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Evaluation of antimicrobial and antioxidant properties of some herbs used in sub-Saharan Africa, Nigeria

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ABSTRACT

Objective: To examine antimicrobial and antioxidant potentials of *Cissus populnea* (*C. populnea*) root and *Securidaca longipedunculata* (*S. longipedunculata*) leaf and root extracts using aqueous, ethanol and petroleum-ether media.

Methods: The free radical 2,2-diphenyl-1-picrylhydrazyl was used to measure scavenging activities of extracts, while phenol and flavonoid contents were estimated by spectrophotometry and the antibacterial screening of extracts was done using disc diffusion method. The extracts were tested against five strains of Gram positive and negative bacteria. Minimum inhibitory concentration of extracts in the screening assay was according to micro-broth dilution, and minimum bactericidal concentration was carried out by agar diffusion method.

Results: The ethanol extract of the *S. longipedunculata* leaf recorded the highest [(83.00 ± 2.97) μg/mL] radical scavenging potential while the least value [(48.50 ± 2.55) μg/mL] was recorded for *C. populnea* root ethanol extract. The highest phenol content was found in the ethanol extract of *C. populnea* [(145.60 ± 0.85) mg gallic acid equivalent/g], while the least value [(87.75 ± 3.46) mg gallic acid equivalent/g] was recorded in petroleum ether extract of *S. longipedunculata*. The highest and lowest values [(81.30 ± 0.99) and (46.60 ± 1.98) mg rutin equivalent/g] of flavonoid were recorded with ethanol extracts of *S. longipedunculata* leaf and root, respectively. The aqueous and ethanol leaf and root extracts of *S. longipedunculata* were active against the growth of *Escherichia coli*, *Staphylococcus aureus*, *Vibrio cholerae*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Similar results were observed with ethanol and aqueous root extracts of *C. populnea* for the same bacteria.

Conclusions: All the crude plant extracts possessed strong antioxidant and antimicrobial activities qualifying them as medicinal herbs that could be included in animal feed production.

1. Introduction

Globally, people from every walk of life, depend on plants as source of food and for medical purposes since time immemorial. Most of the populations in developing nations including Nigeria make use of herbal medicines to meet their health needs. Herbs are often used to provide fundamental health service, to community living in isolated areas, where it is the only available health care and also offering affordable medication[1,2].

Also, in the areas where modern medicine is available, the interest in herbal medicines and their utilization has been ever-increasing due to their efficacy and potency in the cure of numerous bacterial infections and other diseases[2,3].

Moreover, due to the soaring price of effectual antibiotics and the quandary of microbial strains that are resistant to antibiotics all over the world, about 60%–85% of the population of developing world relies on herbs as alternative medicines for their wellbeing[4-6].

Herbal products contain active ingredients of plant materials which manifest varying biological activities. Hence, extracts from herbs that are used as both preventive and curative medicines of several diseases, provide therapeutic modalities with broad range of antimicrobial actions against a variety of pathogenic microorganisms[7-11].

Furthermore, these extracts have antioxidants which restrain the commencement of oxidizing chain reactions by delaying

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the oxidation of other molecules in chemical reactions[12]. Many of these naturally occurring antioxidants found in herbs such as *Cissus populnea* (*C. populnea*) and *Securidaca longipedunculata* (*S. longipedunculata*) demonstrate an array of biological impacts including anti-inflammatory, anti-bacterial, anti-allergic, antithrombotic and vasodilator action[13-17].

In addition, extracts from *C. populnea* (Ogbolo) have been credited with antibacterial properties[14], while the aqueous extract of its stem bark is used as aphrodisiac and to improve fertility among the Yoruba speaking people of South West, Nigeria[15]. Similarly, *S. longipedunculata* (Ipeta) root extracts have shown remarkable benefits as analgesia, anti-inflammatory, antioxidant and anti-depressant[18], anti-malaria, anti-trypanosomiasis and aphrodisiac[19-21].

Therefore, this study evaluates the anti-oxidant and antimicrobial potentials of the aqueous, petroleum ether and ethanolic extracts of *C. populnea* root and *S. longipedunculata* root and leaf.

2. Materials and methods

2.1. Collection of herbs

Samples of *C. populnea* root and *S. longipedunculata* leaf and root, were bought at Oyingbo herbal market, Lagos, Nigeria. These herbs were identified at the Herbarium, Botany Department, University of Lagos. Plant samples were washed with water, spread out and air dried at room temperature. The dried leaves and roots were powdered using an electric blender[22]. The extractions of herbs were carried out with aqueous, petroleum ether (80 ℃) and ethanol (95%) solvents.

2.2. Extraction process with aqueous solvent

20 g of each dried herb was put in conical flask (250 mL) containing 200 mL distilled water and boiled at 75 ℃ for 30 min. It was put on an orbital shaker for 24 h, filtered with muslin cloth and centrifuged at 10 000 r/min for 5 min. The collected supernatant was concentrated in water bath at 75 ℃. An oily material of aqueous extract gotten from each herb was weighed, kept in labeled bottles and refrigerated at 4 ℃[22].

2.3. Extraction process with ethanol

20 g of each dried herb was put in conical flask (250 mL) containing 200 mL ethanol (95%). The mixture was put on an orbital shaker for 24 h and filtered with muslin cloth. The extraction was repeated twice, the filtrates obtained were put together, centrifuged at 10 000 r/m for 5 min while the supernatant obtained was concentrated in water bath at 70 ℃. An oily material, crude ethanol extract collected from each herb was weighed, kept in labeled bottles and refrigerated at 4 ℃[22].

2.4. Extraction process with petroleum ether solvent

20 g of each dried herb was put in conical flask (250 mL) containing 200 mL petroleum ether (80%). The flask was put on an orbital shaker for 24 h and filtered with muslin cloth. The extraction was repeated twice, the filtrates obtained were put together and centrifuged at 10 000 r/min for 5 min.

The extract was heated in an oven (40 ℃) to remove the solvent and an oily material. Crude petroleum ether extract gotten from each herb was weighed, put in labeled bottles and refrigerated at 4 ℃[22].

2.5. Assay for anti-oxidation process

2.5.1. Estimation of scavenging property of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radicals

The free radical scavenging property of the fractions was measured *in vitro* by DPPH test using the following method. 24 mg DPPH and 100 mL methanol solution was prepared to make an absorbance 0.98 ± 0.02 at 517 nm using spectrophotometer and refrigerated at 20 ℃. 3 mL aliquot from the above solution was added to 100 μL of the sample at different concentrations (10–500 μg/mL). The mixture was thoroughly blended and incubated in the dark at room temperature for 15 min. Thereafter, the absorbance was measured at 517 nm and the control test was prepared in above manner without the sample[23,24]. The measurement of the DPPH (%) radical scavenged was presented in the next equation:

Scavenging (%) = (Value of control absorbance – Value of sample absorbance)/Value of control absorbance × 100

2.5.2. Estimation of total phenol

The total phenol content (TPC) was assessed by spectrophotometric method[25]. 1 mL of the sample was mixed with 1 mL of Folin-Ciocalteu's phenol reagent and allowed to stay for 5 min, after which 10 mL 7% Na_2CO_3 solution was added, followed by the addition of 13 mL de-ionized distilled H_2O and thoroughly mixed. The mixture was incubated in the dark for 90 min at 23 ℃, after which the absorbance was measured at 750 nm. The value of phenol was estimated in triplicate with the mean value of absorbance taken, the standard solution of gallic acid was prepared and the calibration line was interpreted. The concentration of phenolics (mg/mL) was expressed in terms of gallic acid equivalent (GAE).

2.5.3. Estimation of total flavonoid

0.3 mL of extract, 3.4 mL methanol (30%), 0.15 mL $NaNO_2$ (0.5 mol/L) and 0.15 mL $AlCl_3.6H_2O$ (0.3 mol/L) were put together in a 10 mL test tube. After 5 min, 1 mL NaOH (1 mol/L) was added to the mixture which was thoroughly blended and the absorbance was determined against the blank reagent at 506 nm. The rutin standard solution (0 to 100 mg/L) was used to determine the standard curve for total flavonoid. The value of flavonoid was estimated in triplicate and expressed as milligram of rutin equivalent per g of dried extract[26].

2.6. Assay for antibacterial procedure

2.6.1. Preparation of standard inoculums for antibacterial assay

A total of five microorganisms were obtained from the Microbiology Division, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria. They were *Staphylococcus aureus* (*S. aureus*) ATCC 25923 (Gram positive), while the remaining microbes were Gram negative: *Vibrio cholerae* (*V. cholerae*) ATCC 14035, *Escherichia coli* (*E. coli*) ATCC 25922, *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC 27854 and *Salmonella typhi* (*S. typhi*) ATCC 700931. The microbes were maintained on nutrient agar slant at 4 ˚C with sub-culturing done every two months. Prior to their use for *in vitro* antimicrobial assay, a loop of each microbial culture on slant was placed in Mueller-Hinton broth (5 mL) and grown for 6 h at 37 ˚C. The turbidity of the resulting broth culture was adjusted to 0.5 McFarland standards to achieve a cell density 1.5×10^8 CFU/mL and labeled as the standard inoculums.

2.6.2. Procedure for disc diffusion test

The extract was screened for bacteria, by establishing the zone of inhibition of bacteria with disc diffusion method[27]. Each plant extract was tested individually against five pathogenic bacteria strains of Gram positive and negative organisms[28].

2.6.3. Preparation of herb extracts solutions

The dried herb samples were individually weighed and dissolved in sterile distilled H_2O to prepare appropriate dilution to get different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg/mL) and were refrigerated before they were used.

2.6.4. Inoculation of test plates

20 mL Mueller-Hinton agar was prepared in Petri dish (100 mm × 15 mm). 100 µL of each standard inoculum of microorganism was plotted on the surface of the Mueller-Hinton agar plate with a sterile cotton swab (HiMedia, readily prepared swabs) that had been dipped into the turbid culture suspension. Preparation of discs (6 mm) was carried out with Whatman filter paper (No. 1) and put in hot air for sterilization. These discs were loaded with broad spectrum antibiotics (ciprofloxacin) and plant extract solutions (*C. populnea* and *S. longipedunculata*) of varying concentrations and placed in refrigerator for 24 h. Paper discs were placed on the surface of the inoculated agar plates, and pressed down properly apart and firmly for complete contact with the agar surface. The agar plates were incubated for 1 h at 5 ˚C to ensure good diffusion and then incubated for 24 h at 37 ˚C. Afterwards, the agar plates were reversed and incubated for 24 h at 37 ˚C[29].

2.6.5. Antibacterial screening of plant extracts

30 mg/mL of each herb extract was prepared; 20 µL of this extract was put on the surface of sterile paper disc (diameter, 6 mm) that was subsequently incubated for 15 min at 4 ˚C. Five plant extract discs were mounted on plates and three replicate plates were used per extract. The plates were reversed and put in an incubator for 24 h at 37 ˚C. The inhibition zone diameter for each disc was estimated after incubation and the average of the triplicate tests was taken. The degree of activity of each of the extracts was expressed in mm according to inhibition zone diameter [no activity (< 7 mm), active (7–11 mm) and very active (> 12 mm)][29].

2.6.6. Estimation of minimum inhibitory concentration (MIC)

The MIC is the smallest amount concentration of the test plant extract that produces no turbidity after incubation for 24 h[29]. The MIC of each herb extract in the screening assay was estimated using the method of micro-broth dilution[30]. Serialized dilutions of each herb extract at different concentrations (0.5–32 mg/mL) in sterile Mueller-Hinton broth were dispensed into the wells of 96-well microtiter plate (50 µL per well). This was followed by the addition of equivalent volume of each of the test microorganism at 10^6 CFU/mL into the well. The plate was covered with aluminum foil and put in an incubator for 24 h at 37 ˚C. The results were compared with standard antibiotic ciprofloxacin (10 µg/disc) from Oxford (UK).

2.6.7. Estimation of minimum bactericidal concentration (MBC)

The MBC is the smallest amount of concentration of the test plant extract that produces no colonies of the test organisms after incubation for 24 h[29]. The MBC of each active or very active medicinal plant was assessed using the method of agar diffusion. 10 µL aliquot of test bacteria culture was taken from the microtiter plates used for the MBC assays. Each aliquot was used in the surface inoculation of Mueller-Hinton agar plate, which was incubated at 37 ˚C for 24 h.

2.7. Statistical analysis

All data collected during the experiment were analyzed by routine statistical analysis: mean, range, and standard deviations which were adopted from Zar[31].

3. Results

The results of free radical scavenging capacity of different plants parts used with different extraction media are shown in Table 1. The results showed that all extracts of the test plants had radical scavenging activity. The ethanol extract of the *S. longipedunculata* leaf recorded the highest [(83.00 ± 2.97) µg/mL] radical scavenging capability followed by *C. populnea* aqueous extract. The least was reported for *C. populnea* ethanol extract at (48.50 ± 2.55) µg/mL.

The TPC is recorded in Table 1. The highest TPC was found in the ethanol extract of *C. populnea* [(145.60 ± 0.85) mg GAE/g], while the least was reported in petroleum ether extract of *S. longipedunculata* [(87.75 ± 3.46) mg GAE/g].

The flavonoid content value was highest in ethanol extract of *S. longipedunculata* leaf [(81.30 ± 0.99) mg RE/g] and the least was also recorded by ethanol extract of *S. longipedunculata* root [(46.60

Table 1

DPPH free radical scavenging activity, total phenol and total flavonoid of *S. longipedunculata* and *C. populnea* extracts (mean ± SD).

Readings	SL root (Aqueous)	SL root (Ethanol)	SL root (PE)	SL leaf (Aqueous)	SL leaf (Ethanol)	SL leaf (PE)	CP root (Aqueous)	CP root (Ethanol)	CP root (PE)
DPPH free radical scavenging activity (µg/mL)	63.75 ± 1.77	65.05 ± 4.17	72.40 ± 2.26	60.6 ± 0.14	83.00 ± 2.97	52.60 ± 3.39	81.30 ± 0.99	48.50 ± 2.55	72.20 ± 0.57
TPC (mg GAE/g)	88.50 ± 2.40	108.65 ± 1.91	87.75 ± 3.46	105.6 ± 1.13	90.20 ± 0.28	108.65 ± 1.91	108.00 ± 3.11	145.60 ± 0.85	102.35 ± 0.92
Total flavonoid content (mg RE/g)	56.15 ± 1.63	46.60 ± 1.98	68.05 ± 3.18	48.10 ± 2.97	46.60 ± 1.98	52.60 ± 3.39	81.30 ± 0.99	50.60 ± 0.57	72.20 ± 0.57

RE: Rutin equivalent; SL: *S. longipedunculata*; CP: *C. populnea*; PE: Petroleum ether.

± 1.98) mg RE/g] (Table 1).

The inhibitory activities of the plant extracts are recorded in Table 2. The aqueous and ethanol leaf and root extracts of *S. longipedunculata* were active against the growth of *E. coli*, *S. aureus*, *V. cholerae*, *P. aeruginosa* and *S. typhi*. Similar results were recorded for ethanol and aqueous root extracts of *C. populnea* for the same set of bacteria.

The aqueous extract of *S. longipedunculata* root elicited MIC mean of (1.50 ± 0.70)–(11.50 ± 4.95) µg/mL and MBC mean of (2.90 ± 1.56)–(23.00 ± 9.90) µg/mL, while the aqueous extract of *S. longipedunculata* leaf elicited MIC mean of (1.35 ± 0.64)–(12.00 ± 5.65) and MBC mean of (3.00 ± 1.41)–(30.00 ± 0.00) µg/mL against the test microorganisms (Table 3).

Table 3

MIC and MBC of aqueous root and leaf extracts of *S. longipedunculata* against the test microorganisms (mean ± SD, µg/mL).

Bacteria	Aqueous root extract of SL		Aqueous leaf extract of SL	
	MIC	MBC	MIC	MBC
E. coli ATCC 25922	2.00 ± 0.24	2.90 ± 1.56	2.38 ± 1.94	5.63 ± 2.65
S. aureus ATCC 25923	3.88 ± 0.18	9.50 ± 7.78	7.75 ± 0.35	11.50 ± 4.95
V. cholerae ATCC 14035	1.50 ± 0.70	3.00 ± 1.41	3.88 ± 0.18	5.75 ± 2.47
P. aeruginosa ATCC 27853	7.75 ± 0.35	15.50 ± 0.71	12.00 ± 5.65	30.00 ± 0.00
S. typhi ATCC 700931	11.50 ± 4.95	23.00 ± 9.90	1.35 ± 0.64	3.00 ± 1.41

SL: *S. longipedunculata.*

Table 2

Bacteria growth inhibitory activities of *S. longipedunculata* and *C. populnea* extracts.

Bacteria	Inhibition zone diameter (mm)								
	SL root (Aqueous)	SL root (Ethanol)	SL root (PE)	SL leaf (Aqueous)	SL leaf (Ethanol)	SL leaf (PE)	CP root (Aqueous)	CP root (Ethanol)	CP root (PE)
E. coli ATCC 25922	13	10	G	8	G	10	3	10	2
	12	12	G	7	G	11	2	11	3
S. aureus ATCC 25923	10	9	2	10	2	13	4	8	2
	12	9	3	11	2	13	3	9	1
V. cholerae ATCC 14035	14	10	G	10	3	9	2	10	3
	12	8	G	11	4	8	2	12	2
P. aeruginosa ATCC 27853	10	10	2	7	2	12	3	13	3
	10	8	3	6	3	13	2	12	4
S. typhi ATCC 700931	5	14	2	12	4	9	4	8	G
	6	12	2	12	3	8	3	10	G

G: Indicates bacteria growth. SL: *S. longipedunculata*; CP: *C. populnea*; PE: Petroleum ether.

Similarly, the ethanol extract of *S. longipedunculata* root elicited MIC mean of (5.75 ± 2.48)–(7.75 ± 0.35) µg/mL and MBC mean of (11.25 ± 5.30)–(12.00 ± 5.66) µg/mL. Also, the ethanol extract of *C. populnea* root elicited MIC mean of (1.15 ± 0.92)–(11.50 ± 4.95) µg/mL and MBC mean of (2.90 ± 1.56)–(23.00 ± 9.90) µg/mL (Table 4).

Table 4

MIC and MBC of ethanol roots extracts of *S. longipedunculata* and *C. populnea* against the test microorganisms (mean ± SD, µg/mL).

Bacteria	Ethanol root extract of SL		Ethanol root extract of CP	
	MIC	MBC	MIC	MBC
E. coli ATCC 25922	7.75 ± 0.35	11.50 ± 4.95	11.50 ± 4.95	23.00 ± 9.90
S. aureus ATCC 25923	5.75 ± 2.48	11.25 ± 5.30	11.50 ± 4.95	23.00 ± 9.90
V. cholerae ATCC 14035	7.75 ± 0.35	12.00 ± 5.66	5.75 ± 2.47	7.75 ± 0.35
P. aeruginosa ATCC 27853	5.75 ± 2.48	11.25 ± 5.30	1.15 ± 0.92	2.90 ± 1.56
S. typhi ATCC 700931	5.63 ± 2.65	11.25 ± 5.30	7.75 ± 0.35	11.50 ± 4.95

SL: *S. longipedunculata*; CP: *C. populnea.*

4. Discussion

The DPPH assay has been used extensively to assess the scavenging actions of numerous natural compounds such as extracts of plants, because of its stability and reproducibility[32]. The assay is habitually used to appraise the capability of antioxidants to remove free radicals which are major factor in biological damage due to oxidative stress[33]. In this study, the solvents (aqueous, ethanol and petroleum ether) have good capacity for extracting antioxidant molecules because all the plant extracts exhibit different levels of antioxidant action as free radical removers.

Phenols are present in large number in the plant materials, and are known to have several biological roles, which include antioxidant action[34]. Previous reports indicated good positive linear correlation between antioxidant capacity and TPC in spices, herbs, and other dietary plants[35]. The results of this study also authenticated that phenolic compounds are responsible for the antioxidant actions of the plant extracts[36,37]. The TPC from this study ranged between (87.75 ± 3.46) and (145.60 ± 0.84) mg GAE/g. These high TPC values recorded in the study illustrated that the plants extracts are probable natural antioxidant agents, which can be used to prevent oxidative rancidity and replace commonly used synthetic

antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole[35,38] in the production of animal feed.

Flavonoid is among the natural compounds that possess wide range of chemical and biological activities, including radical scavenging property[39]. The antioxidant potential of flavonoid depends on their structure particularly their position in the compound and their ability as electron donor to a free radical[40,41]. The total flavonoid content in this study ranged between (46.60 ± 1.97) to (83.00 ± 2.96) mg RE/g indicating the usefulness of the studied plant extracts as indigenous antioxidants for the management of disorders of oxidative stress and, other biochemical functions[42].

Furthermore, these results reflected the potent antimicrobial phytochemicals present in the plant materials used for this study. The capability of the extracts to inhibit several bacterial species is an indication of the broad spectrum antimicrobial potentials of *S. longipedunculata* and *C. populnea*. The antimicrobial actions of the plant extracts could be as a result of the presence of different bioactive compounds[43]. The high antimicrobial action of the aqueous root extracts observed was similar to the reports of earlier authors[44,45], which attributed these activities to the high content of flavonoid present in the extracts. Additionally, it was reported elsewhere that *S. longipedunculata* possessed antitrypanosomal and anti-inflammatory activities[46,47].

These phytochemical components have been documented as major biological active compounds which exhibit their effects on physiological processes[48]. This is further corroborated by Tyler[49] that many phytomedicines wield their impacts through the additive or synergetic action of several compounds performing at a single or several target locations that are related to physiological activities.

Among the studied plants, the ethanol extracts of *S. longipedunculata* and *C. populnea* showed better antioxidant activities in addition to having highest amount of phenols and flavonoids while petroleum ether extracts were least in performance in the measurements of the above parameters. Also, the ethanol and aqueous herbal extracts of *S. longipedunculata* and *C. populnea* were active against the growth of *E. coli*, *S. aureus*, *V. cholerae*, *P. aeruginosa* and *S. typhi*. In view of the above remarkable end results of the studied crude plant extracts, they are better potential antioxidant and antimicrobial agents which make them probable nutraceuticals in animal feed.

Conflict of interest statement

We declare that we have no conflict of interest.

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