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Comparative Study of Antimicrobial Potentials of Leaf and Root Extracts of *Calliandra portoricensis (Jacq)-benth (Fabaceae)* on Some Human Pathogens

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Authors' contributions

This work was carried out in collaboration among all authors. Author NEO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AKA managed the analyses of the study whereas author OEA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The plant *Calliandra portoricesis* had been widely used over the years in traditional medicine. Such uses included; treatment of swollen gum, tooth ache and inflammation, worm expeller, viperean venom antidote and more. This investigation was aimed at screening and anti-microbial evaluation of various leaf and root extracts of the plant. By this, explore substitution of root with leaf as excessive root harvesting could lead to shrub extinction. The dried and pulverized samples were subjected to successive extraction using solvents of varying polarities; n-hexane, ethyl acetate and 70% aqueous methanol. The respective extracts were concentrated *en vacuo* in a rotatory evaporator at temperature not exceeding 40°C. Seven human pathogens were selected comprising the G +ve, G-ve, fungi, group that was known to acquire resistance easily and nosocomial strains namely; *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Streptococus fecalis, Candida albican and Aspergillus nigar.* Ciprofloxacin and fluconazole

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solutions served as the control reference standards. Agar well diffusion assay method was used and the Inhibition Zone Diameters (mm) of growth were measured to assess activities for all the extracts. The Minimum Inhibitory Concenyrations (MIC) and Total Activity (TA) were also determined. The experimental values indicated that both leaf and root materials of this plant exhibited anti bacterial and anti fungal properties on the selected human pathogens especially with respect to the reference control standards at $P \leq 0.05$. Except for anthraquinones, the leaf though exhibited weaker activities than root for same quantity of materials showed close similarity in activity pattern. In this sense, with an appropriate quantitative adjustments, leaf material could effectively substitute the root for antimicrobial purposes.

Keywords: Calliandra portoricensis; antibacterial and antifungal activities; leaf substitute; root; avoid shrub extinction.

1. INTRODUCTION

Since the ground breaking discovery of the penicillins by Alexander Fleming in 1928, antimicrobial agents have proved to be remarkably effective for the control of bacterial and fungal infections. Nevertheless, the emergence of resistant strains of the pathogenic microorganisms over time had gradually rendered the conventional treatment less effective [1]. Bacterial resistance to the action of antimicrobial agents is a challenge of our time. This can be attributable to the inherent structure or physiology of the bacteria (constitutive resistance) or they could develop a mechanism to circumvent the action of the drugs through genetic mutation or through acquisition of genetic elements (acquired resistance) [2].

It has been estimated by the World Health Organization (WHO) that about 80% of the world inhabitants rely mainly on traditional medicine [3]. These levels of embrace might be associated withs high costs of orthodox pharmaceutical medicines and increased degree of acceptability from cultural and spiritual dimensions, accessibility and the perception of it having minimal adverse effects [4,5]. Herbal medicines and raw materials are equally as economically rewarding as the orthodox pharmaceutical products since they can contribute as much as US$ 43 billion with an annual growth rate of between 5 to 15% [5].

The patronage of herbal medicine for healthcare is notably more pronounced in resource – limited and developing countries especially in Africa [6]. The remaining 20% of the world population resides in the developed regions of the globe, however, the prescription data analysis in US indicated up to 25% plant extracts or active compounds that were derived from high plants [7].

Spirituality, religion and traditional medicine were almost a trilogy since there was a natural bond between the three; hence religion has so much influence on African traditional medicine [8].

About 700 different pharmaceutically important compounds and a number of top selling modern medicines from plants have contributed to the compilation of Western Medicinal Pharmacopoeia [9].

African traditional medicine is so vast and touches on; surgery, traditional birth attendance, therapeutic occultism, faith healing, psycho-therapy, bone-setting, divination, therapeutic fasting and dieting, hydrotherapy to mention but a few, nevertheless, the greatest area of interest to the modern collaborative medicine is the phyto-therapy [10]. In recognition of this significant impact of traditional medicine, WHO came up with the Alma Ata declaration which urges an accommodation of proven traditional medicine in National Drug Policies and Regulatory Measures [11].

Currently, plants have a major advantage of being the cheapest and most effective alternative source of medicines [12]. Some of the challenges with traditional medicine revolve around; inability to keep pace with scientific and technological advancement [13]. In this sense, the methodology, techniques, and training are often maintained at utmost secrecy. The diagnosis, dosage of medicaments and preservation methods are often highly inaccurate. [14].

Infectious diseases are ranked second most implicated causes of death all over the world [15]. This is mainly because of the burden of newly emerging infectious diseases, re-emergent diseases and multiple-drug resistant microbial

strains which imply continued necessity for the development of new antimicrobial agents [16,17]. To this effect, plants are known to be a good reservoir of chemicals with antimicrobial properties [18]. There is then a need for increased levels of research in the vast field of medicinal plants. To facilitate this, it means that the relationship with the traditional medicine practitioners must be more organized, officially formalized, bilaterally equitable and collaboratively strengthened [19]. The ultimate goal in this quest being scientific validation of the safety, efficacy, quality and the dosage of the medicinal plant material used [20]. Indeed, plant derived medicines have made a large contribution to human health and well-being [21].

The susceptibility to the microbial infections is very high in most African countries because of the poor levels of sanitation, hygiene, nutritional status and general living conditions [22]. Poor health facilities aggravate this situation, and even when the orthodox healthcare becomes available at all, the affordability should be a mirage to majority of the population [23].

Medicinal plants are those used in order to prevent, relieve or cure a disease or to alter physiological or pathological processes or any plant employed as a source of drugs or their precursors [24]. Usually, numerous organic synthesis of complicated compounds in the laboratory is quite expensive but ironically are executed at far cheaper costs by nature through plant and animal kingdoms.

The pharmacological activity of many natural active principles cannot be fully optimized therapeutically because of various challenges such as stability, solubility, poor absorption, unpredictable distribution in the body, first pass metabolism, short biological half-lives and more. Some of those disadvantages are expected to be overcome by pharmaceutical formulation in order to produce the desirables. Often, the challenges can only be resolved by a chemical modification of the drug molecule [25].

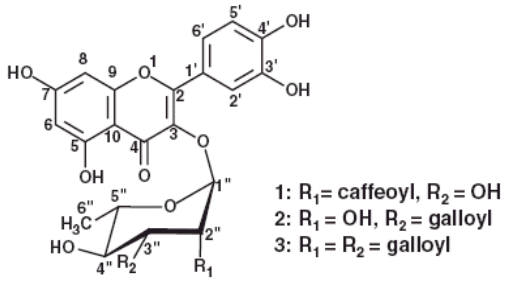
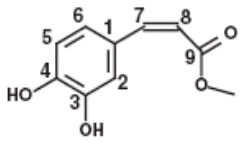
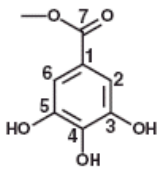
The burden of infectious diseases has been felt much over time. This upsurge can also be linked to high risk pathogens like the nosocomial strains as well as high risk patients as in immuno-compromised hosts (Person whose immunologic system is deficient) [26]. A report has shown that *Candida albicans* has ranked fourth as the most implicated nosocomial blood stream infections in the United States of America. It is the microorganism implicated in candidosis and is the most common invasive fungal infections in critically ill, non-neutropenic patients [27]. The search for a new strategy in anti-fungal treatment and prevention is driving an extensive and intensive exploration of various plant species for validation of claims on them by folkloric practices. A number of reasons had been adduced for the poor performance of the classical antifungal agents which include increased incidence of drug-resistance, high treatment costs and only fungustatic agents are available.

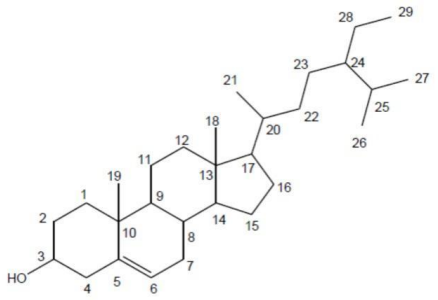
Historically with natural products, moulds and bacteria produce some defensive substances that can prevent attack by other organisms. This concept led to discovery of many semi-synthetic penicillins and other antibiotics such as *Streptomyces*, neomycin, polymixin and more. Microbial fermentation also leads to discovery of a novel drug such as cholcystokimn (CCK) antagonist from *Aspergillus alliaceus* [28]. By closely following the folkloric, the medicinal chemist had made the best out of the following plant materials; alkaloids morphine (from the Opium poppy known in ancient Egypt); atropine and hyoscine (from plants of the *Solanaceae* family known to the ancient Greeks) and reserpine (from *Rauwolfia serpentina*, the snake root, popular in India as a herbal remedy) and non-nitrogenous natural products such as salicylates, example salicin from the Willow tree (Salix species) botanical source (known to Hyppocrates) and glycosides such as digitoxin and digoxin in Digitalis species from the Foxgloves (in folk use in England for centuries) [29].

*Calliandra portoricensis*is under study, is a shrub distributed in tropical regions of America, India, West Indies and West African Nigeria [30]. This plant has a success history of use by the Herbalists for the treatment of various ailments such as; throat and tooth inflammations, swollen tonsil, mouth ulcers, fever, oral fthrush, black tongue, asthma, worm expeller, laxative and as antidote for viperean venom [31,32,33], as well as antimicrobial properties [34].

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

Table 1. Some chemical constituents previously isolated from the genus *Calliandra*

Structural formular	Name of Isolated compound	Morphological part	References
	1.) Quercitrin 2″-*O*-caffeate 2.) Quercitrin 3″-*O*-gallate 3.) Quercitrin 2″,3″-di-*O*-gallate	Leaves and stem of *Calliandra haematocephala*	[35];
 *Z*-caffeoyl			
 galloyl			

	**β-sitosterol**	Leaves of *C. haematocephala.*	[36]

1.1 The Aims of This Research

- To conduct a cooperative investigation of phytochemical constituents of leaf and root extracts of *C. portoricensis*.
- To evaluate the antimicrobial activities of crude extracts of leaf and root of *C. portoricensis* used in Nigerian traditional medicine and
- To verify if antimicrobial activities of both are comparable thereby providing a good alternative to excessive use of the root capable of promoting shrub extinction.

2. MATERIALS AND METHODS

2.1 Plant Material

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

2.2 Phytochemical Screening

Preliminary phytochemical tests were conducted on the pulverized samples of *C. portoricensis* by adopting standard methods [37,38].

2.3 Methods

2.3.1 Preparation of crude plant extracts

100 g each of the dried and pulverized plant samples (leaf and root) were macerated and subjected to successive extraction for 24 x 3 h using organic solvents of varying polarities; n-hexane, ethyl acetate and methanol. The extracts were filtered and the air-dried husks re-packed for successive maceration as described above, with the next solvent for both leaf and root samples. The different filtrates were concentrated *en vacuo* in a rotary evaporator at temperatures not exceeding 40°C. The respective yields were noted and all the three extracts evaluated for antimicrobial activities as described here under.

2.3.2 Preparation of test microorganisms

2.3.2.1 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. The McFarland number 0.5 standard was prepared by mixing 9.95 ml of 1.0% H_2S0_4 in distilled water and 0.05 ml of 1.0% $BaCl_2$ in distilled water, so as to estimate bacterial density by comparison with the prepared bacterial suspension [39].

2.3.2.2 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 sub cultured as described above.

2.4 *In vitro* Antimicrobial Susceptibility Evaluation

2.4.1 Antibacterial susceptibility tests

The cup-plate agar diffusion assay method adopted for the evaluation of the crude extracts

of *Calliandra portoricensis leaf,* root and the reference control samples was as previously described [40]. The bioassays were variously executed in triplicate. Ciprofloxacin was used as reference standard 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 [41]. Fluconazole was used as the reference standard for the fungi s*pecies.* It has been reported to elicit a good activity against *Candida* infections [42].

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 approximately equivalent to 150 – 10^6 C.F.U. per ml. was aseptically 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 sterilized 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 and 20 (mg per ml) of the crude extracts 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 µg / 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 Inhibition Zone Diameters (IZD) of the growth field were measured andrecorded.

The above process for bacterial assay was repeated for the test fungi with the following exceptions; the incubation was at room temperature (25°C) for 72 h and the reference standard sample was fluconazole at the concentration of 1000 µg / ml.

2.5 Determination of Minimum Inhibitory Concentration (MIC)

This was carried out by a modification of standard agar- well diffusion method [43]. The active crude samples of *C. portoricensis* were 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. The MIC values were subsequently determined by observation of the concentrations at which there was no more visible inhibition of microbial growth field.

3. RESULTS AND DISCUSSION

Table 2 on percentage yield indicated that the constituents of both leaf and root had greater affinity to polar solvents (C.p- root 4.98 and leaf 1.63) as against the non- polar n- hexane (C.p- root 1.43 and leaf 0.68) respectively.

The percentage yied of the leaf was observed as close to 50% of those of the corresponding yield of root.

Table 3 on the result of phytochemical screening showed that both leaf and root contained varying quantities of; saponin, flavonoids, cardiac glycosides, steroids, triterpenoids, reducing compounds and alkaloids. Cyanogenic glycosides and tannins were absent in both dry samples, whereas anthroquinones were found present in the leaf only.

Table 4 on the antimicrobial susceptibility tests indicated that ethyl acetate root extracts had highest Inhibition Zone Diameter (IZD) of 28 mm against *Candida albican*, and also with broad activity for both bacteria and fungi. The methanol extracts for root ranked second with 25 mm of IZD on same *Ca*. The corresponding values for leaf were 15 mm and 18 mm respectively. The ethyl acetate extracts of root had IZD of 18 mm against *Bs* just as that of methanol root extracts was 18 mm on *Sf*. The corresponding values for leaf extracts were; 15 mm and 13 mm respectively. The n- hexane extracts exhibited the lowest activities of all the three extracts with even no inhibition at 20 mg/ ml against *Sf*. and *An* for root and *An* for leaf.

The antimicrobial susceptibility test result for leaf and root extracts indicated both performance as being significant with respect to the control reference standards (Ciprofloxacin and fluconazole) at $P \leq 0.05$ The susceptibility result was found to be consistent too with the report which suggested that IZD of 10 mm and above despite the current ease of acquired microbial resistance should be considered to possess some antimicrobial activity; while those equal to

or above 20 mm could be considered as noteworthy [45] Further, the MIC data were in line with report of an investigation which expressed that extracts having activity where MIC values were below 8 mg/ml were considered to possess some antimicrobial activity, as natural products with MIC values below 1 mg/ml should be considered as noteworthy [46].

The MIC range of 5 – 10 (mg / ml for ethyl acetate root extracts against the corresponding values for leaf extracts of 5 -20 (mg /ml) for the same test human pathogens was indicative of better antimicrobial performance of the root extracts.

Highest TA values of 10.0 (root) was observed for methanol extracts against *Sa, Sf* and *Ca* The highest TA value for the leaf extracts was *3.2 against Sa, Bs, Sf* and *Ca.* The TA values reflect a combination of antimicrobial potential and extractability of the biologically active constituents from the plant matrix. The root extracts ranked higher in this resprct.

Table 2. The percentage yield of the plant extracts in different solvents

S/N	Morphological part.	n-hexane	Ethyl acetate	Methanol
1.	Cp-R	1.43	2.30	4.98
2.	Cp -L	0.68	1.61	1.63

Cp-R (Calliandra portoricensis – Root);
Cp-L (Calliandra portoricensis – Leaf);

Table 3. Results of the phytochemical screening for *C. portoricensis* (leaf and root)

Secondary metabolite	Results	
	Root	Leaf
• 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 cyanogenic Glycosides		
• Test for Alkaloids	-	-
Meyer's reagent	++	+
Dragendorff's reagent	++	+
Hager's test	++	

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

Table 4. Result of antimicrobial susceptibility testing of various crude extracts of C. *portoricensis* leaf and root against selected human pathogens

S/N	Morphological part.	Solvent of extraction	Test Organisms used – MIZD (mm)																		
			Sa EXT.		CTR.	Ec EXT.		CTR.	Bs EXT.		CTR.	Kp EXT.		CTR.	Sf EXT.		CTR.	Ca EXT.		CTR.	An EXT.		CTR.
			40	20		40	20		40	20		40	20		40	20		40	20		40	20	
1	Cp (Root)	Hexane	15.00± 0.40	10.00± 0.30	23.00± 0.15	12.00± 0.25	5.00± 0.70	23.00± 0.23	14.00± 0.60	8.00± 0.60	30.00± 0.25	13.00± 10	10.00± 0.50	15.00± 0.85	15.00± 0.30	-	17.00± 0.45	17.00± 0.48	10.00± 0.25	15.00± 0.40	7.00± 0.60	-	13.00± 0.50
		Ethyl acetate	17.00± 0.35	11.00± 0.80	21.00± 0.15	13.00± 0.75	5.00± 0.70	15.00± 0.75	18.00± 0.20	7.00± 0.65	22.00± 0.30	10.00± 0.10	5.00± 0.40	14.00± 0.90	16.00± 0.85	9.00± 0.20	30.00± 0.35	28.00± 0.50	15.00± 0.80	15.00± 0.40	15.00± 0.15	5.00± 0.20	11.00± 0.60
		Methanol	14.00± 0.50	5.00± 0.40	15.00± 0.70	8.00± 0.10	6.00± 0.80	15.00± 0.25	15.000± 0.20	11.00± 0.25	17.00± 0.35	11.00± 0.50	4..00± 0.70	14.00± 0.65	18.00± 0.60	10.00± 0.40	35.00± 0.25	25.00± 0.55	15.00± 0.70	16.00± 0.15	10.00± 0.70	4.00± 0.50	13.00± 0.70
2	Cp (Leaft)	Hexane	12.00± 0.25	9.00± 0.80	35.00± 0.35	9.00± 0.50	11.00± 0.20	21.00± 0.20	15.00± 0.15	5..00± 0.50	21.00± 0.70	10.00± 0.60	5..00± 0.50	13.00± 0.85	15.00± 0.70	4..00± 0.30	23.00± 0.40	16.00± 0.65	11.00± 0.70	11.00± 0.80	6.00± 0.50	-	12.00± 0.30
		Ethyl acetate	15..00± 0.35	11.00± 0.50	24.00± 0.20	10.00± 0.70	4.00± 0.50	16.00± 0.10	15.00± 0.80	7..00± 0.40	10.00± 0.35	9.00± 0.25	4..00± 0.60	12.00± 0.60	14.00± 0.15	9.00± 0.40	35.00± 0.50	15.00± 0.40	9.00± 0.50	13.00± 0.45	8..00± 0.30	4.00± 0.20	11.00± 0.25
		Methanol	10.00± 0.50	6..00± 0.50	17.00± 0.60	11.00± 0.50	5.00± 0.50	21.00± 0.75	18.00± 0.85	5.00± 0.20	30.00± 0.45	12.00± 0.50	6..00± 0.30	10.00± 0.20	13.00± 0.30	7.00± 0.80	8.00± 0.30	18.00± 0.50	10.00± 050	15.00± 0.20	7.00± 0.80	4.00± 0.20	12.00± 0.60

Cp-R (Calliandra portoricensis – Root), Cp-L (Calliandra portoricensis – Leaf), MIC (mg/ ml) = Minimum Inhibitory Concentration.
Sa =Staphylococcus aureus, Ec = Escherichia coli, Sf = Streptococcus fecalis, Bs = Bacillus subtitlis, Kp = Klebsiella pneumoniae Ca = Candida albican, An = Aspergillus niger.
MIZD = Mean Inhibition Zone Diameter. (mm); EXT. = extracts and CTR = Control - Ciproflaxacin (40 µg / ml for bacteria) and Fluconazole (1000 µg / ml for fungi);
(-) = No inhibition of growth field; 10% aqueous DMSO (negative control with no inhibition).
Values were expressed as mean ± SEM; n=3; Analysis was processed with (SPSS-version 20.0) software and a one way (ANOVA) at $P \leq 0.05$

Table 5. Result of Minimum Inhibitory Concentration (MIC) and Total Activity (TA) for the sample extracts (leaf and root) and controls against the selected human pathogens

V	Morphological part	Solvent of extract	Test microorganisms													
			Sa		Ec		Bs		Kp		Sf		Ca		An	
			MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA	MIC	TA
1.	C.p (Root)	n-Hx	10.00±0.15	1.4	10.00±0.75	1.4	5.00±75	2.9	10.00±0.80	1.4	40.00±0.45	0.4	5.00±0.15	2.9	10.00±0.45	1.4
		Eto Ac	5.00±0.45	4.6	5.00±0.30	4.6	5.00±0.50	4.6	5.00±0.45	4.6	5.00±0.85	4.6	5.00±0.50	4.6	5.00±0.20	4.6
		MeOH	5.00±0.60	10.0	10.00±0.60	4.9	10.00±0.25	4.9	10.00±0.15	4.9	5.00±0.20	10.0	5.00±0.85	10.0	10.00±0.60	4.9
2.	C.p (Leaf)	n-Hx	10.00±0.75	0.7	20..00±0.35	0.3	10.00±0.40	0.7	10.00±0.75	0.7	10.00±0.70	0.7	10.00±0.35	0.7	40.00±0.45	0.2
		Eto Ac	5.00±0.45	3.2	10.00±0.80	1.6	5.00±0.45	3.2	10.00±0.60	1.6	10.00±0.40	1.6	10.00±0.80	1.6	20.00±0.70	0.8
		MeOH	10.00±0.30	1.6	20.00±0.45	0.8	10.00±0.70	1.6	20.00±0.40	0.8	5.00±0.20	3.2	5.00±0.70	3.2	40.00±0.30	0.4

Sa =Staphylococcus aureus, Ec = Escherichia coli, Sf = Streptococcus fecalis, Bs = Bacillus subtitlis, Kp = Klebsiella pneumoniae Ca = Candida albican, An = Aspergillus niger.
Cp-R (Calliandra portoricensis – Root), Cp-L (Calliandra portoricensis – Leaf), MIC (mg / ml) = Minimum Inhibitory Concentration
TA (ml/g) = Total Activity (Quantity of material extracted from 1 g of plant material in mg, divided by MIC in mg / ml .[44]
Values were expressed as mean ± SEM; n=3; Analysis was processed with (SPSS-version 20.0) software and a one way

4. CONCLUSION

The experimental data showed that the constituents of leaf and root samples as well as their respective antimicrobial patterns were qualitatively similar except for the presence of anthraquinone in leaf. Some adjustments need to be effected regarding the equivalent weights to approximately 50% in order to substitute root with leaf as antimicrobial medicinal agent. This switching may be necessary so as to avert an imminent extinction of *Calliandra portoricensis* shrub due to excessive harvesting capable of resulting in afforestation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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