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Chromatographic Isolation of Antimicrobial Compounds of *Calliandra portoricensis* (Jacq)-Benth (*Fabaceae*) Root

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Abstract: The plant *Calliandra potoricesis* had been widely used over the years ethnomedicinally. Such uses included; treatment of swollen gum, tooth ache and inflammation, worm expeller, viperean venom antidote and more. This research is aimed at screening and evaluation of various root extracts of the plant, isolation of the antibacterial and antifungal compounds from the plant by use of various chromatographic techniques and then determine the antimicrobial activities of the pure isolates. N-hexane (2.1g), ethyl acetate (4.61g), aqueous methanol (54.3g), extracts were obtained by successive extraction of 0.8 kg of the pulverized root sample. The more bioactive ethyl acetate extract was subjected to chromatographic column fractionation (Silica gel G 200-400 mesh-stationary phase). The mobile phase gradient of n-hexane: ethyl acetate: methanol (4:0:0, 3:1:0, 2:2:0, 1:3:0, 0:1:0, 0:3:1, 0:2:2, 0:1:3, 0:0:4-v/v/v) were used for elution. Agar well diffusion method was adopted for the bioassays susceptibility tests and MIC determination. A wider selection of microorganisms was made including G +ve and G -ve bacteria, fungi, nocosmial groups and those known to acquire resistance easily. The human pathogenic clinically viable isolates were; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Streptococcus fecalis, Candida albican, Aspergillus niger*. On the basis of bioactivity and analytical TLC profiles, the fractions were combined to 4 which were later bulked to two f ractions. The more active pooled fraction FB on further purification with preparative TLC yielded four bands that fluoreced under UV lamp (Light green, light blue, deep purple and light purple). The bands were extracted to yield four bioactive pure commands C_1 (9 mg), C_2 (8 mg), C_3 (6 mg) and C_4 (5 mg). These were to be characterized for structural elucidation. The activity of the isolates especially against the fungi was quite significant with respect to the reference controls (Ciprofloxacin and fluconazole) at $P \leq 0.05$.

Key words: *Calliandra portoricensis* · Antibacterial and Antifungal Activities · Four Pure Compounds

INTRODUCTION

Over the years, humans have depended on natural products for basic needs such as food and medicines. Evidence abounds on how the ancient civilizations of Chinese, Indians and North Africans used plants for the treatment of various diseases [1]. There has been huge burden of the infectious diseases on the populace due to the newly emerging and re-emergent diseases as well as multiple drug-resistant microbial strains that have necessitated search for newer and better antimicrobial agents [2]. About 80 % of world inhabitants patronize herbal medicine [3] and this is most pronounced in the resource-limited countries [4].

Currently, plants are still rated as the most economical and effective alternative source of medicines and 'lead' for novel drug discovery [5, 6]. Traditional medicine has failed to keep pace with the advances in science and technology [7]. Studies are therefore needed to validate scientifically, the safety, efficacy, quality and dosage of medicinal plant used [8].

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Fig. 1: Photograph of *Calliandra portoricensis* showing, twigs, leaves and flowers

Table 1: The taxonomy of *Calliandra portoricensis*:

Kingdom	*Plantae*
Phylum / Division	*Tracheophyta*
Class	*Magnolipsida*
Family	*Fabaceae*
Sub family	*Mimosiodeae*
Genus	*Calliandra*
Specific epithel	*Portoricensis*
Species	*Calliandra portoricensis*

Table 2: Some chemical constituents previously isolated from the genus *Calliandra:*

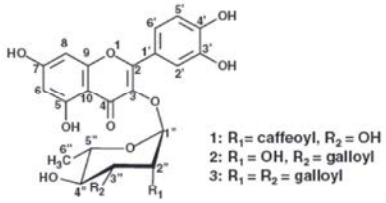
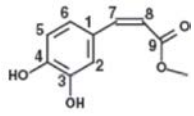
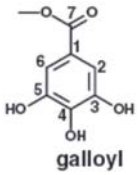
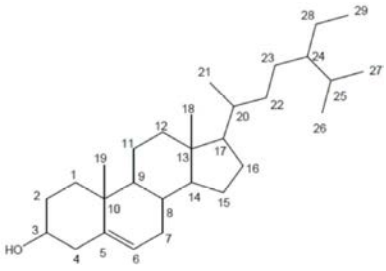
Structural formular	Name of Isolated compound	Morphological part	References
 1: R_1= caffeoyl, R_2 = OH 2: R_1 = OH, R_2 = galloyl 3: R_1 = R_2 = galloyl	1.) Quercitrin 2"-*O*-caffeate	Leaves and stem of *Calliandra haematocephala*	[18]
 *Z*-caffeoyl	2.) Quercitrin 3"-*O*-gallate		
 galloyl	3.) Quercitrin 2",3"-di-*O*-gallate		
	β-sitosterol	Leaves of *C.haematocephala.*	[20]

Table 3: Common pathogenic microorganisms and some of their health implications:

S/N	Microbial strains	Associated diseases	References
1	*Streptococcus fecalis* aerobic -(Gram +ve cocci)	Tonsilopharyngitis, pneumonia, wound, skin infections, sepsis etc	[21]
2	*Staphylococcus aureus*-(Gram +ve cocci)	Cellulitis, endocarditis, food poisoning, septicemia, osteoarticularosis	[22]
3	*Escherichia coli* (Gram -ve rod)	Gastroenteritis, urinary tract infections, adult bacteremia, neonatal meningitis	[23]
4	*Klebsiella pneumoniae* (Gram -ve rod)	Urinary tract infections, pneumonia, blood stream infections and others	[24]
5	*Bacillus subtitis* (Gram +ve rod).	Endocarditis, fatal pneumonia, septicaemia, bacteraemia, food poisoning wounds etc	[25]
6	*Candida albicans* (fungi)	Candidiasis; (mouth, throat, esophagus, vagina, invasive etc	[26]
7	*Aspergillus niger* (fungi)	Especially for the immune-compromised individuals; - lung diseases, allergic reactions etc	[27]

C. portoricensis, was selected for this research and is a shrub distributed in tropical regions of America, India, West Indies and West African Nigeria [9]. This plant has a success history of use by the Herbalists for its antimicrobial activity.

It has been used in ethnomedicine as; Antidote for viparean venom, throat and tooth inflammation, swollen tonsil, oral thrush [10, 11], worm expeller, laxative, abortificient [12] Antidiarrhoea,anticonvulsant and antimicrobial properties of crude extracts have been reported [13-15].

The test bacteria and fungi (Table 3) were selected to reflect fairly wide spread of pathologically viable clinical strains, some of which fall into the nosocomial groups as well as the strains that easily gained resistance over the years to antimicrobial agents, incorporating the G + ve and G-ve bacteria [16, 17].

There are reports on isolates and identification of some phyto constituents particularly from *Calliandra haematocephala* as shown in Table 2. The compounds are mostly acylated quercetin rhamnosides [18]; sterols [19]; quercetrin and their esters [20].

- The aim of study was screening and evaluation of crude root extracts of *Calliandra portoricensis* in different solvent systems for antimicrobial activity, isolate the anti-microbial compounds from *C. portoricensis* under study.
- and determine the antimicrobial (bacteria and fungi) activities of the isolated compounds.

MATERIALS AND METHODS

Plant Material: The root of *Calliandra portoricensis* was collected in the month of June 2016 from Osisioma Local Government Area in Abia State of Nigeria. The plant was identified and authenticated in the Herbarium of Plant Science and Biotechnology, Department in the Faculty of Natural Sciences of University of Port Harcourt, Rivers State, Nigeria by Dr. Chimezie Ekeke with the Herbarium Number: UPH/v/1240. The material was properly washed, air dried, pulverized and stored for subsequent use.

Phytochemical Screening: Preliminary phytochemical tests were carried out on the pulverized root material of *C. portoricensis* by using standard methods [28, 29].

Extraction: The pulverized root material of *Calliandra portoricensis* (0.8 Kg) was subjected to successive cold maceration process with 2 Litres of n-hexane over 24 h with intermittent shaking for a total of 72 h (3 x 2 Litres). After every 24 h period, the extracts were filtered. The marc was air dried, re-packed into the flask and re-extracted with ethyl acetate (3 x 2 Litres) as above. The subsequent air dried marc was finally re -packed and re-extracted with methanol. The respective filtrates after each 24 h period, were combined and there after concentrated *en vacuo using* a rotary evaporator at temperatures not exceeding 40°C. The combined n-hexane, ethyl acetate and methanol extracts were transferred into pre-weighed beakers, dried to constant weights and the respective yields determined.

All the three extracts were evaluated for antimicrobial activities as described below.

Thin Layer Chromatography (TLC): Pre-coated silica gel GF_{254} (0.25 mm) was used for the analytical TLC, whereas GF_{254} (0.50 mm) was used for Preparative TLC. The developed plates were viewed in; daylight, under UV lamp (254 and 365 nm), iodine fumes and 0.5% P-anisaldehyde in Sulphuric acid spray reagent. The analytical TLC plates assisted variously in detecting the crude extracts, chromatographic fractions, pure isolated compounds and in the monitoring of the chromatographic column eluates.

Preparation of Preparative TLC Plates: Slurry of 100 g silica gel G and 250 ml distilled water was prepared in a 500 ml conical flask. The slurry was stirred with a glass rod. The slurry was introduced into the receptacle of the Spreader (adjusted to 0.5 mm thickness) and quickly spread on the plates arranged and secured on the tray. The coated plates were allowed to stand for 3 h in order to set and then activated in the oven for 1h at 105°C.

Determination of Suitable Mobile Phase: The crude extracts and chromatographic column fractions were used for the trial TLC spotted on analytical silica gel GF_{254} pre-coated plates and developed in several mobile phase systems that led to identification of- n-Hexane: ethyl acetate: methanol (14:3:1).

Determination of Suitable Spray Reagent: Several spray reagents were tried on the analytical TLC plates after development, leading to selection of - 0.5% P-anisaldehyde in Sulphuric acid, spray reagent. This is useful in detection of phenolic compounds, steroids, terpenes and sugars.

Anti-Microbial Assays

Test Microorganisms: The test bacteria and fungi were selected to reflect fairly wide spread of human pathogens.some of these fall into the nosocomial groups (Bacteria causing infections of hospital patients) as well as the strains that easily gained resistance over the years to antimicrobial agents [30, 31]. The selected clinical strains of microorganisms were: *Staphylococcus aureus* (Gram +ve cocci), *Streptococcus fecalis* (Gram +ve cocci), *Escherichia coli* (Gram -ve rod), *Bacillus subtitlis* (Gram +ve rod), *Klebsiella pneumoneae* (Gram -ve rod), *Candida albican* (fungi), *Aspergillus niger* (fungi). These were all isolates from the Microbiology Department of University of Port Harcourt Teaching Hospital (UPTH), Rivers State Nigeria.

Preparation of Test Microorganisms

Bacterial Suspensions: A loopful of the isolated bacterial colony from the slant was cultured by inoculating into the 10 ml of peptone water in a test tube and incubated at 37°C for 18 h, prior to the antimicrobial assays. Then 0.5 ml of the actively growing test bacterial suspension was sub-cultured into 9.5 ml of peptone water, the turbidity of which was matched with that of standard of 0.5 McFarland units. McFarland number 0.5 standard was prepared by mixing 9.95 ml 1.0 % H_2S0_4 in distilled water and 0.05 ml 1.0 % $BaCl_2$ in distilled water, so as to estimate bacterial density by comparison with the prepared bacterial suspension [32].

Preparation of Fungi: The isolated fungal test organisms were prepared and maintained in Sabouraud Dextrose Agar (SDA) at the room temperature (25°C) and thereafter subcultured as described above.

***In vitro* Antimicrobial Susceptibility Evaluation**

Antibacterial Susceptibility Tests: The cup-plate agar diffusion assay method adopted for the evaluation of the crude extracts, different chromatographic fractions, the isolated pure compounds of *Calliandra portoricensis* root and the reference control was as previously described [33, 34]. The bioassays were variously carried out in triplicate [35]. Ciprofloxacin was used as reference sample for the bacterial assay. It is a relatively new generation of antibiotics patented in 1983 by Bayer AG and is a fluorinated 4 -quinolone derivative, with a broad based spectrum of activities [36]. Fluconazole was used as the reference sample for the fungi species. It has been reported to elicit a good activity against *Candida* infections [37].

All the glasswares and petri dishes were sterilized in an autoclave at 21°C and under pressure of 15 pounds per square inch (PSI) for 20 minutes. One ml of the sub-cultured standard microbial suspension 150-10^6 C.F.U. per ml. was carefully seeded into Muller-Hinton Agar (MHA) in aliquots of 20 ml each. This molten MHA so impregnated with the test micro-organisms in the 20 ml bottle was them distributed into sterile petri-dishes. The seeded molten agar was left to set. In each of the quadrants of the plate, a cup was made with an 8.0 mm sterilized cork-borer.

The wells on the opposite sides of the quadrants were loaded with 0.2 ml of 40 mg per ml and 20 mg per ml of the crude dissolved in 10 % aqueous Dimethyl Sulphoxide (DMSO) by using micro pipettes. The remaining two cups were loaded with 0.2 ml of 10 % aqueous DMSO alone and 0.2 ml of ciprofloxacin solution containing 40 micro gram per ml. DMSO and ciprofloxacin served as negative and positive reference controls respectively. The loaded petri-dishes (in triplicate) for each sample were allowed to stand at room temperature for 1h for diffusion. Thereafter, the plates were incubated in the upright position at 37°C for 18 h. At the end of the incubation period, the diameters of the growth inhibition zones were recorded.

The above process for bacterial assay was repeated for the test fungi except that the incubation was at room temperature (25°C) for 72 h and the reference sample was fluconazole at the concentration of 200 to 1000 micro grams per ml.

Determination of Minimum Inhibitory Concentration (MIC): This was determined by a modification of standard agar- well diffusion method [38, 39]. The active crude sample of *C. portoricensis* was dissolved in 10 % aqueous DMSO by serial two-fold dilution to concentrations of; (40,20,10 and 5) mg / ml. These were loaded in the nutrient agar wells as described above. This process was repeated in determination of MIC of the isolated pure compounds; (C_1, C_2, C_3 and C_4,) at concentrations of; (1.0, 0.50, 0.25 and 0.125) mg / ml. The MIC values were subsequently determined by observation of the concentration at which there was no visible inhibition of microbial growth field.

Column Fractionation of Bioactive Ethyl Acetate Extracts: Thecolumn (65.0 cm X 3.5 cm) was packed with silica gel G(200-400 mesh) as stationary phase. The silica gel was pre-washed in acetone and dried, in oven at 105°C for activation. 200 g of silica gel was mixed with n-hexane in order to form slurry which was stirred by using a glass stirring rod. The column (40 cm packed) had a vent plugged with cotton wool and 20.0 ml of re-distilled n-hexane added into the column. The solvent was thereafter allowed to flow through the tap of the column so as to enable the gel to settle better. Ethyl acetate extracts (4.60 g) was pre-adsorbed onto (10.0 g) of silica gel. G(200-400 mesh) and loaded on the column bed. Subsequently the surface was covered with cotton wool in readiness for elution. This was eluted successively with gradient mixtures of n-hexane, ethylacetate and methanol in the following pattern; 100% n-hexane expected to elute, pigments, waxes, sterols and others: n-hexane: ethyl acetate (75: 25, 50:50, 25:75), 100% ethyl acetate then, ethylacetate methanol (75:25, 50:50, 25:75) 100 % methanol. Several eluates were collected in aliquots of 50 ml and examined on the analytical pre-coated TLC plates silica gel (GF_{254}). This was developed in the selected mobile phase: n-hexane: ethyl acetate. methanol (14:3:1). On the basis of the TLC profile,, observation in day light and under UV. Lamp(254 and 365 nm) and reaction to 0.5 % P-Anisaldehyde-Sulphuric acid spray reagent, similar fractions were bulked to four (fractions F_1 to F_4). The chromatographic fractions were then subjected to antimicrobial evaluation. On the basis of similar chromatographic pattern and antimicrobial profile, the four fractions were further combined to produce two fractions (FA and FB).

Purification of the More Bioactive Fraction (FB) by Preparative TLC: Column fraction FB was further separated on preparative TLC on silica gel G(0.5 mm thickness). Plates (20 cm X 20 cm) were prepared as described above. These plates were then developed in; n-hexane: ethyl acetate: methanol (14:3:1), as mobile phase.

Four bands were identified by the aid of a UV lamp (wavelength -254 and 365 nm). Thereafter, bands were carefully scrapped and extracted in ethylacetate: methanol (3:1) to yield the pure four compounds C_1(9.0 mg), C_2(8.0 mg),, C_3(6.0 mg) and C_4(5.0 mg) as depicted in Figure 2 below.

The pure isolates were subjected to antimicrobial susceptibility assays as earlier described. Compounds C_1 and C_2 shall be subjected to spectroscopic analysis leading to their characterization and structural elucidation of two of the isolated compounds.

RESULTS

Table 4: Percentage yield of *C. portoricensis*root extractsfrom 0.8 kg of plant sample

Solvent	Total extracts (g)	Percentage yield
n-hexane	2.10	0.26
ethyl acetate	4.61	0.58
methanol	54.35	6.80
Total extractable materials	61.06	7.63

Table 5: Results of the phytochemical screening for *C. portoricensis* root

Secondary metabolite	Results
Test for Saponin	
Emulsification test	+
Frothing test	+
Test for Tannins	
Ferric chloride test	-
Test for Flavonoids	
Shinada test	+
Sodium hydroxide test	+
Test for Anthraquinine derivatives	
Free Anthraquinone	-
Combined Anthraquinine	-
Test for cardiac Glycoside	
Kedde's test for lactone ring	+
Keller-Killiani's test for deoxy sugar	+
Test for steroids and Triterpenoids	
Liebemann Burchardis test	-
Salkwoski's test	+
Test for carbohydrates	
Molisch's test	+
Fehling's test for free reducing sugars	+
Test for cynogenic Glycosides	
Test for Alkaloids	-
Meyer's reagent	++
Dragendorff's reagent	++
Hager's test	++

Key:
Negative = (-)
Positive = (+)
Strongly positive = (++)

Table 6: Minimum Inhibitory Concentration (MIC) values in mg / ml of various crude extracts of *C. portoricensis* root against selected human pathogenic microorganisms

Microorganisms	n-hexane extract MIC	Ethyl acetate extract MIC	Methanol Extract MIC
Staphylococcus aureus	10.00±0.80	10.00±0.75	20.00±0.80
Escherichia coli	-	10.00±0.50	20.00±0.45
Bacillus subtilis	5.00±0.25	5.00±0.30	10.00±0.36
Klebsiella pneumonia	-	5.00±0.20	-
Streptococcus fecalis	10.00±0.60	10.00±0.30	20.00±b0.70
Candida albican	10.00±0.45	5.00±0.60	20.00±0.25
Aspergillus niger	-	5.00±0.40	20.00±0.50

Values are expressed as mean ±SEM; n = 3.

Table 7: Result of antimicrobial susceptibility tests of column fractions F_1 to F_4 against the selected human pathogenic micro-organisms at concentration of 20 mg / ml

	F_1		F_2		F_3		F_4	
Microorganisms	20	CTR	20	CTR	20	CTR	20	CTR
Staphylococcus aureus.	-	10.00±0.30	-	12.00±0.70	*18.00±0.70	23.00±0.30	*21.00±0.75	15.00±0.45
Escherichia coli	-	13.00±0.10	-	10.00±0.40	-	20.00±0.70	-	10.00±0.60
Bacillus subtitis	*17.00±0.4	20.00±0.70	*16.00±0.70	17.00±0.25	*18.00±0.48	25.00±0.80	*24.00±0.70	30.00±0.30
Klebsiella pneumonia	*16.00±0.70	22.00±0.30	*18.00±0.40	22.00±0.65	*15.00±0.54	20.00±0.80	*17.00±0.80	22.00±0.60
Streptococcus fecalis	*17.00±0.30	25.00±0.20	*17.00±0.80	20.00±0.50	*20.00±0.81	25.0±0.60	*22.00±0.45	20.00±0.80
Candida albican	10.00±0.50	24.00±0.80	8.00±0.30	15.00±0.80	*17.00±0.45	14.00±0.30	*24.00±0.65	15.00±0.50
Aspergllus niger	- -	18.00±0.40	-	13.00±0.50	*15.00±0.56	12.00±0.40	*22.00±050	10.00±0.70

Values are expressed as mean ± SEM n = 3; CTR. = Control - Ciproflaxacin (20 µ g per ml for bacteria) and Fluconazole (1000 µ g per ml for fungi);
(-) = no inhibition; 10 % aqueous DMSO (negative control, no inhibition).
* Represent the significant values with respect to the control at $P \leq 0.05$

Table 8: Result of antimicrobial susceptibility tests of pooled column fractions (FA and FB) against the selected humanpathogenic microorganisms at concentration of 20 mg / ml

	Fraction FA		Fraction FB	
Microorganisms	20mg/ml	CTR	20mg/ml	CTR
Staphylococcus aureus	11.00±0.45	13.00±0.71	*17.00±0.94	35.00±0.86
EscherichiaColi	*22.00±0.80	28.00±0.45	*24.00±0.80	30.00±0.70
Bacillus subtilis	*20.00±0.60	26.00±0.40	*18.00±0.74	28.00±0.84
Klebsiella pneumonae	*16.00±0.45	28.00	*20.00±0.80	35.00±0.60
Streptococcus fecalis	*18.00±0.84	30.00	26.00±0.56	32.00
Candida albicans	13.00±0.62	20.00±0.32	*17.00±0.92	25.00±0.72
Aspergillus nigar	14.00±0.75	24.00±0.62	*20.00±0.75	30.00±0.68

Values are expressed as mean ± SEM n = 3; CTR. = Control - Ciproflaxacin (20 µ g per ml for bacteria) and Fluconazole (1000 µ g per ml for fungi);
(-) = no inhibition; 10 % aqueous DMSO (negative control, no inhibition).
* Represent the significant values with respect to the control at $P \leq 0.05$.

Table 9:Result of antimicrobial susceptibility tests of TLC bands (B_1- B_4) on selected human pathogenic microorganisms at concentration of 20 mg / ml:

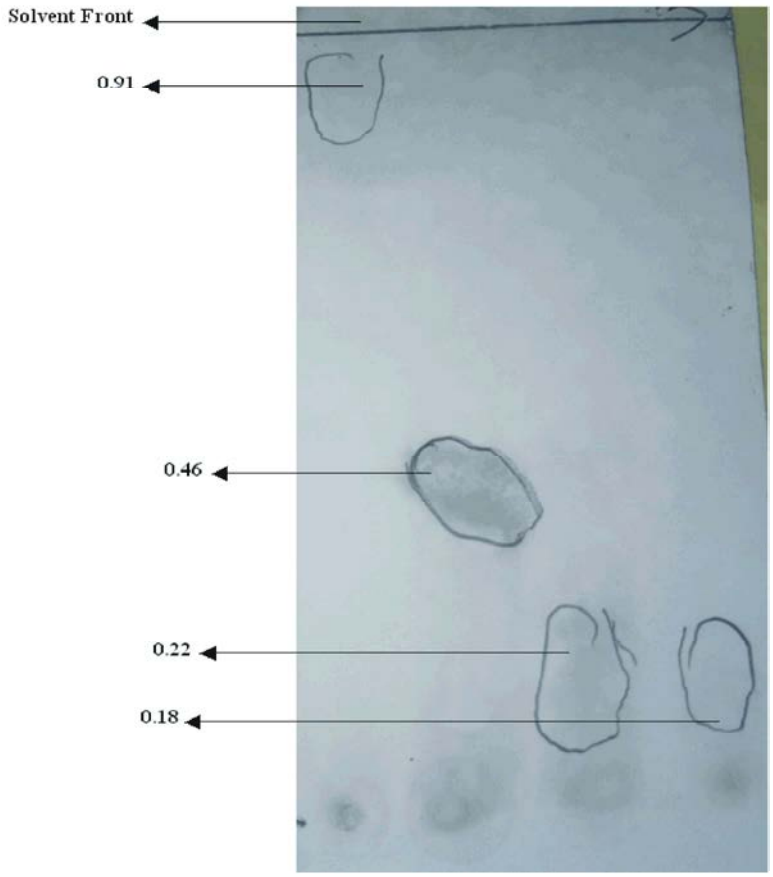
	TLC Band B_1]		TLC Band [B_2]		TLC Band [B_3]		TLC Band [B_4]	
Microorganism	B_{-1}	CTR	B_{-2}	CTR	B_{-3}	CTR	B_{-4}	CTR
Staphylococcus aureus	*25.00±0.40	23.00±0.70	*31.00±0.12	24.00±0.70	*20.00±0.60	22.00±0.40	*20.00±0.60	28.00±0.70
Escherichia coli	*35.00±0.60	38.00±0.90	*20.00±0.60	25.00±0.70	*18.00±0.40	25.00±0.15	*20.00±0.50	28.00±0.70
Bacillus subtitis	*45.00±0.50	40.00±0.80	*22.00±0.50	35.00±0.40	*22.00±0.60	30.00±0.70	*16.00±0.30	25.00±0.40
Klebsiella pneumonae	*35.00±0.70	30.00±0.60	*18.00±0.40	30.00±0.80	*20.00±0.40	25.00±0.80	*18.00±0.40	24.00±0.40
Streptococcus fecalis	*30.00±0.20	35.00±0.10	*20.00±0.80	25.00±0.40	*18.00±0.40	20.00±0.50	*22.00±0.80	28.00±0.10
Candida albicans	*33.00±0.90	35.00±.00	*20.00±0.90	25.00±0.70	*21.00±0.30	25.00±0.40	*18.00±0.40	23.00±0.50
Aspergillus niger.	*25.00±0.50	14.00±0.80	*30.00±0.50	45.00±0.60	*18.00±0.30	40.00±0.90	*18.00±0.40	40.00±0.80

Values are expressed as mean ±SEM ; n = 3; CTR. = Control - Ciproflaxacin (20 µ g per ml for bacteria) and Fluconazole (1000 µ g per ml for fungi);
(-) = no inhibition; 10 % aqueous DMSO (negative control, no inhibition).
* Represent the significant values with respect to the control at $P \leq 0.05$

Table 10: Minimum Inhibitory Concentrations (MIC) values of TLC bands (B_1- B_4) in µ g per ml against the selected human pathogenic microorganisms:

	Micro organisms		TLC Bands	
	B_1	B_2	B_3	B_4
1. *Staphylococcusaureus*	125.00±0.80	125.00±0.10	250.00±0.50	250.00±0.50
2. *Escherichia. coli*	25000±0.30	125.00±0.70	250.00±0.20	250.00±0.80
3. *Bacillus. subtitis*	125.00±0.20	250.00±0.30	125.00±0.30	250.00±0.10
4. *Klebsiella pneumoneae*	250.00±0.60	125.00±0.80	250.00±0.70	125.00±0.80
5. *Streptococcus fecalis*	12500.±0.50	125.00±0.10	250.00±0.20	250.00±0.40
6. *Candida albicans*	125.00±0.70	125.00±0.40	125.00±0.40	125.00±0.60
7. *Aspergillus niger*	125.00±0.20.5	125.00±0.60	125.00±0.20	125.00±0.50

Values are expressed as mean ±SEM; n = 3



Absorbent: Silica gel GF_{254}
Mobile phase: n-hexane: ethyl acetate: methanol (12: 4: 1)
Detection: 0.5 % P-anisaldehyde in conc. Sulphuric acid
Colours under U.V lamp: B_{1-} light green B_{2-} Deep purple; B_{3-} light purple; B_{4-} light blue)

Fig. 2: Chromatogram of the preparative TLC bands (B_1 -B_4) of *C. portoricensis* root with their Rfvalues

DISCUSSION AND CONCLUSION

Result of the percentage yields shown in Table 4 indicated that most of the constituents of C. *Portoricensis* root have affinity to polar solvents hence methanol (6.80 %) while the non-polar n-hexane was (0.26 %).

The result of the phytochemical screening as in Table 5 established the presence of saponins, flavonoids, cardiac glycosides, steroids, triterpenoids, reducing compounds and alkaloids. On the other hand, tannins, free and combined anthroquinones as well as cyanogenic glycosides were absent. Except for the tannins

(found present in fresh samples) and anthroquinones (in leaves), the result of the phytochemical screening was consistent withan earlier report [40] and was also a reflection of the finding that plants with antimicrobial properties were associated with presence of polyphenolic compounds, alkaloids, saponins and steroids [41].

The results of antimicrobial susceptibility tests shown in Table 7 for column fractions F_1 to F_4 and subsequently result of Table 8 for pooled fractions FA and FB (FB ranked higher) and for the pure TLC bands B_1- B_4 (B_1 and B_2 ranked best). The test samples exhibited progressive potency of activities from the extracted crude state to the isolated pure compounds. The column fractions were combined on the basis of their antimicrobial pattern and analytical TLC profiles.

The MIC range of 5-10 mg / ml shown in table 6 for ethyl acetate crude extracts highlighted it as ranking highest of all the three different extracts. However, the n-hexane extracts ranked lowest. The MIC values of 250 µg per ml and below for all the TLC bands established that the pure compounds were remarkable in both antibacterial and antifungal activities.

All the susceptibility testing results against the selected human pathogens, to a larger extent were consistent with the report which suggested that inhibitory zone diameters of 10 mm and above despite the current ease of microbial acquisition of resistance, should be considered to possess some antimicrobial activity. Those with zone diameters equal to 20 mm and above were considered noteworthy [42].

Further, results of MIC values were in line with research which expressed that extracts having activity when MIC values were equal to 8 mg / ml or below were considered to have some antimicrobial activity as natural products with MIC. Values below 1 mg / ml should be considered as being noteworthy [43]. In totality, all the extracts (from crude to pure isolates) also compared quite favorably with ciprofloxacin and fluconazole (Reference controls). These were among the latest generations of antibacterial and antifungal agents.

In view of the burden of infectious diseases on the populace and the current quest for plant products in treatment of infectious diseases by bacteria and *candida albican*, it is highly recommended that the isolates be investigated further.

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