenic micro-organisms such as *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Candida albican* and methicillin resistant *Staphylococcus aureus* (MRSA) have necessitated renewed alternative discovery of potent drugs from plants with antimycobacterial properties [5-8]..

Also, free radicals such as nitric dioxide ($NO_2^{\bullet}$), peroxynitrite ($ONOO^{-}$), nitric oxide (NO•), hydrogen peroxide (H_2O_2); hydroxyl radical (·OH) and superoxide (O_2^{-}) are regularly produced in living tissues where they cause widespread damage to bio-molecules which can lead to diverse disease conditions, such as cancer, neurodegenerative diseases, the aging process and heart diseases [9-11].

Many synthetic drugs have been used to protect against these free radicals but they have unfavourable side effects. Consumption of natural antioxidants obtained from fresh plant produce and other food supplements can be substitutes to these artificial compounds [12-14].

Combretum racemosum (P. Beauv.), also known as Christmas rose, that belongs to the family Combretaceae, is widely distributed in the tropical and pan tropical regions. The plant has been used in African traditional medicine for many years to cure various ailments such as toothache, ulcer, tuberculosis, haemorrhoids, dysentery, male sterility, internal parasites and skin diseases [15-17]. The leaves of the plant have also been used by the people of Bayelsa State, Niger Delta region of Nigeria to treat high blood pressure.

This study thus was aimed at evaluating polyphenolic contents and antioxidant activity of the solvent partitions obtained from *C. racemosum* leaf extract. In addition, the antimicrobial activity of the plant on some microbes such as *Bacillus polymyxa*, *Bacillus anthracis, Bacillus stearothermophilus*, *Clostridium sporogenes*, *Corynebactarium pyogenes, Micrococcus luteus, Klebsiella pneumoniae, Pseudomona flourescens* and *Candida pseudotropicali* which have not been reported in literature was also investigated.

2. MATERIALS AND METHODS

All the solvents used were distilled prior to use. All the reagents used for the antioxidant assays were of analytical grade ordered from Sigma.

2.1 Collection of plant material

Fresh leaves of *Combretum racemosum* were collected from the campus of the Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The plant was identified in the Department of Pharmacognosy, Niger Delta University. A voucher specimen number KKA 33 was deposited at the University herbarium. The fresh leaves were washed, and air dried at ambient temperature (30 ^{0}C) for 3 weeks. The dried leaves were ground into powder with MINGYUE grinding machine (Model Number: MY-20 MY-30 MY-40). Finally, the powder was sealed in a polythene bag and kept in a refrigerator until use.

2.2 Extraction of plant material

500 g of the fine powdered leaves were extracted with 20 % (v/v) aqueous methanol (5 L) at room temperature for 24 hours and filtered using Whatmann No. 1 filter paper. The filtrate was concentrated *in vacuo* at 40 ^{0}C using rotary evaporator to a small volume (200 mL) to give the methanol extract (MEE), which was subsequently partitioned with n-hexane (3 x 1L), dichloromethane (3 x 1L), ethyl acetate (3 x 1L) and n-butanol (3 x 500 mL) [18].

2.3 Qualitative phytochemical tests

Phytochemicals such as alkaloids, reducing sugar, cardiac glycosides, tannins, saponins, terpenoids, steroids, phenols and flavonoids were tested in all the partitions using standard techniques described by Sofowora [19]and Trease and Evans [20].

2.4 Antimicrobial study

The microorganisms used in this study were typed cultures of National Collection of Industrial Bacteria (NCIB)and locally isolated organisms (LIO), which were obtained from the Microbiology Department, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria. These microorganisms include:

NCIB Isolates: *Bacillus cereus* (NCIB 6349), *Bacillus subtilis* (NCIB 3610), *Bacillus stearothermophilus* (NCIB 8222), *Clostridium sporogenes* (NCIB 532), *Staphylococcus aureus* (NCIB 8588), *Enterococcus faecalis* (NCIB 775) and *Micrococcus luteus* (NCIB 196), Escheria coli (NCIB 86) and *Klebsiella pneumoniae* (NCIB 418)
Locally isolated bacteria isolates: *Bacillus polymyxa*, *Bacillus anthracis*, *Corynebactarium pyogenes.*
Locally isolated fungal isolates: *Candida albicans* and *Candida pseudotropicalis*
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2.5 Agar-well diffusion Assay

Agar-well diffusion method as described by Ben Hsouna *et al* [21] with some modifications was used to perform the antimicrobial sensitivity testing of the plant extract and partitions. The bacterial isolates were grown in sterile nutrient broth at 37 ^{0}C for 18 h while the yeasts were grown in malt extract at 25 ^{0}C for 24 h before use. Then 0.2ml of the standardized microbial culture (10^6 cfu/ml, equivalent of 0.5 McFarland standards) was inoculated into molten Mueller-Hinton agar and malt extract agar (Lab M, UK) for the bacteria and yeast respectively. This was poured into Petri dishes and allowed to set before wells were bored into it using sterile cork borer (6 mm). The wells were carefully filled up with prepared concentration of the extract (25 mg/ml) and partitions (10 mg/ml) with the aid of a Pastuer pipette. The plates were then incubated at 37 ^{o}C (bacteria) and 25 ^{0}C (yeast) after which the diameter of zones of inhibition (mm) were measured. The effect of the partitions against microbial isolates was compared with those of standard antibiotics (1 mg/ml), streptomycin (Pfizer Inc., New York), ampicillin (Lab Oftalmiso, Spain), and nystatin (Pfizer Inc., New York). The experiment was carried out in replicate of three.

2.6 Determination of minimum inhibitory concentrations.

The method described by Akinpelu and Kolawole [22] was utilized to determine the MICs of the partitions. A two-fold dilution of the partitions was prepared and 2ml of different concentrations of the partitions was added to 18 ml of pre-sterilized molten nutrient agar (bacterial) and malt extract agar (yeast) to give final concentrations of the partitions ranging between 0.31 and 20.0 mg/ml. The medium was then poured into Petri dishes and allowed to set. The surfaces of the medium were allowed to dry before streaking with 18 – 24 h old standard (10^6 cfu/ml) inoculums, after which the plate was incubated at 37 ^{o}C (bacteria) and 25 ^{0}C (yeast). The MIC was determined as the lowest concentration that inhibits the growth of the isolates. The experiment was carried out in replicate of three.

2.7 Determination of minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC)

Minimum bactericidal/fungicidal concentrations of the partitions were determined by the method described by Gulluce *et al* [23] with slight modification. The test microbial cell samples were taken from plates with no visible growth in the MIC assay and sub-cultured using a sterile loop onto freshly prepared nutrient agar plates and malt extract agar plates respectively for the bacterial and fungal isolates. These agar plates were then incubated at 37 ^{o}C for 48h and 25 ^{o}C for 72 h r for the bacterial and fungal isolates respectively. The MBC and MFC were determined as the lowest concentration of the extract that did not show visible growth on the new set agar plates. The experiment was carried out in replicate of three.

2.8 Total phenolic content (TPC)

The Folin–Ciocalteu assay method as described by Li *et al* [24] was used to determine the total phenolic content of crude extract and partitions. To a mixture of 0.1 ml of sample and 0.9 ml of distilled water, was added 0.2 ml of Folin-Ciocalteu's phenol reagent and the resulting mixture was vortexed. After 5 min, 1.0 ml of 7 % (w/w) Na_2CO_3 solution was added and the solution was then make up to 2.5 ml before incubating for 90 min at room temperature. The absorbance against a negative control containing 0.1ml of water in place of the sample was then taken at 750 nm. Gallic acid (0.1 mg/ml) was used as standard in order to determine Gallic Acid Equivalent (GAE) of sample, after preparing a calibration curve. Distilled water was used as blank.

2.9 Total flavonoid content (TFC)

The aluminium chloride colorimetric assay method described by Neergheen *et al*. [25] was employed to determine the total flavonoid content of the plant extract and partitions. To 0.1 ml of extract/standard was added 0.4 ml of distilled water. This was followed by 0.1 ml of 5 % sodium nitrite. After 5 min, 0.1 ml of 10% Aluminum Chloride and 0.2 ml of sodium hydroxide was added and the volume was made up to 2.5 ml with distilled water. The absorbance at 510 nm was measured against the blank. Standard quercetin with varying concentration (12.5 - 100 µg/ml) were used as standard. The total flavonoid content of the plant extract/partitions, expressed as mg quercetin equivalents per gram of the plant extract/partitions is calculated as:

X=q* Vw

Where X is the total content of flavonoid compound in quercetin equivalents
Q is the concentration of quercetin established from the standard curve
V is the volume of extract/partition (ml)

W is the weight of the extract/partition in reaction mixture

2.10 Total antioxidant capacity (TAC)

The method described by Pellegrini *et al.*, [26] was utilized for TAC assessment of the plant extract. To 0.1 ml of the extracts (1 mg/ml) or standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/ml) was added 1.0 ml of the reagent solution which consisted of 0.6 M sulphuric acid, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate. The tubes containing the reacting mixture were incubated in a water bath at 95 ^{o}C for 90 min. The mixture was then allowed to stand and cool to room temperature and the absorbance measured at 695 nm against a blank which consisted of the reacting mixture containing distilled water in place of the extract. The antioxidant activities of the partitions were expressed as an ascorbic acid equivalent.

2.11 Determination of the radical scavenging ability using the 2,2-diphenyl-2-picrylhydrazyl hydrate assay

DPPH free radical scavenging activity of the extract and partitions were spectrophotometrically determined at 517 nm using the method described by Barros *et al* [27]. To 1.0 ml of different concentrations (31.25 – 125 ug/ml) of the extract or standard in a test tube was added 1.0 ml of 0.3 mM DPPH in methanol. The mixture was mixed and incubated in the dark for 30 min after which the absorbance was read at 517 nm against a DPPH control containing only 1.0 ml methanol in place of the extract.

The percent of inhibition was calculated as follows:
$I\% = [(A_{blank}-A_{sample})/A_{blank}] \times 100$
Where A_{blank} is the absorbance of the control (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration.

2.12 Nitric oxide radical scavenging assay

The nitric oxide scavenging activity of the sample was measured spectrophotometrically according to the method described by Marcocci et al. [28]. The reaction mixture, containing 0.1 ml of different concentrations (10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml) of the partition solutions and 0.9 ml of sodium nitroprusside (2.5 mM) in phosphate buffer saline (pH 7.2, 10 mM) was incubated under illumination for 150 min. After incubation, 0.5 ml of 1% sulphanilamide in 5% phosphoric acid was added and incubated in the dark for 10 min., followed by addition of 0.5 ml 0.1% of NED (N-1-napthylethylenediamine dihydrochloride). The absorbance of the chromophore formed was measured at 546 nm [23]. The percentage inhibition of nitric oxide radical formation was calculated as expressed above in DPPH radical scavenging assay.

2.13 Determination of ABTS+ radical scavenging activity

The 2, 2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid ($ABTS^{+}$) free radical scavenging activity was determined by the $ABTS^{+}$ radical cation decolourisation assay as developed by Re *et al.* [29]
The ABTS radical cation ($ABTS^{+}$) was produced by reacting ABTS stock solution (7 mM) with 7 mM potassium persulphate in a ratio 2:1 and the mixture was allowed to stand in the dark at room temperature for 16 h before use for complete oxidation of the ABTS mixture. Prior to use, the stock solution was diluted with 70% ethanol to an absorbance of 0.75 ± 0.05, to give ABTS working solution. $ABTS^{+}$ radical scavenging analysis was performed by mixing 2.0 ml of the ABTS working solution with 20 µl of the different concentration of partitions/standard solution (trolox). The negative control was prepared by adding 20 µl of 70% ethanol in 2.0 ml of ABTS. The decolourization reaction was complete after 1 min.
Absorbance was measured spectrophotometrically at 734 nm and the readings obtained were used to calculate the % inhibition as follows;
$\%\ ABTS^{+}\ inhibition = [1-(A_{734\ nm\ sample}/A_{734\ nm\ control})] \times 100$
Where:
$A_{734\ nm\ sample}$ is the absorbance at 734 nm of the sample
$A_{734\ nm\ control}$ is absorbance at 734 nm of the negative control.
The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph by plotting inhibition percentage against extract concentration.

2.14 Determination of ferric reducing antioxidant power

Ferric reducing antioxidant power of the plant extract and partitions was determined by the method described by Panda *et al* [30]. The FRAP working reagent consisted of 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tri-(2-pyridyl)-1, 3, 5-triazine and 20 mM $FeCl_3.6H_2O$ mixed together in the ratio of 10:1:1 respectively. A 50 µl aliquot of the partitions at 1 mg/ml and 50 µl of standard solutions of ascorbic acid (20, 40, 60, 80, 100 µg/ml) were added to 1 ml of FRAP reagent in duplicate tubes. Absorbance measurement was taken at 593 nm exactly 10 min after mixing against reagent blank containing 50 µl of distilled water. All measurements were taken at room temperature with plant partition solutions protected from direct sunlight. The Ferric reducing antioxidant power was expressed in ascorbic acid equivalent concentration (EC) which was defined as the concentration of antioxidant that gave a ferric reducing ability equivalent to that of the ascorbic acid standard

3 RESULTS

3.1 Qualitative phytochemical tests

Results of the qualitative phytochemical tests are given in Table 1. The results revealed the presence of reducing sugars, cardiac glycosides tannins, saponins, terpenoids, steroids, and flavonoids in the extract and polar ethylacetate and n-butanol partitions. The percentage yields of the extract and partitions are shown in Table 4.

Table 1: Phytochemical analysis of the partitions of *C. racemosum* leaf extract.

Phytochemicals	MEE	HEP	DCP	EAP	BUP
Alkaloids	-	-	+	+	-
Saponins	+	+	+	+	-
Tannins	+	-	-	+	+
Flavonoids	+	-	-	+	+
Cardiac glycosides	+	-	-	+	+
Terpenoids & steroids	+	-	-	+	+
Phlobatamin	-	-	-	-	-
Ketonic sugar	+	-	-	-	+
Anthroquinones	-	-	-	-	-
Reducing sugar	+	-	-	+	+

MEE= Methanol extract; HEP= n-Hexane partition; DCP = Dichloromethane partition; EAP = Ethylacetate partition; BUP = n-Butanol partition
+ means present; - means not present

3.2 Antimicrobial activity

Susceptibility of the test organisms, in terms of zones of inhibition, against the antimicrobial agents is shown in Table 2. All the test isolates were sensitive to the plant extract and partitions except the n-hexane partition which was only active against *Klebsiella pneumoniae* and the test yeast isolates. The maximum zone of inhibition exhibited by the plant extract (21±0.76 mm) was against a *Bacillus subtilis* which was completely resistant to ampicillin used as standard drug. The The maximum zone of inhibition exhibited by the partitions (22±0.50 mm) was expressed by the n-butanol partition against *Pseudomonas flourescens* which was also completely resistant to both streptomycin and ampicillin. Moreover, the lowest MIC exhibited by the plant extract was 1.57 mg/ml against almost 42% of the test isolates, while the MIC exhibited by the fraction partitioned into dichloromethane was ≤ 0.63 mg/ml against all test isolate except *Candida pseudotropicalis* (2.5 mg/ml). The range of MIC by streptomycin against the bacterial isolates was between 0.03 and 0.5 mg/ml, while that of nystatin against the yeast isolates was between 0.06 and 0.13 mg/ml (table 2). In addition, the minimum bactericidal concentration of 0.63 mg/ml was exhibited by the dichloromethane partition against 34% of the bacterial isolates and as well by the n-butanol partition against *Pseudomonas flourescens* and the test yeast isolates (Table 4).
Both ethyl acetate and n-butanol partitions demonstrated the best antifungal activity with MIC and MFC values of 0.63mg/ml when compared with known antimicrobial agents, streptomycin and ampicillin (Tables 3 and 4).

3.3 Polyphenolic contents and antioxidant activities

Results revealed that the n-butanol partition has the highest phenolic contents (phenolics and flavonoids), followed by the EAP, MEE, HEP and DCP. The total antioxidant capacity assay also follows the same order as shown in Table 5.
Results of the DPPH assay of the plant extract and solvent partitions are shown in Table 6. Positive DPPH test suggests that the partitions are free radical scavengers. All the solvent partitions show strong DPP radical scavenging activity

compared with standards Vitamin C (10.29±0.04 µg/ml) and trolox (10.89±0.17 µg/ml) based on their IC_{50} values of 38.39 ± 7.20; 49.56 ± 1.04; 67.48 ± 1.92; 144.56 ± 4.73 and 162.59 ± 26.74 µg/ml for n-butanol, crude, ethyl acetate, hexane and DCM partitions respectively

The antioxidant potency of the partitions to reduce the pre-formed radical cation to ABTS is in the following order ethyl acetate>DCM> butanol>crude>hexane, according to the IC_{50} values of 464.12 ± 0.63; 464.45 ± 2.39; 494.79 ± 0.45; 614.20 ± 2.72 and 2095.21 ± 0.90 µg/ml respectively. Results as shown in Table 5 were compared with one obtained using trolox (with IC_{50} of 337.49 ± 2.75 µg/ml) to demonstrate that the partitions and extract are potent antioxidants. The order of NO scavenging activity is as follows: butanol>crude>ethyl acetate> DCM >hexane with their IC_{50} of 0.11 ± 0.03; 0.14 ± 0.02; 0.26 ± 0.06; 0.50 ± 0.26 and 0.63 ± 0.01 µg /ml respectively; while the standard ascorbic acid gave IC_{50} of 64.15 ± 2.52 µg/ml. Table 6 shows that the ferric reducing power of the partitions exhibited the following order: BUP > MEE>EAP > HEP > DCP with the values180.52±0.86; 148.27±0.75; 114.49±0.05; 64.65±0.36 and 60.77±0.32 mg/g respectively. BUP exhibited the greatest antiradical activity, IC_{50} for DPPH, and NO are 38.39±7.20; and 0.11±0.03 µg/ml respectively and 180.52±0.86 mg/ml for FRAP

Table 2: Sensitivity patterns of zones of inhibition exhibited by partitions obtained from *C. racemosum* leaf extract on micro-organisms isolates

Bacterial Isolates	**Zones of inhibition (mm) ****						
	MEE (25mg/ml)	**HEP** (10mg/ml	**DCP** (10mg/ml)	**EAP** (10 mg/ml)	**BUP** (10 mg/ml)	**STREP** (1 mg/ml)	
***B. cereus* (NCIB 6349)**	18	0	18±0.29	20±1.00	18	29	20±0.58
***B. polymyxa* (LIO)**	20±1.00	0	18	16±0.50	18±1.00	26±0.58	0
***B. anthracis* (LIO)**	18±1.15	0	14±1.00	20	16±0.58	30±0.29	30±1.12
***B. subtilis* (NCIB3610)**	21±0.76	0	19±0.50	20±0.76	18±1.15	31±1.00	
***B. stearothermophil* (NCIB 8222)**	20	0	18±1.00	22±1.00	20	30±0.58	26
***C. sporogenes* (NCIB 532)**	18±0.58	0	0	22	20±1.15	28±0.58	18
***C. pyogenes* (LIO)**	0	0	16±0.76	16±0.58	20	26±0.00	36±1.00
***Staph. aureus* (NCIB 8588)**	18±1.00	0	18	14	18±1.00	30±0.58	28
***E. faecalis* (NCIB 775)**	20±0.29	0	16	18±0.29	22±0.76	32±1.00	26±0.58
***M. luteus* (NCIB 196)**	16±0.58	0	0	14±0.76	16±0.58	26±0.58	29±1.15
***E. coli* (NCIB 86)**	20	0	16±1.00	16±0.50	20±1.00	26	24±1.00
***K. pneumoniae* (NCIB 418)**	20	16±0.58	0	12±1.00	18	25±0.29	20
***Ps. aeruginosa* (NCIB 950)**	18±0.76	0	18	18±1.00	22±0.50	0	0
***Ps. flourescens* (NCIB 3756)**	18±0.58	0	19±1.15	16	20±0.50	29±1.00	24±0.29
***P. vulgaris* (LIO)**	16±0.76	0	0	18±0.58	18	28	18±0.76
Fungal Isolates	**MEE** (25mg/ml)	**HEP** (10mg/ml)	**DCP** (10mg/ml)	**EAP** (10 mg/ml)	**BUP** (10 mg/ml)	**NYST** (1 mg/ml)	
Candida albicans	20	18±0.76	0	18±1.32	20	28±1.00	
C. pseudotropicalis	18±1.00	16	13±1.15	16±0.58	20±1.00	24	

Key: LIO = Locally Isolated Organism; NCIB = National Collection of Industrial Bacteria; MEE= Methanol extract; HEP= n-Hexane partition; DCP = Dichloromethane partition; EAP = Ethylacetate partition; BUP = n-Butanol partition; mm ** = mean of three replicates; 0 = not sensitive; STREP = Streptomycin; AMP = Ampicillin; METH = Methanol; NYST = Nystatin.

Table 3: The minimum inhibitory concentrations (mg/ml) of the partitions of *C. racemosum* leaf extract and standard antimicrobials exhibited against susceptible microorganism isolates.

Bacterial isolates	**MEE**	**HEP**	**DCP**	**EAP**	**BUP**	**STREP**	**AMP**
***B. cereus* (NCIB 6349)**	1.57	ND	0.31	0.63	0.63	0.06	0.06
***B. polymyxa* (LIO)**	1.57	ND	0.31	1.25	0.63	0.13	ND
***B. anthracis* (LIO)**	12.50	ND	0.63	1.25	1.25	0.25	0.03
***B. subtilis* (NCIB 3610)**	1.57	ND	0.31	1.25	0.63	0.25	ND
***B. stearothermophilus* (NCIB 8222)**	3.13	ND	0.63	1.25	1.25	0.06	0.06
***C. sporogenes* (NCIB 532)**	12.50	ND	ND	0.63	1.25	0.06	0.13
***C. pyogenes* (LIO)**	ND	ND	0.63	1.25	1.25	0.13	0.03
***Staph. aureus* (NCIB 8588)**	3.13	ND	0.63	1.25	0.63	0.06	0.03
***E. faecalis* (NCIB 775)**	1.57	ND	0.63	0.63	0.31	0.03	0.06
***M. luteus* (NCIB 196)**	3.13	ND	ND	1.25	0.63	0.25	0.06
***E. coli* (NCIB 86)**	12.5	ND	0.63	1.25	0.63	0.25	0.25
***K. pneumoniae* (NCIB 418)**	1.57	3.13	ND	0.63	0.31	0.50	0.25
***Ps. aeruginosa* (NCIB 950)**	1.57	ND	0.31	1.25	0.63	ND	ND
***Ps. Flourescens* (NCIB 3756)**	1.57	ND	0.31	1.25	0.31	0.03	0.13
***P. vulgaris* (LIO)**	3.13	ND	ND	0.63	1.25	0.50	0.25
Fungal isolates	**MEE**	**HEP**	**DCP**	**EAP**	**BUP**	**NYST**	
Candida albican	3.13	0.63	ND	0.63	0.63	0.06	
C. pseudotropicalis	3.13	1.25	2.50	0.63	0.63	0.13	

Key: ND means Not Determined

Table 4: The minimum bactericidal/fungicidal concentrations (mg/ml) of the partitions of *C. racemosum* leaf extract and standard antimicrobials exhibited against susceptible microorganism isolates.

Bacterial isolates	**MEE**	**HEP**	**DCP**	**EAP**	**BUP**	**STREP**	**AMP**
B. cereus (NCIB 6349)	3.13	ND	0.63	1.25	1.25	0.13	0.13
B. polymyxa (LIO)	3.13	ND	0.63	2.50	1.25	0.25	ND
B. anthracis (LIO)	12.50	ND	1.25	2.50	2.50	0.25	0.06
B. subtilis (NCIB 3610)	3.13	ND	0.63	2.50	1.25	0.25	ND
B. stearothermophilus (NCIB 8222)	3.13	ND	1.25	2.50	1.25	0.13	0.13
C. sporogenes (NCIB 532	12.50	ND	ND	2.50	2.50	0.13	0.25
C. pyogenes (LIO)	ND	ND	1.25	2.50	2.50	0.25	0.06
Staph. aureus (NCIB 8588)	6.25	ND	1.25	2.50	2.50	0.25	0.06
E. faecalis (NCIB775)	3.13	ND	1.25	1.25	1.25	0.06	0.13
M. luteus (NCIB 196)	6.25	ND	ND	2.50	1.25	0.25	0.13
E. coli (NCIB 86)	12.50	ND	1.25	2.50	1.25	0.25	0.25
K. pneumoniae (NCIB 418)	3.13	2.50	ND	1.25	0.63	0.50	0.25
Ps. aeruginosa (NCIB 950)	3.13	ND	0.63	2.50	1.25	ND	ND
Ps. flourescens (NCIB 3756)	3.13	ND	0.63	2.50	0.63	0.06	0.25
P. vulgaris (LIO	3.13	ND	ND	1.25	2.50	0.50	0.50
Fungal isolates	**MEE**	**HEP**	**DCP**	**EAP**	**BUP**	**NYST**	
Candidalalbican	3.13	0.63	ND	0.63	0.63	0.06	
C. pseudotropicalis	3.13	1.25	2.50	0.63	0.63	0.13	

Key: ND means Not Determined

Table 5: Percentage yields, total phenolic and flavonoid contents and total antioxidant capacity of the different partitions of *Combretum racemosum* leaf extract.

Partitions	% Yield	Total phenolic content (mg GAE/g)	Total flavonoid content (mg QUE/g)	Total antioxidant capacity (µg AAE/g)
Methanol Extract	7.38±1.67	187.02±1.02	700.57±65.23	195.43±0.87
n-Hexane	0.82±0.98	109.31±6.92	662.90±8.02	173.84±1.75
Dichloromethane	0.87±0.45	108.83±2.69	852.91±12.26	151.96±2.46
Ethylacetate	0.18±1.03	205.70±6.59	821.79±16.22	145.29±0.89
n-Butanol	0.50±0.62	249.60±6.45	1845.54±108.13	220.65±8.61

Values are expressed as mean± SD of three parallel measurements

Table 6*: In vitro* free radical scavenging activities of the different partitions of *Combretum racemosum* leaf extract**.**

Partitions	DPPH	ABTS	NO	FRAP
	----------IC_{50} (µg /ml)----------			mg AAE/g
Methanol Extract	49.56 ± 1.04	614.20 ± 2.72	0.14 ± 0.02	148.27±0.75
n-Hexane	144.56 ± 4.73	2095.21 ± 0.90	0.63 ± 0.01	64.65±0.36
Dichloromethane	162.59 ± 26.74	464.45 ± 2.39	0.50 ± 0.26	60.77±0.32
Ethylacetate	67.48 ± 1.92	464.12 ± 0.63	0.26 ± 0.06	114.49±0.05
n-Butanol	38.39 ± 7.20	494.79 ± 0.45	0.11 ± 0.03	180.52±0.86
L-ascorbic acid	10.29±0.04	ND	64.15 ± 2.52	
Trolox	10.89±0.17	337.49 ± 2.75	ND	

Values are expressed as mean± SD of three parallel measurements
ND – not determined.

4. DISCUSSION

The presence of tannins, saponins, alkaloids, terpenoids, steroids, reducing sugars, cardiac glycosides and flavonoids in the plant extract and polar ethylacetate and n-butanol partitions show that the plant is rich in phytochemicals and this is in agreement with the results obtained by other researchers who had carried out phytochemical studies on *Combretum species* [17,31,32].

4.1 Antimicrobial activity

The extract of *C. racemosum* exhibited significant antimicrobial potentials across bacteria with different Gram reactions as well as the test yeast isolates. When the extract was partitioned into different fractions, the activity increases and compared favourably with the standard antibiotics used considering the level of purity of the two different agents. The fraction partitioned into n-hexane was not potent against test bacterial isolates but able to inhibit the growth of the test yeast. This suggest that n-hexane may not be a good solvent in extracting antibacterial agent from *C. racemosum*, thus indicates the polar components of the phytochemicals expected to be embedded in this fraction is only active against the yeast isolates. On the Other hand, the remaining partitions were able to inhibit the growth of bacteria isolates such as *Bacillus polymyxa, B. anthracis* and *Pseudomonas aeruginosa* which were resistant to one or both of the antibiotics used in this study. Hence, the leaf extract of *C. racemosum* could be probable source of antimicrobial agents of natural origin that may be of help in combating multidrug resistance problems currently impeding success in clinical services against infectious diseases. *P. aeruginosa* as one of the susceptible isolates, has been implicated in urinary tract infections, pneumonia, bacteraemia and as well as causing high morbidity and mortality rate in patients suffering from cystic fibrosis [33, 34]. Likewise, *E. coli* and *E. faecalis* which are the causal agents of diarrhoea and urinary tract infections further validate the use of this plant in folklore remedy against urinary tract and gastro-intestinal infections [35] as previously reported by Kau et al. [36]

4.2 Polyphenolic contents and antioxidant activities

The total phenolic, flavonoid contents and antioxidant capacity assay show that the n-butanol partition has the highest phenolic compounds (phenolic acids and flavonoids), followed by the EAP, MEE, HEP and DCP as shown in Table 5. Phenolic antioxidants are considered powerful free radical scavengers [37]. These biologically active compounds release

hydrogen to free radical thereby disrupting the lipid oxidation in cells. Presence of phenolic hydroxyl groups in these compounds have been attributed to their ability to remove free radicals from tissues [38, 39]. The FTIR spectrum confirmed the presence of these phenolic, hydroxyl and carbonyl groups in the polar partitions

Results indicate that the plant extract and its various partitions contain reasonable amounts of polyphenolic compounds such as flavonoids and condensed tannins. It is well established that these compounds considerably affect human nutrition and health due to their antioxidant potentials [13,15].

Production of nitric oxide is poisonous to tissues which is associated with various inflammatory conditions such as arthritis, juvenile diabetes, and ulcerative colitis [40]. Increased toxicity of NO has been envisioned to be due to the production of the highly reactive peroxynitrite anion, when it reacts with superoxide radical [41]. In addition, phenolic compounds in plants are strong inhibitors of NO and peroxynitrite productions [42]. Result in this study proved that the extract and fractions have more potent nitric oxide scavenging activity than that of the reference ascorbic acid (AA) as shown in Table 6. The n-butanol partition exhibits higher reducing power 180.52±0.86mgAAE/g, compared to other fractions. Presence of antioxidant compounds in the plant samples causes the Fe^{3+}/ferricyanide complex to be reduced to Fe^{2+} ions which quenches the radical chain reactions that may otherwise impair the surrounding tissue [43,44].

The antioxidant activity demonstrated by the partitions in DPPH, NO, ABTS and FRAP assays may be attributed to their hydrogen-releasing capabilities of the phenolic compounds [45, 46]. This supports the report by Masoko and Eloff [47] who measured the antioxidant activities of some species African combretum.

5. CONCLUSION

In this study, we have successfully extracted different solvent partitions with n-hexane, dichloromethane, ethyl acetate and n-butanol from crude aqueous metabolic leaf extract of *Combretum racemosum*. On the basis of the results obtained, it can be concluded that the leaf extract contains large amounts of flavonoids and phenolic compounds, demonstrated high antioxidant and free radical scavenging. In addition, the plant solvent partitions exhibited broad spectrum antimicrobial activities which compared favourably with the standard antibiotics, streptomycin and ampicillin. These *in vitro* assays indicate that the leaf of *Combretum racemosum* is a good source of affordable antimicrobial agents of natural origin and could as well assume a vital role in the removal of free radicals in living tissues.

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7. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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