British Journal of Pharmaceutical Research
4(12): 1552-1567, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antimicrobial and Cytotoxic Activities and GC-MS Analysis of Phytocomponents of Methanolic Extract of *Curculigo pilosa* (Schum and Thonn) Engl. (Hypoxidaceae) Rhizomes

Elijah Y. Shaba[1], Abdullahi Mann[1*] and Jonathan Yisa[1]

[1]Department of Chemistry, Federal University of Technology, Minna, P.M.B. 65, Minna, Niger State, Nigeria.

Authors' contributions

Author EYS has contributed significantly to acquisition of data, analysis, drafting of the manuscript. Author AM has made substantial contribution to conception and design, interpretation of data, drafting and revising the manuscript for intellectual content. Author JY has participated in the research design, data analysis and revising the manuscript for intellectual content. All authors read and approved the final manuscript.

Original Research Article

Received 18th March 2014
Accepted 8th May 2014
Published 18th June 2014

ABSTRACT

Objectives: To investigate the antimicrobial and cytotoxic activities of crude methanol extract of *Curculigo pilosa* (Schum and Thonn) Engl. (Hypoxidaceae) rhizomes and its solvent soluble fractions and to analyze the most active fraction by Gas Chromatography Mass Spectrometry (GC-MS) technique.
Place and Duration of Study: Department of Chemistry and Department of Microbiology, School of Natural and Applied Sciences, Federal University of Technology, Minna, Nigeria in June, 2012.
Methodology: Shade air-dried powder of *Curculigo pilosa* rhizomes was extracted with methanol by Soxhlet extraction. Crude methanol extract of *Curculigo pilosa* root (CCPM) and the solvent soluble fractions namely: *n*-hexane (CPH), chloroform (CPC), ethylacetate (CPE), *n*-butanol (CPB) and residue (CPM) were obtained. Phytochemical constituents of the most active fraction (methanol residue) of *Curculigo pilosa* rhizomes were determined using GC-MS technique.
Results: The antimicrobial activity of some solvent fractions tested except *n*-hexane

**Corresponding author: Email: abdumann@yahoo.com;*

fraction appears to be promising with Minimum Inhibitory Concentration (MIC) ranging from 0.09–6.25 mg/mL. However, ethyl acetate and *n*-butanol fractions showed antibacterial activity against highest number of bacterial strains. The results revealed that methanolic residue was more potent than derived fractions. Cytotoxicity assay results indicated weak cytotoxic activity of the crude *C. pilosa* methanol root extract as displayed by its LC_{50} (764.07µg/mL). The qualitative phytochemical evaluation indicates the presence of chemical constituents such as flavonoids, terpenoid, saponins**,** tannins**,** alkaloids**,** cardiac glycosides, steroid and anthraquinone. The quantitative analysis of its metabolites depicts alkaloids (12.80±0.49), saponins (54.49±0.33), flavonoids (44.88±0.36), tannins (69.49±0.65), phenols (50.40±0.34), oxalates (10.95±0.63), cyanides (44.87±0.70) and phytate (15.00±0.05). The major phytocomponents identified by GC-MS analysis in the combined fractions of *C. pilosa* methanolic residue indicated that there were different types of high and low molecular weight compounds. In particular, it revealed the presence of 3-eicosyne (8.98%), pentadecanoic acid (2.41%) hexadecanoic acid (31.18%), octadecanoic acid (1.52%) 9-octadecenoic acid (24.42%), linoleic acid ethyl ester(3.93%), androstan-3-one (5.90%), 1-phenanthrenenmethanol (5.78%), 1,2-benzenedicarboxylic acid (4.59%), hexanedioc acid (13.38%), 8,11-octadecadienoic acid (9.02%), nonadecane (3.52%), ethanol-2, 2-oxybis (20.75%), propane-1-(1-methylethoxy) (8.05%) and 2, 6,10-dodecatriene-1-ol (5.14%).
Conclusion: In this study, some of the phytocompounds identified are biologically important and may have contributed to the observed antimicrobial activity, hence their therapeutic significance which may support the ethnomedicinal uses of *C. pilosa* in the treatment of venereal diseases in humans.

Keywords: Curculigo pilosa; cytotoxicity; GC-MS analysis; brine lethality bioassay; antimicrobial activity.

1. INTRODUCTION

Globally, plants are used medicinally and often are good sources of many potent and powerful drugs to treat microbial infections [1,2]. About 25% of prescribed drugs in the world originate from plants [3]. Nigerian biodiversity has been described as rich sources of medicinal plants for indigenous uses and practices [4, 5] and it could be a potentially source for innovative and sustainable solutions to drug discovery and development in modern society [6,7]. Medicinal plants of genus *Curculigo* have emerged as a good source of the traditional medicines. Some uses of these plants in the traditional medicines have been validated by pharmacological investigation [8]. *Curculigo pilosa* is used in the management and treatment of venereal diseases and candidiasis in humans in Nupe ethnomedicine. *Curculigo pilosa* (Schum and Thonn) Engl. belongs to the family of Hypoxidaceae (Common name: – Golden eye grass, African crocus); Local Nigerian names: – [Nupe – Echidungi; Hausa – Baka, ekuaku, Dooyar kureegee; Yoruba – Epakun]. It is an herbaceous plant with stout, erect vertical, cylindrical rhizomes bearing a cluster of grass-like leaves to 60 cm long and yellowish solitary flower shoots to 20 cm at the end of the dry season [9]. *Curculigo pilosa* is a plant that has up to 45 cm high with a vertical, cylindrical rhizome, 0.9-9.4 x 0.7-1.8 cm as shown in Fig. 1, leaves pseudopetiolate, lanceolate to ovate, erect or reflexed, 5.0-26.0 mm broad. Leaf lamina plicate thinly pilose with whitish to yellowish hairs. Scape terete, sparsely pilose, subterranean, 0.5-1.5 cm long. Flowers solitary, 1.2-2.5 cm in diameter are as shown in Fig. 2. Perianth segments acute, 8.8-15.0 x 2.5-3.5 mm; Filaments filiform, 1.84.0 mm long. Anthers 2.5-3.0 mm long.

Fig. 1. *Curculigo pilosa* plant with flowers

Fig. 2. Rhizomes of *Curculigo pilosa* obtained from Edozhigi forest, Niger State, Nigeria

2. MATERIALS AND METHODS

2.1 Collection of Plant Samples

Fresh rhizomes of *C. pilosa* were collected based on ethnobotanical information from Edozhigi forest along Bida-Wuya Road, Niger State, Nigeria. The sample was collected in month of June, 2012. Plant was identified and authenticated by Dr. Jemilat A. Ibrahim, a plant taxonomist at the National Institute of Pharmaceutical Research Development, Idu-Abuja.

2.2 Preparation of Samples

The fresh rhizomes of *C. pilosa* collected from the forest were thoroughly washed with distilled water, air-dried at room temperature under shade and prepared according to methods described by Edeoga et al. [10]. It was then pulverized into uniform powder manually. The sample was then sieved (40 mesh sizes), weighed, bottled, sealed, labeled and kept for analysis.

2.3 Extract Preparation

The pulverized *C. pilosa* rhizome (300 g) was place in a thimble before it was placed inside a soxhlet extractor and extracted with methanol (9500 mL) 48 h at 68ºC. Resulting solution was then concentrated under reduced pressure 35ºC using a rotary evaporator (Büchi Rotavapor, R-205; Quickfit, England) and evaporated to dryness under vacuum. The extract was weighed and labeled as the crude methanol extract of *C. pilosa* (CCPM) and then used to calculate percentage recovering.

2.4 Qualitative and Quantitative Analyses of the Crude Methanolic Extract of *C. pilosa*

Standard methods for qualitative analysis were used to screen the crude methanol extract for their phytoconstiteunts as described as follows: reducing sugar [11]; flavonoids [12]; cardiac glycosides, steroids, tannins, saponins and terpenoids [13]; steroidal nucleus [14]; and anthraquinones, alkaloids [12]. Standard methods were used for quantitative analysis of tannins and saponins [15]; flavonoid [16]; alkaloids [17] and oxalate [14].

2.5 Partitioning of the Crude Methanolic Extract of *C. pilosa*

The crude methanol extract was partitioned into *n*–hexane, chloroform, ethylacetate and *n*–butanol; the fractions were collected [18].

2.5.1 Activity of the solvent soluble fractions of *C. pilosa*

The antimicrobial activities of the various solvent soluble fractions of the crude methanolic extract of the rhizomes of *C. pilosa* were carried out in Department of Microbiology, Federal University of Technology Minna, Niger State.

2.5.2 Media preparation and maintenance of bacteria

The organisms were maintained on nutrient agar plates at 4ºC in the refrigerator and were revived for bioassay by sub culturing in fresh nutrient broth (Oxoid Ltd, Basingstoke, Hampshire, England) for 24 h before being used.

2.5.3 Antimicrobial screening by agar well diffusion technique

The agar well diffusion method was employed for the screening of antimicrobial activities of the soluble fractions [19-22]. In this method nutrient agar medium was prepared and poured into sterile Petri dishes (90 mm diameter) and allowed to solidify and labeled properly. Sterile cork borer (4 mm) was use; five holes will be bored on the surface of the agar medium equidistant from one another. Various concentrations of the soluble fractions (200, 100, 50, 25 mg) were reconstituted in 5.0 ml of sterile distilled water and vortexes for homogeneity to make final concentrations of 2,000, 1,000, 500 and 250µg/ml respectively. 0.5 ml of the soluble fractions and control solution at different concentrations was each introduced into the holes and allowed to fully diffuse at room temperature, after which was inoculated with the bacterial culture. The plates will be incubated at 37ºC for 24 h. The extract and the control of the radial growth were then observed after incubation. The resulting zones of inhibition were being measured with a millimeter ruler, surrounding bacterial growth. For each bacterial strain, methanol was use as negative control.

2.5.4 Determination of minimal inhibitory concentration (MIC)

The minimum inhibition concentration (MIC) values were studied for the bacterial strains which were sensitive to the extract in disc diffusion assay. MIC values were determined using micro-well dilution assay method [19-22]. A serial doubling two-fold dilution of either extract was prepared in a micro liter tray over the range 10-0.075 mg/ml in 100 µl nutrient broth. The broth was supplemented with ethanol absolute at a concentration of 0.5% in order to enhance extract solubility. Overnight broth cultures of each strain were prepared from 18 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. An aliquot 100 µl of the inoculum was added to diluted extract. The final volume in each well was 200 µl. The plate was covered with a sterile plate sealer. Positive and negative growth controls were included in every test. The tray was incubated aerobically at 30°C according to bacteria for 18-24 h. The MIC is defined as the lowest concentration of the extract at which the microorganism tested does not demonstrate visible growth in the broth. Bacterial growth was indicated by turbidity.

2.6 Brine Shrimps (*Artemia salina* L) Lethality Bioassay

Brine shrimp lethality bioassay was use to predict the presence of cytotoxic activity in the extract [23,24]. Brine shrimp eggs were obtained from Prof M.O. Fatope, Department of Chemistry, Sultan Qaboos University, Muscat, and Sultanate of Oman. They were hatched in natural seawater obtained from the Bar Beach, Ikoyi, and Lagos, Nigeria and incubated for 48 h in 3.8 g/l seawater under light. The stock solution was prepared by dissolving 0.02 g extract in 2 ml dimethyl sulphoxide (DMSO). 1.8 ml of the brine was added to 0.2 ml of the stock to give 1000 ppm solution. One milliliter of each stock solution (50, 500, and 5000 µg/ml) was put into a test tube and made up to 5 ml with filtered sea water to give overall concentrations of 10, 100 and 1000µg/ml (ppm). After hatching, the larvae of brine shrimp nauplii were collected and they were used for the brine shrimps lethality test using the method described by McLaughlin [25] with slight modification. Ten brine shrimp larvae were

then placed in each of the test tubes. Ten nauplii were drawn into each vial using Pasteur pipettes and placed in a test tube containing 4.0 ml of brine solution and 0.5 ml of plant extract concentration and made up to 5 ml with brine solution. They were not given food because the hatched brine shrimp can survive up to 48 h without food [26], as they still feed on their yolk during this period [27]. The control vials containing 5 ml of brine solution with two drops of DMSO and ten nauplii and all experiments were done in triplicates. After 24 h of incubation at room temperature under light, the content of each vial was then examined, the number of surviving larvae counted using a magnifying lens and recorded and the percentage of death calculated. Larvae were considered dead if they did not exhibit any form of movement during several seconds of observation. Extracts are regarded as nontoxic if its LC_{50} is greater 100µgmL^{-1} in brine shrimp lethality assay [25]. The mortality percentage and LC_{50} (lethal concentration for 50% of the population) were determined using statistical analysis and the graph of Logarithm of concentration against percentage lethality [28].

2.7 Data Analysis

The percentage lethality of the extracts and fractions on brine shrimps was calculated from the mean of larvae that survived in the treated tubes and controls. The numbers of dead nauplii was divided by initial number of nauplii (10) and multiply by 100. Finney's probit analysis [29] was used to determine 50% lethal concentration (LC_{50} value) at 95% confidence interval as the measure of toxicity of the plant extract.

2.8 Column Chromatographic Fractionation

The silica gel was first activated in the oven for 2 h at 105°C. The Pasteur pipette was setup in such a way that it is straight and supported with a clamp. A small amount of glass wool was put into the base of the pipette using a clean wire to tamp it down lightly to prevent any of the fine particles from going through. 200 ml of n-hexane were used to slurry of silica gel (100 g) which was then pour from the Pasteur pipette. The solvent flows slowly down the column. 1 g of the extract was re-dissolved in small amount of methanol (10 ml) and 20 g of activated silica gel was added. The mixture was swirl until the solvent evaporates and only a dry powder remains. The dry powders (silica gel and sample) were place on a folded piece of weighing paper and transfer it to the top of the prepared column. A fresh eluting solvent mixture was added to the top of the column increasing the polarity of the solvent mixture. Equal sized fractions were collected sequentially into vials and carefully labelled for thin layer chromatography analysis [30]. The bioactive MeOH fraction (1.0 g) previously embedded in silica gel was then mounted and fractionated by column chromatography (8 mm x 10 Cm) on silica gel (70-230 mesh, Merck, Darmstadt, Germany) and successively eluted using *n*-hexane: EtOAc (95: 1→ 0: 100, v/v); EtOAc: MeOH (95: 1→ 0: 100, v/v) in a stepwise solvent system [30]. The fractions were pooled into 3 combined fractions (F_{10}-F_{32}); (F_{33}-F_{48}) and (F_{49}-F_{64}) based on their TLC Profiles were used for GC-MS analysis.

2.9 GC/MS Analysis of the Combined Fractions

GC-MS analysis was carried out on a Shimadzu (Kyoto, Japan) GC-MS model QP 2010 at National Research Institute for Chemical Technology, Zaria, according to the EN 14103 standard method [31,32]. The GC column oven temperature (70ºC), injecting temperature (250ºC), flow control mode (linear velocity), total flow (40.8 ml/min) column flow (1.80 ml/min), pressure (116.9 kpa), linear velocity (49.2 cm/sec) and purge flow (3.0 ml/min) were employed for this analysis. A sample volume of 8.0 µL was injected using split mode (split

ratio of 20.0). The peak area, that is, the % amount of every component was calculated by comparing its average peak area to the total areas. Software was used to handle mass spectra and chromatogram. Interpretation of mass spectrum GC-MS was conducted by comparing the database peaks of National Institute Standard and technology (NIST) library with those reported in literature, the mass spectra of the peaks with literature data [33]. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Component relative percentages were calculated based on GC peak areas without using correction factors. The Name, Molecular weight and structure of the components of the test materials were ascertained.

3. RESULTS

3.1 Quantitative Analysis of Crude Methanolic Extract of *Curculigo pilosa*

The results of the quantitative analysis of the crude methanolic extract of *Curculigo pilosa* (Table 1) shows that the plant contained some metabolites.

3.2 Partitioning of the Crude Methanolic Extract of *Curculigo pilosa* into Solvent Soluble Fractions

Crude methanol extract was partitioned into different solvent soluble fractions starting with a least polar solvent, *n*-hexane; all the soluble compounds were dissolved into different solvent based on the polarity (Table 2).

Table 1. Quantitative analysis of crude methanolic extract of *Curculigo pilosa*

Phytoconstituents	Result (mg/100g dry wt.)
Alkaloids	12.80±0.49
Saponins	54.49±0.33
Flavonoids	44.88±0.36
Tannins	69.49±0.65
Phenols	50.40±0.34
Oxalates	10.95 ±0.63
Cyanides	44.87±0.70
Phytate	15.00± 0.05

Analyses were mean of three replicates ± standard deviations

Table 2. Percentage yield of the partitioned solvent soluble fractions of *Curculigo pilosa*

Solvent soluble fraction	Percentage yield
n-Hexane soluble fraction	4.72
Chloroform soluble fraction	17.04
Ethylacetate soluble fraction	20.97
n-Butanol soluble fraction	25.63
Residue	29.38

Table 3. Antimicrobial activity of *C. pilosa* extract (mm)

Extracts	*Escherichia coli*				*Staphylococcus aureus*				Pathogens *Streptococcus feacalis*				*Salmonella typhii*				*Candida albicans*			
	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000	250	500	1000	2000
CPH	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_	_
CPC	_	_	4	4	_	_	_	4	_	_	_	_	_	_	_	4	_	_	_	3
CPE	_	_	5	5	_	_	2	6	_	_	7	11	_	_	_	_	_	5	10	15
CPB	_	_	8	11	_	5	9	10	_	_	6	12	_	_	4	9	5	8	17	21
CPM	_	3	10	13	_	8	10	13	_	_	5	12	_	_	6	11	7	11	19	25
CLO																				30
CIP																				28

Key: CPH = n-Hexane fraction, CPC = Chloroform fraction, CPE = Ethyl acetate fraction, CPB = n Butanol fraction, CPM = Methanolic residue, CLO = Clotrimazole, CIP = Ciprofloxacin

3.3 Antimicrobial Activity of *C. pilosa* Extract

The solvent soluble fractions were tested for their antimicrobial activity. The bacterial properties are expressed in millimeter (mm) zones of inhibit of ion against some pathogens as showed in (Table 3 above). The present investigation proved that residue showed more activity against all tested pathogens with maximum activity (25 mm) against *Candida albicans.* The present investigation proved that, the residue portion of the plant extract showed activity against all tested pathogens with maximum activity (25 mm) against *Candida albicans* as shown in (Table 3). The ethyl acetate extract of *C. pilosa* showed highest activity against *Candida albinca* (15 mm) followed by *Staphylococcus faecalis* (11 mm), with *Staphylococcus aureus* (6 mm) and *E. coli* (5 mm). Generally the activity of the ethyl acetate soluble portion was not active at lower concentration of the extracts. The fraction does not show any activity against *Salmonella typhi.* The result from this investigation has showed that the methanol soluble portion extract was more active against the isolates compared to the other extracts.

3.4 Cytotoxic Activity of *C. pilosa* Extract

The (Table 4) shows the result of Brine shrimp lethality bioassay of crude methanolic extract after 48 h which revealed that the *C. pilosa* root exhibit weak cytotoxic activity against brine shrimp nauplii (larvae) with LD_{50} (50% mortality) value found to be 764.07, 602.62 and 664.23ppm respectively. The 90% mortality (LD_{90}) calculated for the crude extract was 2682.57, 3335.89 and 2141.02ppm respectively.

Table 4. Cytotoxicity of the crude methanolic extract of *C. pilosa*

Concentration	% Mortality
1000ppm	
1	50
2	60
3	50
500ppm	
1	20
2	30
3	40
100ppm	
1	20
2	20
3	10
10ppm	
1	0
2	0
3	0

3.5 Column Chromatographic Fractionation of the Methanolic Residue of the Rhizomes of *C. pilosa*

Based on the most significant antimicrobial activity results obtained for the *C. pilosa* methanolic residue, it was then fractionated using column chromatography to give three combined fractions namely: (F_{10}-F_{32}), (F_{33}-F_{48}) and (F_{49}-F_{64}) which were analysed using GC-MS spectrometer (Tables 5-7). The constituents identified in the present study belong to the

class of saturated and unsaturated fatty acids and to the categories of aliphatic acid esters, fatty esters, aldehydes, aliphatic hydrocarbons and the terpenes family.

Table 5. Analytical parameters deduced from GC-MS spectrum first combined fractions (F_{10}-F_{32}) of *C. pilosa* rhizomes

Line No.	IUPAC Name	Molecular formula	Molar mass	R.T	Area %	Fragmentation peaks
1	3-Eicosyne	$C_{20}H_{38}$	278	17.467	8.98	(43), 95, 109, 123
2	Pentadecanoic acid	$C_{17}H_{34}O_2$	270	19.207	2.41	57, (74), 87,101, 115, 129, 143
3	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	20.640	31.18	(75), 115 129, 143, 157,171, 185, 213
5	Octadecanoic acid	$C_{19}H_{38}O_2$	298	22.760	1.52	43, 57, 74, (87), 171, 185, 199, 213, 241, 255
6	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282	23.472	24.42	41, 55, (69), 83, 97
7	7 Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	308	24.524	3.93	41, (67), 81, 95, 263
8	Androstan-3-one	$C_{19}H_{30}O_3$	306	25.854	5.90	41, 55, 69, (83), 97, 111, 125, 273, 288
9	1-Phenanthrenenmethanol	$C_{20}H_{30}O$	286	26.382	5.78	157, (253), 271
10	1, 2-Benzenedicarboxylic acid	$C_{24}H_{38}O_4$	390	27.235	4.59	70, 112, (149), 261

Key: () is the base peak

Table 6. Analytical parameters deduced from GC-MS spectrum second combined fractions (F_{33}-F_{48}) of *C. pilosa* rhizomes

Line No.	IUPAC Name	Molecular Formula	Molar Mass	R.T	Area %	Fragmentation Peaks
1	Nonadecane	$C_{19}H_{40}$	268	18.467	3.52	43, (57), 71, 85, 99
4	8, 11-Octadecaienoic acid	$C_{19}H_{34}O_2$	294	22.402	9.02	67, 81, 95, 109, 262
5	Hexanedioic acid	$C_{22}H_{42}O_4$	370	26.069	13.38	57, (129), 241, 327, 341

Key: () is the base peak

Table 7. Analytical parameters deduced from GC-MS spectrum third combined fractions (F_{49}-F_{64}) of *C. pilosa* rhizomes

Line No.	IUPAC Name	Molecular Formula	Molar Mass	R.T	Area %	Fragmentation Peaks
1	Ethanol-2,2oxybis	$C_{19}H_{40}$	106	5.265	20.75	(45), 75
2	Propane-1-(1-methylethoxyl)	$C_6H_{14}O$	102	7.384	8.05	(43), 87
5	2, 6, 10-dodecatriene-1-ol	$C_{15}H_{26}O$	222	15.750	5.14	(69), 81

Key: () is the base peak

4. DISCUSSION

4.1 Phytochemical Screening of *C. pilosa* Extract

The present finding is in conformity with the result of the analysis of Gbadamosi and Egunyomi [34] who reported the presence of saponins, tannins, alkaloids and a trace of

anthraquinone in the same plant. Some researchers have also reported the presence of these components in different families of medicinal plants to possess biological activities [35]. Some of these compounds have been associated to antibacterial activities and thus have curative properties against pathogenic microbes [36,37]. The presence of these secondary metabolites in plant is responsible for their therapeutic effectiveness against wide range of diseases. Due to this fact it is not surprising that *C. pilosa* is active against some of these tested pathogens. Secondary metabolites present in *C. pilosa* have been reported to have medicinal advantages to cure diseases. Many physiological activities in wide range of anti-infective action have been assigned to tannins [38].

4.2 Quantitative Analysis of *C. pilosa* Extract

The results of the quantitative analysis of the crude methanolic extract of *C. pilosa* (Table 2) shows that the plant contained some anti-nutritional properties. Saponins (54±0.33), tannins (64.49±0.65), Phenols (50.40±0.34), cyanides (44.87±0.076), oxalates (10.95±0.34), alkaloids (12.80±0.49) and phytates (15.00±0.05) respectively. The result of different soluble fraction obtained from the fractionation of the crude methanol extract revealed small value of percentage yield of n-hexane soluble fraction which may be probably due to the fact that *n*-hexane is a non-polar solvent and so does not have affinity for highly polar materials such as tannins, alkaloids, saponins, flavonoids and carbohydrates. This result is in agreement with the finding of Rangari [39]. The result revealed that residue have high percentage yield of 29.38%, follow by the *n*-butanol soluble fraction (25.63%), Chloroform soluble fraction (17.04%) with *n*-hexane having the least percentage yield of 4.72%. The results of the quantitative analysis of the crude methanolic extract of *C. pilosa* as shown in (Table 2) revealed that the plant contained significant concentration of tannins, phenols, saponins, cyanides, oxalates, alkaloids and phytates which are comparable to the report of Okwu and Nnamdi [40]. The results show high concentration of tannin 69.49±0.65 among all the antinutrient value determined. The concentration of the value of saponin from the analysis was 54.49±0.33.The alkaloid content was 12.80±0.49. The result showed very low concentration of oxalate (10.95±0.63).The concentration of flavonoids (44.88) in the plant extract.

4.3 Antimicrobial Activity of *C. pilosa* Extract

Curculigo pilosa is a medicinal herb that has a wide range of ethnomedical applications in Nupe traditional medicine especially for the treatment of different diseases such as candidiasis in humans are said to be effective. This finding also agrees with the earlier report of this plant's inhibition of *C. albicans* by Gbadamosi and Egunyomi [34]; this result supported the use of *C. pilosa* in herbal medicine for disease prevention and treatment of infections. The chloroform soluble portion was not active against *Staphylococcus faecalis* it inhibit the growth of *Escherichia coli, Staphylococcus aureus, Salmonella typhi,* and *Candida albicans* and have least activity compare to other extracts that shows zone of inhibition. The present study has shown that extract from *C. pilosa* rhizomes can suppress the growth of these organisms. Generally, the activity of chloroform soluble portion extract was active against all the organisms. The *n*-hexane soluble portion of plant did not show any activity against all the tested isolates. This result is different from the findings of analysis of same plant by Oliver [41] which reported that oil extracted from *C. pilosa* have slight antibiotic action and are used in the treatment of infections [41]. The ethyl acetate extract of *C. pilosa* showed highest activity against *Candida albicans* (15 mm) followed by *Staphylococcus faecalis* (11 mm), with *Staphylococcus aureus* (6 mm) and *E. coli* (5 mm). Recent reports have shown that there is

reduction in the discovery of new antimicrobials agents globally [42], coupled with alarming cases of drugs resistant to available antimicrobials. Minimum inhibitory concentration (MIC) is used as an index for measuring the efficacy of antibacterial agents [43]. The fraction does not show any activity against *Salmonella typhi.* The result from this investigation has showed that the methanol soluble portion extract was more active against the isolates compared to the other extracts. The control (clotrimazole) show maximum activity at 30 mm against *Candida albicans*. The activity of the residue portion extract compared favourably for *Candida albicans*. This indicates that the plant extract may be better at treating candidiasis. The extracts may be better than the control tablet since it is still in its crude form and due to the process of extraction the plant undergo and presence of other metabolites which may not be active, will mask some of the active compounds in the plant. Adenike et al. [44] made a similar observation that the crude plant preparations have generally been reported to exhibit lower antimicrobial activity than pure antibiotic substance such as ciprofloxacin.

4.4 Cytotoxic Activity of *C. pilosa* Extract

For uniformity of the result, the tests were repeated three times. The *in vivo* lethality in a simple zoological organism, such as brine shrimp lethality test might be used as a simple tool to guide screening and fractionation of physiological active plant extracts, where one of the simplest biological responses to monitor is lethality, since there is only one criterion; either dead or alive [23-28]. Therefore, this test has been successfully used by many researchers as a simple biological test in order to detect anti-tumour compounds [23]. There is a general toxicity test agreement that LC_{50} above 100µgmL^{-1} is non-toxic while that below 100µgmL^{-1} is indicative of toxicity [23-28]. The present result shows that the extract of *C. pilosa* root exhibited no cytotoxic activity against the brine shrimp larvae at 10ppm. LD_{50} values < 1000 ppm have been considered significant for crude extracts [24]. A close looking at the LD_{50} of the current results indicate values that are close to 1000ppm. This is an indication that the extract of *C. pilosa* root is not very active against brine shrimp nauplii. According to Meyer et al. [45] several naturally extracted products which had LC_{50}<1000µg/mL using brine shrimp bioassay were known to contain physiologically active principles. Although toxicity test using BST does not give a clear depiction on cytotoxicity against cancer cell, however this method has been reported useful for screening anticancer from plant/natural sources. The results of the percentage mortality show that the methanolic extract had the highest percentage mortality (60%) activity at 1000ppm. At 500ppm the plant was not very active against brine shrimp nauplii with 40% percentage mortality compared to 60% for 1000ppm. The plant shows just a slight activity of 20% percentage mortality against brine shrimp nauplii at 100ppm. There was not activity against brine shrimp nauplii at 10 ppm since all the brine shrimp larvae survived and the percentage mortality at that concentration recorded zero percent after 24 h. The non-significant lethality of the extract from the root of *C. pilosa* may be an indication of the absence of potent cytotoxic compounds. The outcome of the present study depicts C*. pilosa* root as lacking in anti-tumor compounds. However, the antimicrobial results from this study have shown that extract from this plant exhibit broad spectrum activity by inhibiting the growth of several human pathogenic bacteria and candida species at relatively low concentrations of soluble fractions. This is indication of the broad spectrum antimicrobial potential of *C. pilosa* could make this species a candidate for antibiotic bioprospecting. The ability of the extracts from this plant to effectively suppress the growth of both human pathogenic bacteria and fungi at relatively low concentrations further validates the folkloric use of the plant species for the treatment of various human diseases. The observed antimicrobial activities in this study could be attributed to the presence of phytochemicals like saponins, tannins and phenolics [46]. Since these compounds may not have any discernible physiological roles in the plants in which they occur, many of them have significant biological

effects on animals. However, it is generally accepted that they play an essential role of stress reduction in plant physiology through the interaction between the plant and its environment [46].

4.5 Chemical Constituents of *C. pilosa* Methanolic Residue by GC-MS

For the identification of phytocompounds Gas Chromatography-Mass Spectrometry (GCMS) is a reliable tool [28]. The GC-MS spectra gave a complete fragmentation pattern of the constituents contained in the combined fractions. In present study 16 different compounds were identified with the help of standard library search software. The major peaks are fatty acids such as pentadecanoic acid, hexadecanoic acid and octadecanoic acid. Fatty acid derivative such as linoleic acid ethyl ester was also found to be present in considerable amounts. The fraction also contained hydrocarbons 3-eicosyne and nonadecane. Fatty acids are reported to be biologically significant in the diagnosis and control of venereal diseases such as gonorrhea and candidiasis. The presence of these fatty acids in a considerable amount might serve to recognize the potential pharmacological importance of this plant in disease control.

5. CONCLUSION

The plant contain various phytochemical constituents such flavonoids, terpenoid, saponins**,** tannins**,** alkaloids**,** cardiac glycosides**,** steroids and anthraquinone. Secondary metabolites from plants are considered as potential sources of novel antimicrobial compounds. The GC-MS of semi-purified fraction indicate that the plant contain some chemical constituents which may be responsible for the antimicrobial and weak cytotoxic activities reported in this work. However, the mechanism of their actions should be further elucidated; the actual constituents responsible for bioactivity should be isolated, identified, purify and structural elucidated. The results indicate that *C. pilosa* is an important source of fatty acids and their derivatives which are reportedly found to be active against various venereal diseases like gonorrhea and candidiasis in human.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

ACKNOWLEDGEMENTS

The Authors are thankful to the authority of Federal University of Technology, Minna, Niger State, Nigeria for providing financial support and other research facilities in conducting this research work. The Authors are also grateful to Prof M.O. Fatope, Department of Chemistry, Sultan Qaboos University, Muscat, Sultanate of Oman for supplying the brine shrimp eggs for carried out this project. We appreciate the assistance the Department of Microbiology, Federal University of Technology Minna, Niger State for supply of microbial isolates used and conduct of antimicrobial experimental work and Mr. Gabriel F. Ibikunle for his continuous assistance during the BST experimental work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. 3rd ed. Spectrum Books, Ibadan; 2008.
2. Farnsworth NR. Screening plants for new medicines. In Wilson, E.O. (Ed). Biodiversity. National Academy Press: Washington, DC. 1988;83-97.
3. Rates SMK. Plants as source of drugs. Toxicon. 2001;39:603–13.
4. Iwu MM. African Medicinal Plant in the Search for new drugs based on ethnobotanical leads. In: D.J. Chadwick and J. Marsh (Eds) Ethnobotany and the search for new drugs. Ciba Foundation Symposium, Wiley, Chichester. 1994;185:116-129.
5. Iwu MM. Handbook of African Medicinal Plants. C.R.C Press, Florida. USA. 1993:26–267.
6. Cragg GM, Newman DJ. Snader KM. Natural Products in Drug Discovery and Development. J Nat Prod. 1997;60:52-60.
7. Sofowora A, Ogunbodede E, Onayade A. The Role and Place of Medicinal plants in the Strategies for Disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-229.
8. Nie Y, Dong X, He Y, Yuan T, Han T, Rahman K, et al. Medicinal plants of genus *Curculigo:* Traditional uses and a phytochemical and ethnopharmacological review. J Ethnopharmacol. 2013;147(3):547-563.
9. Sofidiya MO, Oduwole B, Bamgbade E, Odukoya O, Adenekan S. Nutritional composition and antioxidant activities of *Curculigo pilosa* (Hypoxidaceae) rhizome. Afri J Biotechnol. 2011;10(75):17275-17281.
10. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afri J Biotech. 2005;4(7):685-688.
11. Trease E and Evans WC. Pharmarcognosy Bailliere Indall London. 1983;(3):21-22.
12. Evans WC. Trease Evans Pharmacognosy, (15th ed), W.B. Saunders Company Ltd., London; 2002.
13. Amadi RA, Agomuo EN, Irebuem CO. Research Methods in Biochemistry. 2004;90– 93.
14. Association of Official Analytical Chemists (AOAC). Official methods of analysis (15th ed). Washington D.C USA. 400–2200 Wilson Boalevard, Arlinton Virginia USA. 1990;2:910-928.
15. Oloyed OI. Chemical profile of unripe pulp of *Carica pagaya.* Pak. J. Nutr. 2005;(4):379-381.
16. James DB, Abu EA, Wurochekk AU, and Orji GN. Phytochemical and antimicrobial investigations of aqueous and methanolic extracts of *Ximenia americana*. J Med Sci. 2007;7(2):284-288.
17. Harbone JB. Phytochemical methods: A guide to modern techniques of plant analysis. (3rd ed), Chapman and Hall Ltd, London; 2008.
18. Mann A. Survey of Ethnomedicine for the treatment of Tuberculosis: Chemistry Perspective. Ayanwola Printing Works, Minna, Niger State, Nigeria. 2007;117.
19. Bartner A, Pfeiffer KP, Batner H. Applicability of disc diffusion methods required by the pharmacopoeias for testing antibacterial activity of natural compounds. Pharmaz. 1994;49:512-516.
20. Satish S, Mahesh B. Antimicrobial activity of some important medicinal plants against plant and human pathogens. World J Agric Sci. 2008;4:839–843.

21. National Committee for Clinical Laboratory Standards (NCCLS). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Sixth Edition. NCCLS document M7-A6 (ISBN 1-56238-486-4). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2003.
22. Bauer AN, Kirby WMM, Sherries JC, Truck M. Antibiotic susceptibility testing by single disk method. Amer J Clin Pathol. 1996;45:493-496.
23. Solis PN, Wright CW, Anderson M, Gupta MP, Phillipson JD. A microwell cytotoxicity assay using *Artemia salina* (brine shrimp). Planta Med. 1993;26:250-252.
24. Lieberman MA. Brine shrimp bioassay for measuring toxicity and remediation of chemicals. J Chem Ed. 1999;76:1689-1691.
25. McLaughlin JL. Crown gall tumors on Potato disc and brine shrimp lethality. Two simple bioassays for higher plant screening and fractionation. In: Hostettmann, K. (Ed.), Assays for Bioactivity, vol. 1. Academic Press, London; 1991.
26. Lewis GE. Testing the toxicity of extracts of Southern African plants using brine shrimp (*Artemia salina*). S. Afri J Sci. 1995;91:382-384.
27. Moshi MJ, Innocent E, Magadula JJ, Otieno DF, Weisheit A, Mbabazi PK, et al. Brine shrimp of some plants used as traditional medicine in Kagera Region, North West Tanzania. Tanzania J Health Res. 2010;12:63–67.
28. Gupta MP, Monge A, Karitas G, Lopez de Cerain A, Solis PN, Leon E, et al. Screening of Panamanian medicinal plants for brine shrimp toxicity, crown gall tumor inhibition, cytotoxicity and DNA interaction. Internat J Pharmacol. 1996;34:123–127.
29. Finney DJ. Probit analysis. 3rd ed. Cambridge: Cambridge University Press; 1971.
30. Mann A. Phytochemical studies and Evaluation of *in vitro* Antimycobacterial activity of bioactive constituents of the root bark extract of *Terminalia avicennioides* Guill. & Perr. A Ph.D Thesis of the Department of Chemistry, Faculty of Science, Ahmadu Bello University, Zaria. 2010;245.
31. Orishadipe AT, Okogun JI, Mishelia E. Gas chromatography - mass spectrometry analysis of the hexane extract of *Calliandra portoricensis* and its antimicrobial activity. Afri J Pure and Appl Chem. 2010;4(7):131-134.
32. Adams RP. Identification of Essential Oil Components by Gas chromatography/Quadrupole Mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois, USA; 2007.
33. Stein S, Mirokhin D, Tchekhovskoi D, Mallard G. The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectra Library. Standard Reference Data Program of the National Institute of Standards and Technology. Gaithers-burg, MD, US; 2002.
34. Gbadamosi IT, Egunyomi A. Phytochemical screening and *in vitro* anticandidal activity of extracts and essential oil of *Curculigo pilosa* (Schum and Thonn) Engl. Hypoxidaceae. Afri J Biotech. 2010;9:1236-1240.
35. Ayyapa MP, Dhanabakan R, Doss A. Pulaniswamy M. Phytochemical Screening and Antibacterial Activity of Aqueous and Methanolic Leaf Extracts of two Medicinal Plants against Bovine Bacterial Pathogens. Internat J Ethnobot. 2009;13:131-139.
36. Kudi AC, Umoh JU, Eduvie LO, Gefu J. Screening of some Nigerian medicinal plants for antibacterial activity. J Ethnopharmacol. 1999;67:225-228.
37. Abubakar S, Ahmed QU, Samah OA, Omar MN. Bacteriostatic and bactericidal activity of the polar and non-polar extracts of *Andrographis paniculata* against skin disease causing pathogenic bacteria. J Med Plants Res. 2011;5:7-14.
38. Okwu DE, Okwu ME. Chemical composition of *Spondias mombin* linn plant parts. J Sustain Agric Environ. 2004;6(2):140-147.
39. Rangari VD. Pharmacognosy : Tannin Containing Drugs, J.L. Chaturvedi College of Pharmacy 846, New Nandanvan Nagpur- 440009; 2007.

40. Okwu D. E, Nnamdi FU. Evaluation of the Chemical Composition of *Dacryodes edulis* and *Raphia hookeri* Mann and Wendl Exudates Used in Herbal Medicine in South Eastern Nigeria. Afr J Trad CAM. 2008;5(1):194 – 200.
41. Oliver B. Medicinal plants in Nigeria. The Nigeria College of Arts, Science and Technology.1960:93-124
42. Khan AV, Ahmed QU, Mir MR, Shulka I, Khan AA. Antibacterial efficacy of the seed extracts of *Melia azedarach* against some hospital isolated human pathogenic bacteria strains. Asia Pac J Trop Biomed. 2012;1:452–455.
43. Yakubu MT, Mostafa M, Ashafa AOT, Afolayan AJ. Antimicrobial activity of solvent fractions from *Bulbine natalensis* tuber. Afri J Trad Comp Altern Med. 2012;9:459–464.
44. Adenike ST, Babatunde A, Adewoyin OA. Evaluation of pharmaceutical and microbial qualities of some herbal medicinal product in Western Nigeria. Trop J Pharm Res. 2007;6(1):661-670.
45. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Med. 1982;45:31-34.
46. Whiting DA. Natural phenolic compounds 1900-2000: A bird's eye view of a century's chemistry. Nat Prod Rep. 2001;18:583-606.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=548&id=14&aid=4962